Chapter 1

Introduction

1.1. History of Agriculture

In relation to the history of human life, it is interesting to note the proposition of Lee and DeVore (1968) that:

> "Cultural Man has been on earth for more than 2,000,000 years; for over 99% of this period he has lived as a hunter-gatherer. Only in the last 10,000 years has man begun to domesticate plants and animals, to use metals and to harness energy sources other than human body."

At the time people adopted their earliest lifestyle, that of hunter-gatherer, they probably occupied no more than about one third of the planet's land surface (Myers 1984). As cited by Harland (1975), Childe in 1925 reported that the period of agriculture's birth was known as "the Neolithic revolution", when the first shift from hunting and gathering to food production occurred. As people gradually improved the techniques they used in agriculture the transition from medieval to modern practices occurred (Janick, Schery, Woods, and Ruttan 1974). The emergence of new agricultural techniques in the seventeenth and eighteenth centuries can be traced to new crops, technology and economic changes. Consequently, during the eighteenth century the value of good farming rose tenfold. This increase was responsible for the growth of the urban population.

1.2. Pressures on agriculture

Since the late eighteenth-century human population increases has been dramatic, placing pressure on food production. The rate of increase of the latter must limit the rate of population growth (Janick, *et. al.* 1974), presaging famine for an overpopulated world with insufficient food supplies (Marini-Bettolo 1987). The need to increase food production to feed the ever-growing world population has been emphasised by many workers including Martin and Woodcock (1983); Kaeman (1984); Reed (1987).

In addition to the high rate of population growth, which in turn reduces the amount of land available for agricultural practices, the impact of pests, diseases and weeds contribute further to pressures on agriculture. These circumstances urge people to find appropriate means to minimise the problem to achieve the goals of sustaining and increasing yield while causing minimum damage to the environment. This means taking greater advantage of the biological interactions and natural cycles that are already at work or available to work in production systems. Changes in agricultural practices are shown in Figure 1.1.

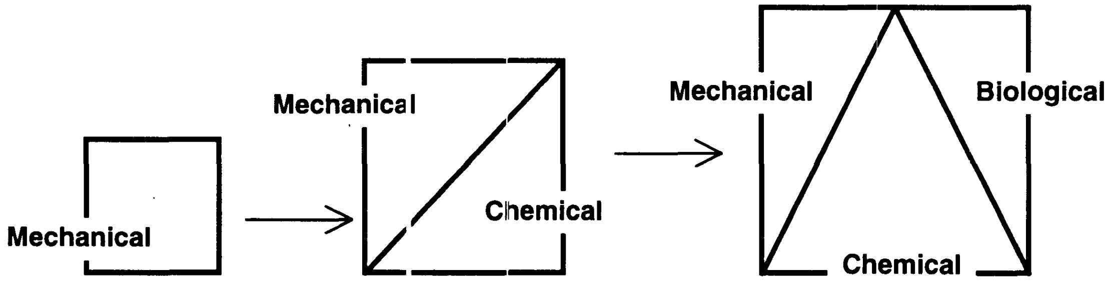

Figure 1.1. Evolution and current perception of the relationship among agricultural technologies (from Gardner *et al.* 1991).

The necessity to raise agricultural productivity has involved greater levels of input, greater intensity of cultivation and a simplification of cropping systems. All these changes can, and usually do, lead to increase pest problems, because organisms such as insects are efficient exploiters of simplified ecological situations (Olembo, 1987)

Methods of controlling pests and diseases have been devised including agricultural chemicals. These chemicals in the form of herbicides, fungicides, insecticides and other pesticides burgeoned following the spectacular commercial success of the insecticide DDT and, later, the broadleaf weed killer 2,4-D. Zadoks and Schein (1979) noted that application of agricultural chemicals had been chosen as the best choice to protect crops and Weinteringham (1984) added that, as a result of the use of pesticides, together with fertiliser, food quality has improved and the cost of food is less than it would otherwise be.

Despite their economic benefits, agricultural chemicals may also have negative impacts. Questions arise about the undesirable effects, direct and indirect, of new agricultural practices on the environment and on food (Lovett 1990). Chemicals can, for instance, lead to the emergence of resistant strains of pests and the resurgence of treated populations, outbreak of formerly secondary pests and population reductions of beneficial insects.

Pragmatically, pest and disease controls should conform to certain basic criteria related to effectiveness, safety and acceptability. Effective prevention or control of plant pests and diseases is essential for maximal crop productivity. Crop protection, ideally, should prevent damaging effects from pests, diseases and weeds economically, safely and in an environmentally sound manner. Every practical means available must be used to minimise the environmental impact of chemical use in agriculture. To date, Integrated Pest Management (IPM) is the best choice in crop protection. Olembo (1987) stated IPM as:

> "a pest management system that, in the context of the associated environment and population dynamic of the pest species, utilises all suitable techniques and methods in as compatible a manner as possible with a view to maintaining the pest populations at levels below those causing economic injury".

Even though IPM is the best choice, it retains chemical substances as one of its components because it is almost impossible to completely eliminate pesticides. Pryce (1983) and Saxena and Khan (1987) reported that new bioactive products which are pest-specific, nontoxic to humans and beneficial organisms, biodegradable, less prone to pest resistance and less expensive will be needed. Research found that the use of these products is very effective because the target sites are known and, eventually, they have less deleterious effects on nontarget species.

Compounds such as secondary metabolites, produced by various plant species, could play an extremely important role against pests (Menn 1983; Rosenthal 1986; Lovett 1990). These compounds might reduce pest injury by rendering plants unattractive or unpalatable (Bernays 1983; Menn 1983; Noris 1986; Swain 1977).

Moreover, it has been demonstrated that secondary metabolites affect microbial activities such as inhibition of oxygen consumption and inhibition of growth (Harborne 1990; Lovett 1991; Schutt and Netzly 1991; Boufalis and Pellissier 1994; Ryan and Jagendorf 1995).

This natural defence of plants involves several group of chemicals which are highly variable, from simple to complex molecules (Saxena and Khan 1987). The greater diversity of these chemicals is found in higher plants as compared to primitive ones, and is attributed to a vast array of secondary compounds such as terpenes, flavonoids and alkaloids. The latter are the most common.

Many plants produce alkaloids as their secondary compounds. Barley, for instance, has two major alkaloids, gramine and hordenine (Overland 1966) which have been proven have allelopathic effect on some plant species (Overland 1966; Lovett and Liu 1987; Liu and Lovett 1989; Reed and Jensen 1989; Lovett and Ryuntyu 1992). Moreover, Kianmatee (1993, personal communication) found that gramine decreased head width and body weight of armyworm (*Mythimna convecta*). It is not, therefore, unreasonable to suggest that gramine and hordenine may be used as plant self-defence agents against a range of pests, including mammals. While the effect of barley allelopathy has been widely studied with a range of organisms, vertebrates have been neglected.

The objective of these experiments was to examine the effect of gramine and hordenine as self-defence factors against vertebrates, exemplified by mice and broiler chickens as animal models. A positive outcome could enhance the long term use of self defence by crop plants as an alternative to pesticides.

Chapter 2

Literature Review

2.1. Allelopathy

The growth and development of plants are generally affected by both biotic and abiotic factors. The former include pests, diseases and weeds whereas the latter include soil, light, water and temperature.

Human activities in cultivating land have altered the environment and among plant species, weeds have proven to have very good adaptation to the disturbed environment in which they grow (Muzik 1970). Weeds have specific characteristics which enable them to survive unfavourably manipulated environments. Firstly, they appear year after year and their persistence is due, primarily, to the ability to produce numerous, long-lived, and easily transportable seeds (Muzik 1970; Duke 1985; Ross and Lembi 1985). In addition, weeds have the ability to compete successfully with crops for light, water, and nutrients (Aldrich 1984; Ross and Lembi 1985).

Competition occurs both above and below ground. Both weeds and crops compete for light, carbon dioxide, oxygen and space above ground. On the other hand, they compete for water and nutrients below ground (Harper 1977). Weeds may also release biological toxins which inhibit crop growth in the soil (Muzik 1970). These interaction between plants are known as interference (Harper 1977).

As cited by Harper (1977), Hall in 1974 proposed interference as an interaction between plants which may occur between individuals of the same species (intra-specific) or between individuals of different species (inter-specific). Harper (1977) further noted interference as the manner in which alterations in the environment are produced. Interference includes neighbour effects due to the consumption of resources in limited supply, the production of toxins or changes in conditions such as protection from wind.

Hall (1974) distinguished two types of interference between species, namely, "competitive interference" and "non-competitive interference". The condition in which one species directly affects the growth of the other by competing for a resource or resources is known as "competitive interference". Meanwhile, "non-competitive interference" means that resources available are used by one species in the early stages of its growth but not in other stages.

The word "allelopathy" was coined by Molisch in 1937 to describe biochemical interactions among all plants direct or indirect (including fungi and bacteria), both stimulatory and inhibitory influences (Rice 1984; Putnam and Tang 1986; Einhellig 1995a). Molisch's definition of allelopathy implies that allelopathy is a chemical process, dependent upon the addition of chemicals to the environment. Aldrich (1984) discusses one feature that distinguishes allelopathy from competition, in that something is being added to the environment, as opposed to something being removed from it. Putnam and Tang (1986) further clarified that it must be emphasised that allelopathy is caused by chemical substances, which are plant metabolites or their products present in the micro environment, such as the rhizosphere.

However, such substances are often involved in plant resistance against pest and disease and this offers a further dimension to allelochemicals (Bentley 1987). Interaction between plant allelochemicals and other organisms has broadened the appreciation of studying the possibility of these substances as chemical communicators that generate associations between plants and other organisms (Lovett, Ryuntyu and Liu 1989; Lovett 1991; Einhellig 1995a). Frequently, allelochemicals can be interpreted as agents of self defence in plants (Mizutani 1989; Petterson, Harris and Allen 1991).

2.2. The history of allelopathy

Allelopathic phenomena have been recorded for over 2000 years. As cited by Rice (1984), this was firstly documented by Theophrastus (300 BC) who observed that chick pea (*Cicer arietinum*) destroyed weeds surrounding it. Moreover, chick pea did not reinvigorate the ground as other related plants (legumes) do. Three centuries later, Pliny (Plinius Secundus, 1 AD) found that chick pea, barley, fenugreek (*Trigonella foenum-graecum*) and bitter vetch (*Vicia ervilia*) "scorched up" corn land. Another phenomenon was the "heavy shade" of black walnut (*Juglans nigra*) which causes headaches in people and injury to anything planted in the vicinity. This effect was due to an alkaloid, juglone, produced by the walnut tree.

DeCandolle, in 1832, found that thistle in the field injured oats; *Euphorbia* sp. and *Scabiosa* sp. injured flax, and rye plants injured wheat. He also suggested that exudates from crops and the effect of crop rotation contributed to the "soil-sickness" problem in agriculture (Rice 1984).

Even though the phenomena of allelopathy have been observed for over 2000 years, intensive studies on this subject have been carried out only since the early 1900s. During the twentieth century scientists have intensively broadened their appreciation of allelopathy between crops and weeds (Barnes and Putnam 1987; Harrison, Jr and Peterson 1991; Einhellig and Souza 1992); within crops (Hicks, Wendt, Gannaway and Baker 1989; Kato-Noguchi, Kosemura, Yamamura, Mizutani and Hasegawa 1994; Yu and Matsui 1994); and between plants and animals (Carlson and Breeze 1984; Cheeke 1988; Bourke and Carrigan 1993; Launchbaugh, Provenza and Burrit 1993).

2.3. Allelochemicals : secondary metabolites

Allelochemicals are identified as those non-nutritional compounds, synthesised by a plant species and able to affect growth, health, behaviour or population biology of another species either as a stimulator or an inhibitor (Whittaker 1970; Whittaker and Feeny 1971) and mediate broad-spectrum toxic reactions (Downum 1986). Allelochemicals are secondary metabolites (Swain 1977, Aldrich 1984) including an array of nonnutritive compounds (Cheeke and Shull 1985) which have no essential physiological function (Geissman and Crout 1969; Aldrich 1984). That is, they do not appear to play a role in the basic metabolism of organisms (Whittaker and Feeny 1971;

Rosenthal 1986), and are thus distinguished from the primary plant metabolites whose physiological functions have been elucidated (Beck and Reese 1976).

As secondary metabolites that evolved from physiologically unimportant to biologically important compounds (Mothes 1976; Mann 1987), allelochemicals are biosynthetically derived from the metabolism of carbohydrates, fats, and amino acids (Einhellig 1985) as side products of the processes leading to the synthesis of primary metabolites (Aletor and Res 1993). In discussing the origin and diversity of allelochemicals, Einhellig (1985) states that it is believed that allelochemicals arise from either the acetate or shikimate pathway, or their chemical skeletons come from a combination of these two.

Most secondary products are metabolically inert but high concentrations can be toxic to cells and organisms (Legg 1984) and there is considerable evidence that allelochemicals, as secondary metabolites, form part of the plant's chemical defence mechanism (Molyneux and Ralphs 1992) against diseases and/or pests (Swain 1977; Aletor and Res 1993).

Secondary metabolites cannot be considered as waste products because some of them function as key intermediates in the metabolism of other compounds, some play a role in embryo development, and others have great importance as mechanical constituents of cell walls (Legg 1984). It is acknowledged that these substances play an important role in plant ecology and are, therefore, important for plant survival in inappropriate environments (Swain 1977; Wink and Twardowski 1992). In addition, they function as growth regulators, insect repellents, or insect attractants (Harborne 1973).

Allelochemicals can affect the growth or aspects of function of the receiving species (Einhellig 1995b). These effects may be manifested through a wide variety of metabolic activities, which include cell division and elongation, action of inherent growth regulators, mineral uptake, photosynthesis, respiration, stomatal opening, protein synthesis, lipid and organic acid metabolism, membrane permeability and action of specific enzymes (Rice 1984; Chou 1989b). The main functions of allelochemicals seem to be chemical defence against micro organisms, phytophagous animals and other competing plant species (Legg 1984; Mizutani 1989; Petterson *et al.* 1991; Wink and Twardowski 1992).

Chemical defence is due to the inability of plants either to change the location of their existence or to the limitations of physical defence. To adapt themselves to the given environment they are equipped with defensive and offensive mechanisms. One defensive mechanism is releasing chemical substances as secondary metabolites (Mizutani 1989) which play an important role in the selection of foods by herbivores (Freeland 1991) and reduce the risk of predation (Petterson *et al.* 1991). The physiological effects of defensive compounds on herbivores have been classified relative to toxicity, inhibition of digestion and deterrence (Feeny 1976).

Whittaker and Feeny (1971) stated that the secondary compounds could be classified on biosynthesis grounds into five major categories *viz.* phenylpropanes, acetogenines, terpenoids, steroids and alkaloids. They pointed out that the phenylpropanes and alkaloids originate from a small number of amino acids and the rest originate, generally, from acetate. Generally, secondary metabolites contain one or more nitrogenous atoms that are part of cyclic systems (Harborne 1973).

2.4. Release of allelochemicals from plants

Allelochemical substances are present in virtually all plant tissues, including leaves, stems, roots, rhizomes, flowers, fruits and seeds (Rice 1984; Duke 1985), but the most important sources are leaves and roots (Rice 1984). They may be released from plant tissues in a variety of ways including volatilisation, root exudation, leaching and decomposition of the plant residues (Aldrich 1984; Rice 1984; Chou 1989a).

The release of allelochemicals from plant to the environment is illustrated in Figure 2.1. and the methods of release will be discussed in turn.

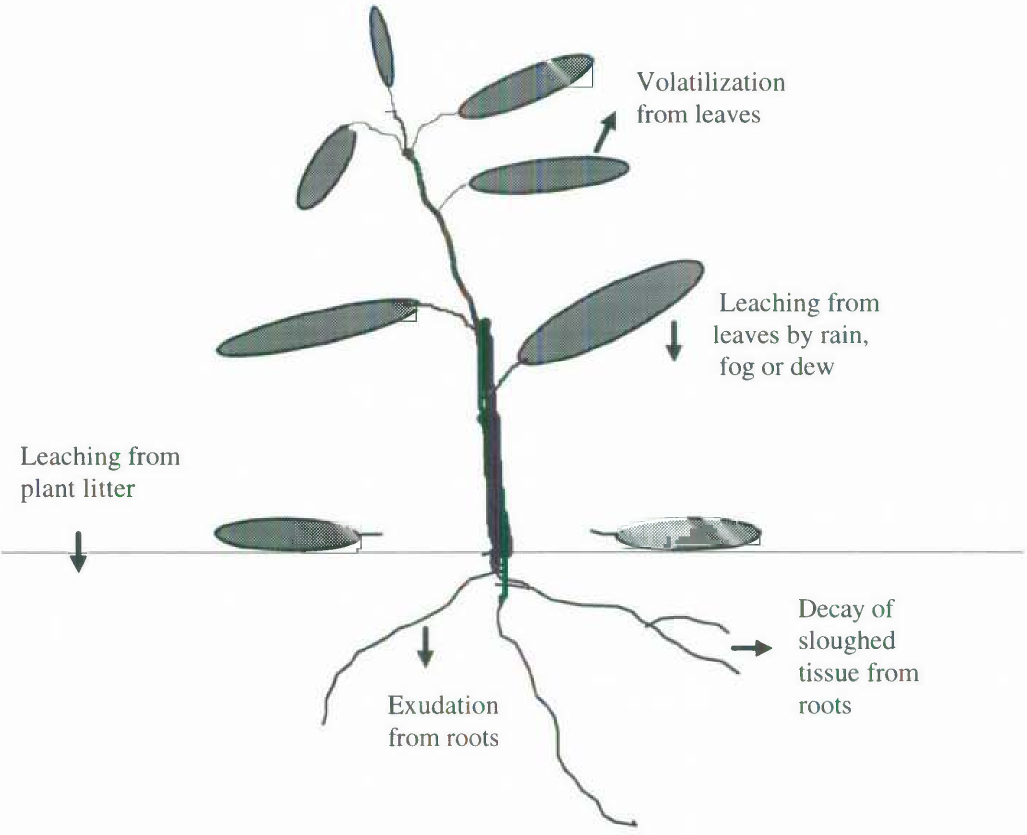

Figure 2.1. The production and release of allelochemicals from plant to the environment (from Burke 1987)

2.4.1. Volatilisation

Volatile allelochemicals may move through the atmosphere from a donor plant to a receiving species. Alternatively, compounds are adsorbed on soil particles and solubilised in the soil solution (Einhellig, 1985).

Rice (1984) cites the work of Molisch in 1937 in his *Der Einfluss einer Pflanze auf die andere-Allelopathic* in which ethylene, a volatile substance released from apple fruits, was shown to inhibit the growth of many plants. Plant leaves are able to produce volatile organic compounds that have allelopathic effects. In the genus *Artemisia,* five major terpenoids and three minor ones have been identified in the leaves. Of these compounds, camphor and 1,8-cineole were the most toxic (Halligan 1975). Species of *Salvia* produce similar volatile organic compounds which have allelopathic effects (Lovett 198[illegible]).

del Moral and Muller (1970), worked on *Eucalyptus camaldulensis* to identify volatile compounds which have allelopathic effects. They found four volatile terpenes, which are toxic to plant growth, produced by fresh leaves of *E. camaldulensis.* They also found that two of the compounds, α-pinene and 1,8-cineole, were in soil under the *Eucalyptus* canopy.

Volatile compounds from some species have also been observed in relation to their effect on micro organisms. For example, Menzies and Gilbert (1967) reported that some fungi responded by spore germination, increased respiration and growth, when they were treated with volatile components from alfalfa tops, corn leaves, wheat straw, bluegrass (*Poa pratensis*) clippings, tea (*Thea sinensis*) leaves and tobacco leaves.

2.4.2. Root Exudation

Research has also shown that roots of some living plants exude allelopathic substances. As cited by Fay and Duke (1977), Martin and Rademacher reported in 1960 that scopoletin and its glucoside (glucose-7-scopoletin) were exuded from oat roots after radicle protrusion. Fay and Duke (1977) reported that three thousand accessions of the United States Department of Agriculture World Collection of *Avena* germplasm were screened for their ability to exude scopoletin (6-methoxy-7-hydroxy coumarin), a naturally occurring compound shown to have root growth inhibiting properties. They found twenty-five accessions which exuded more blue-fluorescing materials than a standard oat cultivar (*Avena sativa* L. cv. 'Garry').

Overland (1966) found that gramine, the alkaloid identified from barley, had an inhibitory effect on the growth of *Stellaria media* (L.) Cyr. Aqueous leachates of seeds and roots of barley also caused inhibition of germination and growth of *Capsella bursa-pastoris* (L.) Medic and *Nicotiana tabacum* L. Living plants and aqueous leachate of living roots were more inhibitory than preparation from dead plants.

Giant foxtail (*Setaria faberii* Herrm.) has also been noted as having inhibitory effects on corn growth through root exudation. Bell and Koeppe (1972) reported that, when corn was seeded into pots with 6-week-old giant foxtail, corn height, fresh weight and dry weight were reduced by as much as 90%. Further, mature giant foxtail inhibited the growth of corn approximately 35% through an allelopathic mechanism. Another weed, *Pluchea lanceolata* (DC) C. B. Clarke, exuded phenolic compounds through the soil and significantly affected asparagus bean (*Vigna unguiculata* var. *sesquipedalis*). These water-soluble compounds reduced seed germination, number of nodes, internode lengths, nodule number and weight and chlorophyll *a* and *b* and chlorophyll *a/b* ratio of the asparagus bean plants (Inderjit and Dakshini 1992).

Sunflower (*Helianthus annuus* L.) has also been reported to have allelopathic effects. Leather (1983) investigated the potential of sunflower to suppress the germination and growth of wild mustard [*Brassica kaber* (D.C.) L.C. Wheeler var *pinnatifida* (Stokes) L.C. Wheeler]. Even though they were less effective than leaf and stem tissue leachates, sunflower root exudates inhibited seedling growth of wild mustard. Leather (1983) also cited the work of Dzubenko and Petrenko in 1971 who reported that root exudates of *Lupinus albus* L. and *Zea mays* L. inhibited the growth of common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.).

2.4.3. Leaching

Allelochemicals which occur in living plants or in residues of such plants can leach out in appreciable amounts due to rain, dew, or fog. Bell and Koeppe (1972) investigated the allelopathic potential of giant foxtail (*S. faberii* Herrm.) on the growth of corn. They used a stairstep apparatus to determine the interaction between corn and giant foxtail seedlings, mature giant foxtail, whole dead giant foxtail plants, and macerated dead giant foxtail leaf and root material. They found that phytotoxins leached from the root of mature giant foxtail resulted in reduction in weight of corn plants by 35% and that chemicals leached from dead giant foxtail reduced corn growth by as much as 50%.

Workers in India investigated the allelopathic effects of *Prosopis juliflora,* a plant which is highly allelopathic and does not allow the growth of any other species around it (Goel, Saxena and Kumar 1989). They found that leaf extracts and leaf leachate of *P. juliflora* were inhibitory due to phenolic compounds leached from the plant. del Moral and Muller (970) found ten phenolic inhibitors in leachate of the litter from *Eucalyptus camaldulensis* and succeeded in identifying five of them as caffeic, chlorogenic, *p*-coumaric ferulic and gallic acid.

In Taiwan, Chou (1989a) evaluated the phytotoxicity of plant leachate from the following grasses: *Brachiaria mutica*, *Digitaria decumbens*, *Imperata cylindrica* var. Major and *Panicum repens*. Aqueous leachates of each grass was used to water plants of the four grasses in pots. It was found that the leachates exhibited variable inhibition of grass growth as compared to a tap water control. Laboratory bioassay showed that the leachates had a significant phytotoxic effect also on early root growth of ryegrass (*Lolium multiflorum*) and lettuce (*Lactuca sativa* var. Great Lakes 366).

2.4.4. Decomposition of plant residues

Chemical compounds can be released by plant residues into the environment during the process of decomposition. The chemicals either stimulate or inhibit the growth and development of other plants. Overland (1966), in examining allelopathic effects of barley seed leachates, found that the germination of *Stellaria media* (L.) Cyr. was distinctly inhibited by barley leachates. In addition, dead barley root leachates inhibited *Stellaria* germination. There was a positive correlation between age of dead barley root with percentage of inhibitory.

Bhowmick and Doll (1982) reported their finding that residues of common ragweed (*Ambrosia artemisifolia* L.), velvetleaf (*Abutilon theophrasti* Medic.), and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] inhibited corn and soybean growth in the greenhouse. The inhibition was greater in a double-pot watering method than with sub surface and surface watering methods. In the field, Bhowmick and Doll (1982) found that residues of barnyardgrass and giant foxtail reduced corn yield. Soybean yield reductions occurred, ranging from 14 to 19%, with common lambsquarters (*C. album* L.), redroot pigweed (*A. retroflexus*), velvetleaf, soybean, and sunflower (*H. annuus* L.) residues.

Five allelochemicals *viz.* ferulic, *p*-coumaric, *p*-OH benzoic, syringic, and vanillic acids were identified from the wheat mulch and its associated soil. Various concentrations of ferulic and *p*-coumaric acid significantly reduced germination and radicle growth of radish (*Raphanus sativa* L.) in bioassay (Lodhi, Bilal, and Malik 1987). Lodhi *et al.* (1987) cited the work of Wang *et al.* in 1967 who isolated five different phenolic acids from sugarcane-associated soils. The concentration of inhibitors found in many soils was sufficient to decrease the growth of wheat, corn and soybean when applied to plants growing in nutrient culture solutions.

Read and Jensen (1989) extracted soluble substances from alfalfa soils, fallow soil and soil where a cereal was the previous crop. In germination chamber experiments they found that water-soluble substances present in methanol extracts of soil cropped to alfalfa or barley decreased seedling root length of alfalfa L-720, winter wheat (*Triticum aestivum* L. Nugaines) and radish (*R. sativa* L.).

Lovett and Jessop (1982) investigated the residual effects of the following crops: field pea (*Pisum sativum* L. var. *arvense* (L.) Poir), field bean (*Vicia faba* L.), soybean (*Glycine max* (L.) Merril), lupin (*Lupinus angustifolius* L.), chickpea (*Cicer arietinum* L.), safflower (*Carthamus tinctorius* L.), sunflower (*H. annuus* L.), rapeseed (*Brassica napus* L.), sorghum (*Sorghum bicolor* (L.) Moensch), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and wheat (*Triticum sativum* L.). They used wheat as the test species and found that crop residues significantly reduced wheat growth in glasshouse, laboratory and field experiments. Moreover, phytotoxicity was increased when crop residues were incorporated into soil rather than being left on the surface.

Einhellig and Leather (1988) reported that strip cropping which included sorghum showed that in the subsequent year weed density and biomass were significantly lower in the previous-year sorghum than in soybean strips. From these experiments it is clear that crop residues in the field may suppress weed growth.

The converse may, of course, occur. Thus, Bhowmick and Doll (1979) reported that water extracts of barnyardgrass (*E. crus-galli* L.) residues inhibited hypocotyl growth of soybean by 5 to 24% and reduced the increments in shoot fresh weight by 25 to 49%. They also observed that water extracts of residues of redroot pigweed (*A. retroflexus*), common weed (*A. artemisifolia*), hoary alyssum (*Barteroa incana*), white cockle (*Lychnis alba*), fall panicum (*Panicum dichotomiflorum*) and green foxtail (*Setaria viridis*) inhibited growth of one or more of the following: radicle growth of corn, coleoptile growth of corn, or hypocotyl growth of soybean.

Bitter sneezeweed (*Helenium amarum*) has also been noted for its allelopathic effects. Aqueous extracts of bitter sneezeweed leaves reduced alfalfa and Italian ryegrass (*L. multiflorum* L.) seedling growth by as much as 50% at a concentration of

0.5% (w/v). Furthermore, leaf extracts were more phytotoxic than either stem or root extracts and seedling growth was reduced more than seed germination (Smith 1989).

2.5. Transport of allelochemicals in the environment

As addressed in the previous section, allelochemicals are released from plants in different ways. A key element in allelopathy is the transfer of chemicals through the environment from one organism to another (Einhellig 1995b). The transport of the allelochemicals and the interactions with other factors in the environment add more to the complexity of allelopathy. Once the chemicals enter into the environment a number of interacting processes such as retention, transformation and the transport process take place (Cheng 1992).

The retention process implies a restriction of the mobility of allelochemicals in the soil. It either removes the chemicals from being transported or merely retards chemical transportation (Cheng 1992; Cheng 1995). Accordingly, the transformation process can render a chemical ineffective in its allelopathic functions (Cheng 1995) and implies change to the allelochemical structure of the original molecule. It is possible that the transformed product may be more toxic or less toxic (Cheng 1992).

Transportation which can be either through the air as a vapor or droplet, or in the soil solution, is a key of allelopathic process. The process is affected by many factors, such as the stability of the allelochemical and the properties of the soil. The integrated process of transportation of allelochemicals from the site of production to the target plant is diagramatically presented in Figure 2.2.

2.6. Factors affecting the production and activity of allelochemicals

The mechanisms of allelopathy are not well understood. Although there is an extensive phenomenological literature there is still a lack of knowledge and understanding on how exactly allelochemicals alter growth of receiving plants or other organisms (Einhellig 1995b).

Allelochemicals can interfere with the growth of receiving plants through different ways such as photosynthesis (Patterson 1981; Mersie and Singh 1993), chlorophyll content (Ramirez-Toro, Leather and Einhellig 1988; Lotina-Hennsen, Bernal-Morales, Romo De Vivar, Perez-C, Castro-R and Aguilar Martinez 1992; and Hejl, Einhellig and Rasmussen 1993) and respiration (Stenlid 1970; Demos, Woolwine, Wilson and McMillan 1975; Rasmussen, Hejl, Einhellig and Thomas 1992; Hejl *et al.* 1993; and Pellessier 1993). The complexity of these phenomena involves other abiotic and biotic factors as well.

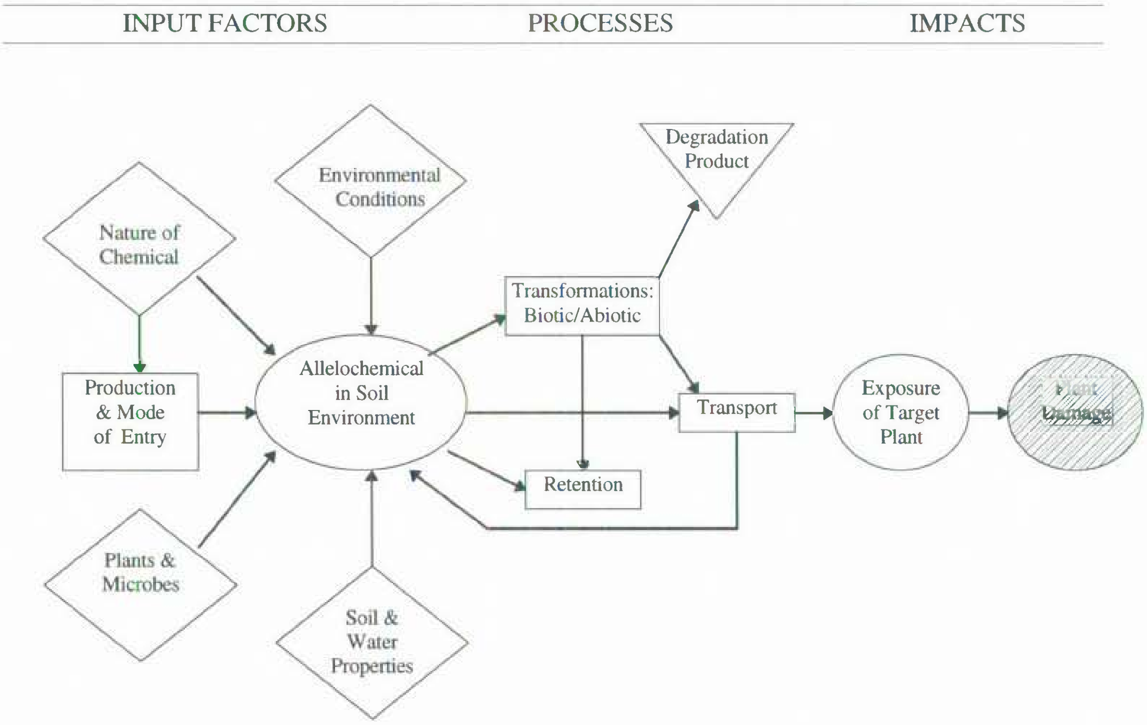

Figure 2.2. A relationship of the factors and processes affecting the transport of allelochemicals from the site of production to the target plant. (from Cheng 1995).

The concentration and activity of allelochemicals in plants can vary with plant phenology and several factors in the environment in which they grow (Klein and Miller

1980; Aldrich 1984; Rice 1984; Duke 1985 and Launchbaugh *et al.* 1993). Tang, Cai, Kohl and Nishimoto (1995) noted that biotic and abiotic stress may enhance the effect of allelopathy. Their work indicated that water stress enhanced phytotoxicity of allelochemicals in both plant tissue of purple nutsedge (*Cyperus rotundus* L.) and in the rhizosphere. Similarly, water deficit increased the amount of DIBOA and its precursor, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (DIMBOA) in germinating corn (Richardson and Bacon 1993). Likewise, the glucosinolate (mustard oil glycoside) content of *Cleome serrulata* in drought-stressed plants was three or four times greater than those of normal plants (Louda, Farris and Blua 1987). Gramine content of barley, however, was not greatly affected by water stress (Hanson, Traynor, Ditz and Reicosky 1981; Salas and Corcuera 1991).

The effects of temperature, alone or with other factors, have been documented. Gramine content, for example, increased when barley was grown at high temperature (Hanson *et al.* 1981; Hanson, Ditz, Singletary and Leland 1983; Salas and Corcuera 1991). This might be an adaptive metabolic response to high temperature. In addition, when barley seedlings were subjected to a mixture of phenolic acids and grown at different temperature, the inhibition of root growth was greatest at the extremes of low and high temperatures (Glass 1976). Kennedy and Bush (1983) demonstrated that the high and low temperature regimes depressed alkaloid accumulation in tall fescue (*Festuca arundinacea* Schreb.).

Nitrogen availability in the soil determines the production of allelochemicals. For instance, the amount of gramine in barley leaves increased with higher nitrogen fertilisation (Marten, Simon and Frelich 1974). Similarly, NO_3^- concentration in the nutrient solution was positively correlated with the gramine content of barley (Salas, Corcuera and Argandona 1990). In addition, aphid population growth rate, reared on barley leaves, decreased with the increase of NO_3^- concentration. However, Marten *et al.* (1974) found that an ammonium source of nitrogen (NH_4Cl or urea) induced greater alkaloid, including gramine, concentrations in reed canarygrass than did a nitrate source (Na NO_3) due to direct use of NH_4^+ for alkaloid synthesis by plants. In contrast, NH_3^- was more functional for overall growth.

Similarly, Bryant, Clausen, Reichardt, McCarthy and Werner (1987) demonstrated that nitrogen fertilisation decreased concentration of condensed tannins and phenolic glycosides in quaking aspen (*Populus tremuloides* Michx.) which in turn increased larval growth of the large aspen tortrix [*Choristoneura conflictana* (Walker)]. This result indicated that plant carbon/nutrient balance influences the quality of aspen leaves as food for the insect in two ways, by increasing the concentration of positive factors such as nitrogen and decreasing the concentration of negative factors (carbon-based secondary metabolites) in leaves.

As cited by Einhellig (1987), the work of Einhellig and Eckrich in 1984 demonstrated that soybeans were more sensitive than sorghum to both temperature and ferulic acid. The weight of soybeans grown under day temperature 34°C and 0.1 mM ferulic acid were significantly reduced by 37%. Therefore, relatively hot environmental conditions enhanced allelochemical inhibition (Einhellig 1987). Lovett, Hoult and Christen (1994) reported that lower light intensities increased hordenine production of barley.

Apart from abiotic factors, biotic factors such as involvement of other organisms also influence the production and activity of allelochemicals. For example, many cases of allelopathy either directly or indirectly involve microbes (Rice 1984; Lovett 1987). In addition, herbivory or mechanical damage which stimulates herbivory can significantly increase the levels of allelochemicals in plants within a few hours or days (Karban and Myers 1989).

Tall fescue (*F. arundinacea*), infected with the fungal endophyte *Acremonium coenophialum*, contains both ergot-type and saturated-pyrrolizidine (loline) alkaloids. The infestation of the endophyte has been associated with insect pest resistance of tall fescue due to the presence of the alkaloids (Petroski and Powell 1991). Neither type of alkaloid is found in tall fescue when the endophyte is absent.

Harborne (1988) demonstrated that the concentration of the *p*-hydroxycinnamic and ferulic acids rise in the salt grass (*Distichlis stricta*) changed when consumed by the herbivorous rodent *Microtus montanus*. However, the phenolic acids, in turn, could have dramatic effects on animal reproduction.

The alkaloid contents of wild tobacco (*Nicotiana sylvestris* Spegazzini and Comes) subjected to damage by larvae of *Manduca sexta* were significantly higher than the alkaloid contents of undamaged plants (Baldwin 1988 and 1989) and these alkaloidal responses reduced the growth of attacking herbivores (Baldwin 1991). Similarly, the concentrations of cucurbitacins B and D in *Cucurbita pepo* 'Black' leaves significantly increased within 3 hours after mechanical injury by squash beetles (*Epilachna borealis*). This finding suggested that the alteration of leaf chemistry has deleterious effects on *E. borealis* fitness (Tallamy 1985).

The allelopathic effects of *Eucalyptus* trees can be mediated by insect grazing on foliage through modification or transmitting toxic allelochemicals which present in leaves (Silander, Trenbath and Fox 1983). This biochemical changes could influence the feeding and growth of phytophagous insects (Baldwin and Schultz 1983) such as the accumulation of an inhibitor of the animal proteinase chymotrypsin and trypsin which may be regulated to make the plant less palatable, and perhaps lethal, to the invading insects (Green and Ryan 1972).

However, changes in allelochemical concentration in plants due to living-organism invasion could weaken the plant defence. For example, physiological and chemical alteration in birchs (*Betula pendula* and *B. pubescens*) occured in the twigs after being browsed by moose (Danell and Huss-Danell 1985). This decrease in defence was reflected by these birchs having more ants, psyllids, leaf-galls and other leaf-eating insects compare to control birchs (unbrowsed).

Another case reported was foliage of heavily browsed *Acacia nigrescens* which was higher in nutrients and lower in condensed tannins than foliage of lightly browsed trees (Du Toit, Bryant and Frisby 1990). It is assumed that severe pruning by browsing reduces intershoot competition for nutrients, promoting rapid shoot regrowth. Carbohydrate demands of rapid regrowth reduce carbon-base secondary metabolites synthesis which, in turn, result in higly palatable leaves that attract further browsing (Danel and Huss-Danell 1985; Du Toit *et al.* 1990).

2.7. Activity of allelochemicals

In discussing allelopathy it is worth referring to Aldrich (1984) who proposed two types of allelopathy, namely, true and functional allelopathy. The former refers to toxic chemicals once they have been released from plants, whereas the latter refers to toxic chemicals once they have been released and transformed by microorganisms. In allelopathic interactions, however, it is not clear whether reduced crop yield is due to direct effects of released toxins or whether the toxins may precondition the crop plant to be invaded by plant pathogens (Duke 1985).

Allelopathy, which is postulated to be one mechanism by which weeds affect crop growth (Bell and Koeppe 1972; Rice 1984), is also one of the various components that determines the pattern of vegetation which, in turn, leads to change in the environment (Aldrich 1984). The change which occurs depends upon concentration of the chemicals, the nature of the chemicals and the period of time the chemicals are in the environment (Hale and Orcutt 1987). These changes may, therefore, be important for plant survival (Swain 1977; Wink and Twardowski 1992). In addition, allelochemicals influence plant succession, inhibit nitrogen fixation and nitrification, determine the pattern of plant species, prevent seed decay and germination and lead to replanting problems (Duke 1985).

2.7.1. Effects of allelochemicals on weed management

Concerns about using pesticides (including insecticides, fungicides and herbicides) because of their effects on the environment have been addressed in Section 1.2. These chemicals may destroy both the target and non-target organisms and also have residual effects in water and soil. Accordingly, much research has been carried out in an effort to minimise the use of chemicals in agriculture. The possibility of using allelochemicals from some crop species to suppress weed growth is an example of such work.

Klein and Miller (1980) cited the work of Funke in 1941 who pioneered the work of investigating crop phytotoxins as "natural herbicides". Funke conducted his studies on beet (*Beta vulgaris* L.) seeds and concluded that beet seeds produce allelopathic agents which inhibited the growth of corn cockle (*Argostemma githago*). Further studies by Funke indicated that allelopathic activity of crops may be involved in determining the presence or absence of certain weed species.

Barnes and Putnam (1987) isolated two phytotoxic compounds [2,4-dihydoxy-1,4(2H)-benzoxazine-3-one (DIBOA) and 2(3H)-benzoxazolinone (BOA) from rye (*Secale cereale* L.). They reported that these compounds showed allelopathic activity by suppressing growth of redroot pigweed and inhibiting emergence of barnyardgrass, when applied to soil. Even though barnyardgrass emerged from the soil it appeared to be stunted and chlorotic. Einhellig and Leather (1988) cited the work of Schilling *et al.* in 1985 who found that prior conditioning with either rye mulch or root residue before tobacco, soybean and sunflower were planted, without tillage, into desiccated rye mulch (fall-planted, spring-killed) gave over 90% reduction in the biomass of common lambsquarter, redroot pigweed and common ragweed (*Ambrosia artemisifolia* L.). Perez and Ormeno-Nunez (1991) reported that rye root exudates inhibited root growth of wild oats (*Avena fatua* L.).

Sorgoleone (ρ-benzoquinone), which derives from root exudates of *Sorghum bicolor,* has an inhibitory activity on weed growth. Einhellig and Souza (1992) reported that bioassay indicated 125 μM sorgoleone reduced radicle elongation of *Eragrotis tef* (Zucc) Trotter, a forage crop commonly known as teff. Fifty μM sorgoleone treatments stunted the growth of *Lemna minor* L. It was also reported that sorgoleone reduced the growth of velvetleaf, redroot pigweed, jimsonweed, crabgrass and barnyardgrass.

2.7.2. Effects of allelochemicals on other organisms

Studies have proven that allelochemicals can affect other organisms. Inderjit and Dakshini (1991) investigated allelopathic effects of cogongrass [*Imperata cylindrica* (L.) Beauv.] in its environment. They demonstrated that phenolic acids produced by cogongrass decreased the number of colonies of the soil fungi *Aspergillus fumigatus*, *A. niger* and *A. candidus*. Moreover, they found that inhibition of nitrogen fixation of *Melilotus parviflora* Desf. occured. Another study indicated that root exudates of tobacco inhibited the growth of black shank (*Phytophthora parasitica* var. nicotianae) fungus. Chlorogenic acid caused 25% inhibition whereas scopoletin produced 39% inhibition of fungal growth at 4000 ppm and 1000 ppm dosage, respectively (Snook, Chortyk and Csinos 1991).

Another significant finding was reported by Miles, Meideros, Chen, Chittawong, Swithenbank, Lidert, Payne and Hedin (1991) who demonstrated that matteucinol (5,7-Dyhydroxy-6,8-dimethyl-4-methoxyflavone), isolated from the root of *Miconia cannabina*, showed significant (135%) antifungal activity against *Pythium ultimum*. Miles *et al.* (1991) also reported that medicarpin, which was extracted from the wood of *Dalbergia monetaria*, possessed an antifungal activity against *Rhizoctonia solani* and *Helminthosporium teres.*

Wink and Twardowski (1992) demonstrated the ability of some secondary metabolites to inhibit bacterial growth. They found that antibiotic activity *of Bacillus* *subtilis* and *Escherichia coli* was clearly exhibited at 0.1% concentration by some alkaloids such as quinidine, quinine and ephedrine.

When a plant is attacked by a predator it can not run. (Rosenthal 1986). Different mechanisms have been found in plants as ways to protect themselves against pathogens and herbivores, such as anatomical structures and the accumulation of secondary metabolites (Freeland and Janzen 1974; Levin 1976 and all related references quoted therein; Ryan and Jagendorf 1995). These defensive chemicals affect different animals in different ways.

One common form of self-defence mechanisms is that the plant renders itself unpalatable to and/or indigestible by the herbivores when the leaves are consumed (Levin 1976; Rosenthal 1986; Ryan and Jagendorf 1995). Rosenthal (1986) suggested that the customary way of determining the defensive capacity of a higher plant's allelochemicals is to demonstrate their toxicity. He further suggested the use of one or more of a variety of insects that have come to be accepted as standard reference species in evaluating biological toxicity, such as the black swallowtail butterfly (*Papilio polyxenes*).

Defence against herbivorous insects is among the roles of allelochemicals. For instance, Westcott, Hinks and Olfert (1992) reported that some secondary plant compounds affected nymphs of the migratory grasshopper, *Melanoplus sanguinipes* (F.). Among 22 compounds tested in a 5-day bioassay they found that saponin, a terpenoid from *Gypsophila* spp., significantly decreased the survival of grasshopper nymphs. Other workers found that the weight of tobacco budworm [*Heliothis virescens* (L.)] larvae was reduced by 90% when animals were fed diets containing 0.2% - 1% of eight alleged allelochemicals of cotton (Jenkin, Hedin, Parrot, McCarty, Jr. and White 1983).

Pyrrolizidine alkaloids, which are found mainly in plants of the families Boraginaceae, Compositae and Leguminosae cause poisoning in vertebrates. Livestock which consumed *Crotalaria* spp. experienced intoxication leading to pulmonary damage, whereas impaired liver function and irreversible liver damage occured when livestock ate *Senecio* spp. (Sharrow and Mosher 1982). Both *Crotalaria* and *Senecio* spp. produce pyrrolizidine alkaloids (Cheeke 1988). Other forage grasses, such as *Phalaris* spp., produce tryptamine and carboline alkaloids which cause neurological damage in livestock (Cheeke 1995).

Pyrrolizidine alkaloids have been demostrated to be involved in toxicity in different species of animals such as hepatic damage in livestock (Cheeke 1988) and rats (Peterson and Jago 1984), significant mortalities of cattle and horses (Harper, Walker, Krahenbuhl and Christie 1985; Lessard, Wilson, Olander, Rogers and Mendell 1986; Mendell, Witt, Gitchell, Gribble, Rogers, Segall and Knight 1988), hepatic damage in horses (Lessard *et al.* 1986), liver megalocytosis and skin lesions in yaks (*Bos grunnies*) (Winter, Seawright, Mattocks, Jukes, Tshewang and Gurung 1990) and clinical sign of depression, anorexia and inactivity in broiler chickens (Pass, Hogg, Russell, Edgar, Tence and Rikard-Bell 1979).

Peterson and Jago (1984) incorporated food pellets containing *Echium plantagineum* into rat diets. They found that at 20 and 40% level of *Echium*, a high mortality of rats from pyrrolizidine alkaloid poisoning occured from acute haemorrhagic necrosis of the liver. Other workers demonstrated that pyrrolizidine alkaloid of *Heliotropium europaeum* caused degenerative lesions in the liver of chickens and ducks (Pass *et al.* 1979). The alkaloid riddelliine, a member of a class of pyrrollizidine alkaloids, reduced body weight gains in both rats and mice and caused developmental toxicity in rodents (Chan, Mahler, Bucher, Travlos and Reid 1994).

McKenzie, Newman, Rayner and Dunster (1988a) reported that prickly paddy melon *(Cucumis myriocarpus*), an annual vine native to sourthen Africa but widely distributed in subtropical and temperate mainland Australia, caused a serious mortality in cattle which ate the fruit of *C. myriocarpus*.

Grazing animals consumed *Solanum glaucophyllum* and *Cestrum diurnum*, native plants to the West Indies, led to the identification of related calcinogenic, the ability to induce vitamin D intoxication. Consumption of *S. glaucophyllum* leaves has been shown to be the cause of calcinotic desease of cattle. Similar form of toxicity in horses and cattle occured in Florida when the animals consumed *C. diurnum* (Weissenberg 1989).

2.8. Interpretation of the effects of allelochemicals

As discussed in Section 2.3, allelochemicals do not have essential physiological functions (Geissman and Crout 1969; Aldrich 1984), that is they do not appear to play a role in the basic metabolism of organisms. In addition, their very diversity of structure and distribution among living organisms has led the idea that they are waste products of metabolism (Muller 1969; Luckner 1972). However, this classic view of waste products has been replaced over recent years by one in which they are regarded as being essentially beneficial to the producer (Harborne 1990). Accordingly, there are substantial data supporting the view that secondary metabolites exist in plants in a state of dynamic equilibrium and are not static end products of metabolism (Seigler and Price 1976) and play a key role in survival of producing plants (Bell 1981).

Allelopathy is a complex phenomenon because it is not only a direct interaction between allelochemicals of the donor plant and the recipient organism but involves other biotic and/or abiotic factors. Accordingly, many chemical ecologists have addressed the role of allelochemicals in ecological processes in which allelochemicals affect the biotic environment (Rosenthal 1986; Harborne 1990; Klein and Blum 1990; Lawrence, Colwell and Sexton 1991; Mahdavi, Solomon and Hubert 1991) and the abiotic environment such as availability of essential minerals and mineral uptake by plants (Klein and Miller 1980; Cheng 1992; Rice 1992).

In discussing the effects of allelochemicals on the biotic environment, it is worth emphasising allelochemicals as self defence agents. Many workers have studied these phenomena as they are aware of the ecological impact of using agricultural chemicals intensively. Evidence has proven that allelochemicals protect the producing plants, either directly or indirectly, by reducing the growth and development of other plants (Barnes and Putnam 1987; Einhellig and Souza 1992; Stobiecki, Ciesiotka, Peretiatkowicz and Gulewick 1993; Kato-Noguchi *et al.* 1994; Yu and Matsui 1994) and protecting them from other organisms (Swain 1977; Rosenthal 1986; Harborne 1988; Krischick, Barbosa and Reichelderfer 1988; Harborne 1990; Cheeke 1995; Stegelmeier, James, Panter and Molyneux 1995).

It has been widely proven that various secondary metabolites, allelochemicals, contribute an important value to the producing plants and have a broadly defensive role (Harborne 1990). Australian native species of *Eucalyptus globulus* ssp. *bicostata* affected the growth of chewing fescue, *Festuca rubra* var. *fallax*, (Silander *et al.* 1983). In addition, del Moral, Willis and Ashton (1978) demonstrated that *E. baxterii* inhibited the growth of *Leptospermum myrsinoides* and *Casuarina pusilla* due to several secondary compounds such as gallic and caffeic acids, glycosides and terpenoids.

The allelochemicals present maintain the overall integrity of the plants against competitors, predators and pathogens (Swain 1977). In discussing secondary metabolites as protective agents, Swain (1977) further emphasised that they may only enhance the survival value of the plant by a very small percentage, however, such small changes could lead to survival or extinction in nature. Having accepted this concept, it is expected that the perception of allelochemicals being a waste product would be subsumed by a better understanding of exploring their beneficial value.

2.9. Occurrence of allelopathy in crop plants

There are many data showing that crop plants, both broad-leaved and narrow-leaved, produce secondary metabolites which allelopathically affect other organisms such as weeds, other crops and animals. Sweet potato, for instance, reduced the growth of yellow nutsedge by more than 50% at 8 and 12 weeks after planting. In contrast, the presence of yellow nutsedge did not markedly affect sweet potato growth (Harrison, Jr. and Peterson 1991). These workers found that the most polar fraction of serially extracted sweet potato periderm tissue was higly inhibitory to yellow nutsedge root growth.

Putnam and Duke (1974) tested the ability of different accessions of cucumber on two different weeds, proso millet (*Panicum miliaceum* L.) and white mustard (*Brassica hirta* Moench). They found that secondary metabolites produced by cucumber caused severe inhibition of weed growth. The inhibition of cucumber PI 169391 on proso millet germination decreased with maturity of the cucumber seed and fruit (Lockerman and Putnam 1981). In addition, the seeds were both autotoxic and allelopathic during early seedling development.

Other broad-leaved crops have been reported to have allelopathic properties, such as tobacco (*Nicotiana tabacum* L.) for its alkaloids nicotine, nornicotine, anatabine and anabasine (Bush and Crowe 1989); potato (*Solanum tuberosum* L.) which contains glycoalkaloids solanine, chaconine and solamarine and aglycon alkaloid solanidine (Sharma and Salunkhe 1989); and radish (*Raphanus sativus*), cabbage (*Brassica oleracea* L. var. *capitata*), brussels sprouts (*B. oleracea* L. var. *gemmifera*), pak-choi (*B. chinensis* var. *chinensis*) for their content of glucosinolate (Fenwick, Heaney, and Mawson 1989). In Australia, McKenzie, Reichmann, Dimmock, Dunster and Twist (1988b) reported that castanospermine, an indolizidine alkaloid produced by *Castanospermum australe* seeds caused haemorrhagic gastroenteritis in cattle.

Narrow-leaved crops such as corn (*Zea mays* L.), grain sorghum (*Sorghum bicolor* L.) and winter wheat (*Triticum aestivum* L.) showed allelopathic activity by reducing fresh weight of jointed goatgrass (*Aegilops cylindrica* Host). The reduction was 70 to 85% when the crop residue was incorporated into soil (Anderson 1993). The results suggested that one way of controlling jointed goatgrass was by incorporating the crop residue in soil with tillage before planting.

Barnes and Putnam (1987) reported the role of benzoxazinones in allelopathy by rye on both dicot and monocot species. They found that dicot species including lettuce (*Lactuca sativa* L.), tomato (*Lycopersicon esculentum* Mill.) and redroot pigweed (*Amaranthus retroflexus* L.) were 30% more sensitive than the monocots tested, proso millet (*Panicum miliaceum* L.) and large crabgrass (*Digitaria sanguinalis* L. Scop.). A general finding of their work was that BOA was more toxic to dicot species, while DIBOA had greater activity on germination of monocots.

Hemken and Bush (1989) reported that tall fescue (*Festuca arundinacea* Schreb.), a grass with excellent agronomic characteristics, produce the alkaloids perloline and perlolidine, which have allelopathic activity. The level of these alkaloids increased during the late summer months in Kentucky and reduced the performance of grazing animals. Interestingly, Cheeke (1995) noted that grasses are generally not as well defended chemically compare to herbaceous plants which are often rich in chemical defences such as alkaloids and glycosides. Most grasses have coevolved with grazing animals and, by growth habit, are able to survive frequent defoliation.

2.10. Common features of allelopathy in crops

Allelopathic activity of different crop species, indigenous and introduced, has been broadly studied. Putnam and Duke (1974) reported that unselected accessions of cucumber (*Cucumis sativus* L.) were capable of severely inhibiting some weeds under controlled conditions. Comparing a hybrid cucumber, cv. Pioneer, and an unselected accession, Lockerman and Putnam (1981) demonstrated that the latter was more well-equipped for chemically-based interference.

When the unselected line PI 266281 of oat (*Avena sativa*) and wild mustard [*Brassica kaber* (D.C.) L.C. Wheeler var *pinnatifida* (Stokes) L.C. Wheeler] were grown together, wild mustard exhibited severe chlorosis, stunting, and twisting. In addition, PI 266281 caused greater effects than the oat cultivar Garry (Fay and Duke 1977).

Yoshida, Tsumuki, Kanehisa and Corcuera (1993) studied the release of gramine from the surface of barley leaves of several lines. They found that gramine content of wild type *Hordeum vulgare* spp. *spontaneum* was about 6-fold that of cultivated *H. vulgare* spp. *vulgare.* This finding was consistent with the work of Lovett *et al.* (1994) which demonstrated that gramine and hordenine content of *H. spontaneum* was higher than that of *H. vulgare* cv. O'Connor.

In general, contemporary cultivars of barley (*H. vulgare*) produced lower amounts of gramine compare to ancestral types (*H. spontaneum* and *H. agriocrithon*) (Lovett and Hoult 1992). However, the production of hordenine by the latter was slightly lower than the former (Lovett *et al.* 1994). Greater light intensity and higher temperatures outdoors may enhance the production of secondary metabolites.

Hanson *et al.* (1981) reported that *H. spontaneum* Koch contained at least 2,000 µg/g dry weight of barley leaves which was enough to depress performance in ruminants. Gramine content, therefore, represents a potential anti-quality factor for barleys otherwise suited to grazing or other forage uses.

Allelochemicals from crop plants have the potential to protect the producing crops against insects and herbivores. For instance, α-tomatine, a glycoalkaloid presents in solanaceous plants, decreased larval survival of Mediterranian fruit fly, *Ceratitis capitata*, (Chan and Tam 1985). Furthermore, this glycoalkaloid lowered pupal weights, extended the pupation period and prolonged the period of adult emergence.

Other solanaceous glycoalkaloids, α-solanine and α-chaconine, were highly fungitoxic to *Helminthosporium carbonum* and these compounds accounted for at least 90% of the fungitoxic activity of potato peel extracts at pH 5.6 (Allen and Kuc 1968). Solanine, chaconine and solanidine inhibited the radial growth of *Alternaria solani* (Ell. & G. Martin) Sor., which causes early blight disease in potatoes and tomatoes, on potato-dextrose agar (Sinden, Goth and O'Brien 1973). These workers found that an increased susceptibility of leaves to *A. solani* was correlated with a decrease in glycoalkaloid concentration in leaves as the potato plants aged. Accordingly, Sharma and Salunkhe (1989) reported, when administered intraperitonially at doses 42 ± 1.8 mg/kg body weight, α-solanine caused 50% death of mice in 7 days. The lethal dose was ≥ 50 mg/kg.

Many workers have demonstrated that antipalatability which, in turn, reduces livestock performance, is related to secondary metabolites (e.g. Gallagher, Koch, Moore and Steel 1964; Arnold and Hill 1972; Pfister, Provenza and Manners 1990; James, Nielsen and Panter 1992; Molyneux and Ralphs 1992). Poisonous plants, which contain secondary metabolites can impair ingestive and reproductive behaviour, thus reducing animal productivity (Pfister, Cheney and Provenza 1992).

Pfister *et al.* (1990) reported that tall larkspur (*Delphinium barbeyi*) which contains 17 individual alkaloids decreased feed consumption in cattle by 41% relative to a control group. Blow away grass (*Agrostis avenacea*) pasture has been associated with tunicaminyluracil toxicosis (Bourke, Carrigan and Love 1992). Cattle dosed with 2 mg tunicamycin/kg died on the sixth day after dosing whereas sheep dosed at 1.5 mg/kg developed clinical signs on the fifth day and died 24 h later.

Terminal liver disease developed in calves fed tansy ragwort (*Senecio jacobaea*)-contaminated pellets and vacuolar changes occured in the liver due to the pyrrolizidine alkaloids (Craig, Pearson, Meyer and Schmitz 1991). Poisoning in ruminants from ingestion of the plant *Lantana camara*, which contains the triterpene acids lantadene A and lantadene B has been reviewed (Pass 1986; McKenzie 1991). Jaundice (liver injury), photosensitisation and ruminal stasis have been recorded as a consequence of lantana poisoning (Pass 1986). Other workers reported that *Castanospermum australe* seeds at the rate of 0.15 g/kg body weight reduced lymphocyte alpha-glucosidase activity of cattle by at least 90%. The absence of lysosomal alpha-glucosidase is a characteristic of Pompe's disease (Reichmann, Twist and McKenzie 1989).

Working on meadow vole (*Microtus pennsylvanicus*), a convenient model for animal work, Kendall, Hill, Jr. and Shenk (1979) demonstrated that alkaloids which occur in reed canarygrass affected feed intake. Likewise, the meadow vole was deterred from feeding on conifer tree bark as soon as the phenolic content rose above 2.6% dry weight (Roy and Bergeron 1990).

Gallagher *et al.* (1964) reported that different alkaloids, such as *N,N*-dimethyltryptamine, gramine and hordenine have been isolated from *Phalaris arundinacea*, a pasture grass. They also noted that the administration of a solution of pure 5-methoxydimethyltryptamine, dimethyltryptamine or gramine killed sheep, guinea-pigs, rats and mice due to acute heart failure. However, some allelochemicals protect the producing plants not by poisoning or repelling herbivores but by interfering with the predators' normal cycle of growth and development (Rosenthal 1986).

Other forage grasses, *Phalaris* spp., cause neorological damage known as *Phalaris* staggers in livestock (Kennedy, Cregan, Glastonbury, Golland and Day 1986; Cheeke 1995). Reed canarygrass (*P. arundinacea* L.), has at least nine alkaloids, including gramine and hordenine, which cause diarrhoea in grazing steers and lambs (Marten, Jordan and Hovin 1976). In addition, total basic alkaloid content of reed canarygrass is negatively correlated with palatability of the grass to ruminant animals

(Marten, Barnes, Simons and Wooding 1973). Williams, Barnes and Cassady (1971), for instance, demonstrated that unpalatable clones had an amount of 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), 18 times the amount of that found in palatable clones.

Grain sorghum (*Sorghum bicolor* L.) has been reported as containing relatively high levels tannins, secondary metabolites which were toxic to young chickens as measured by reduction in growth rate and slightly elevated liver lipids (Chang and Fuller 1964). The gain in weight of chickens and the feed consumption decreased as tannic acid content of the diet was increased, and mortality increased as the tannic acid level increased (Vohra, Kratzer and Joslyn 1966).

Berenbaum and Neal (1985) stated that synergisms among chemical defences occur where the deterrent effects of a plant secondary metabolite can be increased because the plant also produces small quantities of nondeterrent compound that inhibits an insect's ability to alter the deterrent compound.

Studies have shown the value of synergists in insecticide application. Working on Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] larvae, Mahdavi *et al.* (1991) mixed the insecticides permethrin and fenvalerate with piperonyl butoxide, a synthetic synergist. They demonstrated that, 48 h after application, toxicity increased, especially for the fenvalerate + piperonyl butoxide treatment. They also found that both insecticides, when mixed with piperonyl butoxide, increased LD_{50}s for Colorado potato beetle larvae. Mahdavi *et al.* (1991) assumed that this was probably due to the higher concentration of allelochemicals leading to either more induction of the insecticide detoxifying enzymes or selection of more tolerant individuals.

There is also a possibility of using plant allelochemicals as synergistic agents. For instance, myristicin, a methylenedioxyphenyl compound which is found in many umbelliferous crops, is as effective as a synergist for the insecticide carbaryl as is piperonyl butoxide (Berenbaum and Neal 1987). The use of an endogenous synergist in crop plants may be used to enhance the efficacy of externally applied pesticides. Therefore, lower amounts of pesticides need to be applied to effect equivalent control as well as reducing the impact of synthetic pesticides on nontarget organisms.

Similarly, phenolic acid mixtures may increase the respiration of fungi in forest soils. The following phenolic acids are produced by bilberry leaves (*Vaccinium myrtillus* L.), fern fronds [*Athyrium filix-femina* (L.) Roth] and spruce needles (*Picea abies* L. Karst): *p*-hydroxyacetophenone, *p*-hydroxybenzoic acid, cathecol and protocatechuic acid (Pellessier 1993). The mixture of these phenolic acids at 10^{-3} M caused a dramatic increase of the respiration of the fungi *Cenococcum graniforme* (Sow). Ferd. and Winge, and *Laccaria laccata* (Scop. ex Fr.) Berk and Br., two spruce mycorrhizal fungi. However, at 10^{-7} M the mixtures induced a significant decrease in oxygen consumption for both mycorrhizal species (Boufalis and Pellessier 1994). This phenomenon might support the idea that the effect of a phenol is never more or less toxic according to its concentration.

Working on tropical seaweeds, *Rhipocephalus phoenix*, *Udotea cyathiformis* and *Halimeda goreauii*, Hay, Kappel and Fenical (1994) demonstrated that secondary metabolites from these seaweeds and $CaCO_3$ acted synergistically and deterred feeding of herbivores more than the sum of the effects of each compound. Hay *et al.* (1994) tested these synergisms on different types of herbivores, the sea urchin *(Diadema antillarum)*, the amphipod (*Cymadusa filosa*) and a mixed-species group of small parrotfishes.

Metcalf, Lampman and Deem-Dickson (1995) found that combinations of plant volatile kairomones are quantitatively more attractive to insects than the individual components. The mixture of 1,2,4-trimethoxybenzene, indole and (*E*)-cinnamaldehyde was strongly synergistic. Other workers demonstrated that the mixture of betaine hydrochloride and tannic acid was more growth depressing in chickens than either compound alone (Vohra *et al.* 1966).

Kendall *et al.* (1979) reported that feed intake of meadow voles (*M. pennsylvanicus*) was inhibited by allelochemicals. Inhibition by gramine and tryptamine were similar, and greater than the inhibition with hordenine. However, a synergistic effect on intake was obtained with diets that contained a mixture of gramine and β nitropropionic acid.

Combinations of phenolic compounds strongly inhibited seedling growth (the elongation of roots and hypocotyls) and seed germination of lettuce. The inhibition was stronger than that of individual compounds (Li, Inoue, Nishimura, Mizutani and Tsuzuki 1993). For example, chlorogenic acid alone did not inhibit seed germination, however, it increased the inhibition of *trans*-cinnamic, *o*-,*m*-,*p*-coumaric acids and coumarin on lettuce germination.

The growth of grain sorghum and soybean seedlings subjected to ferulic acid and atrazine together was suppressed more than with either alone. This suggests the possibility that allelopathic interference may be the result of the simultaneous action of several compounds (Einhellig 1987).

2.11. Barley as a case study for allelopathy in crops

Two alkaloids, gramine (N,N-dimethylindolemethylamine) and hordenine (N,N-dimethyltyramine) have been isolated from leaves and roots of germinating barley (Bowden and Marion 1951; Leete, Kirkwood and Marion 1952; Frank and Marion 1956; Massicot and Marion 1957; Gower and Leete 1963; Hanson *et al.* 1981; Lovett and Liu 1987; and Liu and Lovett 1990).

Gramine (Figure 2.3), a member of the indole group of alkaloids, is biosynthesised from tryptophan (Bowden and Marion 1951; Gower and Leete 1963). It is not present in barley seeds and roots (Schneider and Weightman 1974) but appears in the leaves within 4 days after planting and then persists for at least 50 days (Mudd 1961) and is toxic to plants, insects and mammals (Yoshida *et al.* 1993).

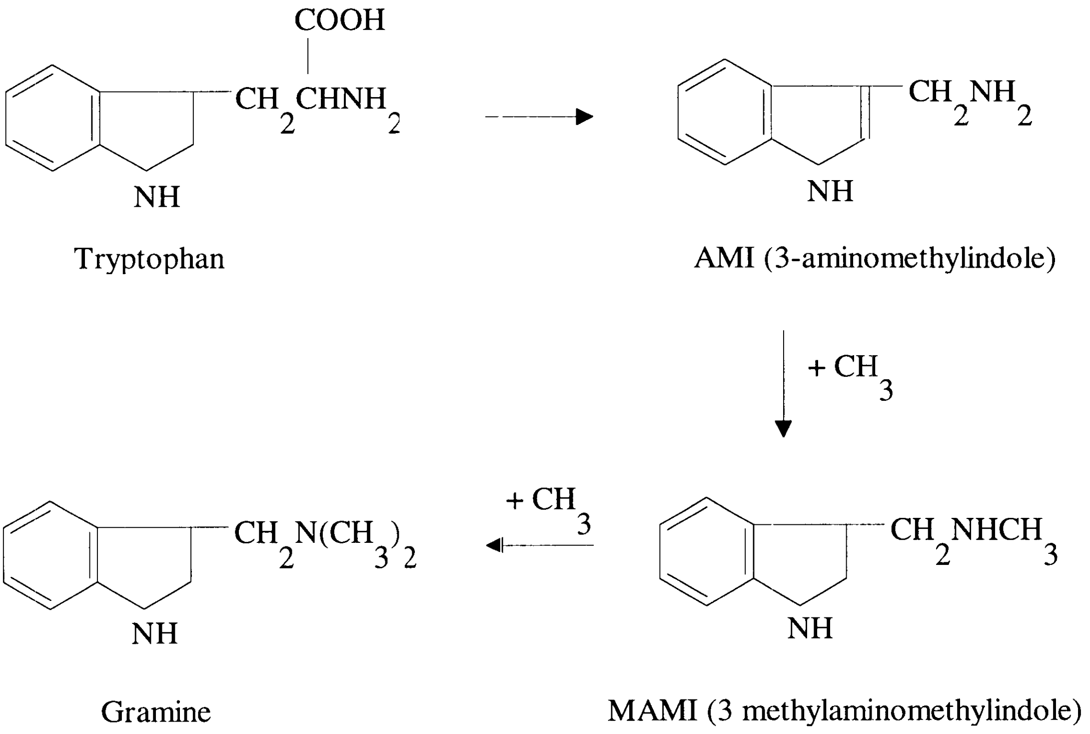

Figure 2.3. The biosynthesis pathway of gramine from tryptophan (from Hanson *et al.*, 1983)

Hordenine, a member of the phenol group of alkaloids, is a result of a decarboxylation and methylation process from tyrosine (Kirkwood and Marion 1950; Leete and Marion 1953; Frank and Marion 1956; Massicot and Marion 1957). Hordenine is absent from barley seeds but appears in the roots from the first day of seed germination (Mann, Steinhart and Mudd 1963). In an hydroponic system, hordenine was released from the roots of barley for up to 60 days. The amount reached a maximum, 2 μg/plant/day, at 36 days, then declined (Liu and Lovett 1993a). The formation of hordenine from tyrosine is presented in Figure 2.4.

Many works have been conducted to examine the role of gramine and hordenine on other organisms (Zuniga, Varanda and Corcuera 1988; Sepulveda and Corcuera 1990; Lovett and Hoult 1993). These lead to the possibility of exploring gramine and hordenine for self-defence agents by barley against other organisms. The roles of the two alkaloids will be addressed in turn.

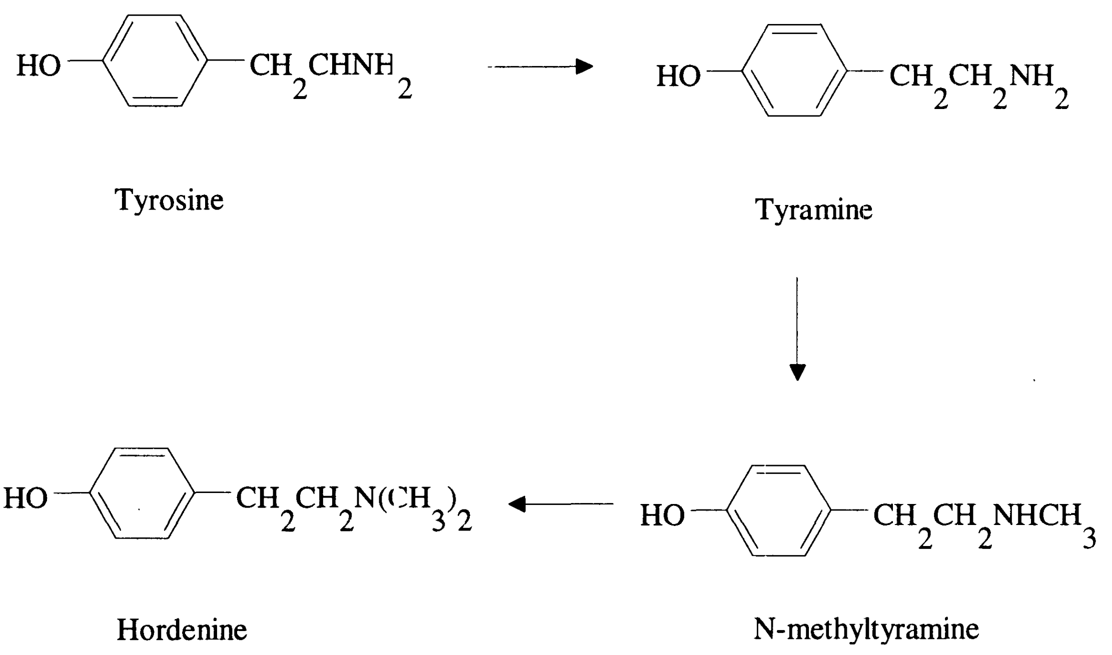

Figure 2.4. The formation of hordenine from tyrosine (from Kirkwood and Marion, 1951)

2.11.1. Activity of barley allelochemicals against weeds

It has been widely documented that barley has the ability to suppress the growth of different weed species due to its alkaloids gramine and hordenine. For example, germination of *Stellaria media* was suppressed by germinating barley by 77% when they were sown simultaneously and by 87% when *S. media* was sown four days after barley (Overland 1966). Brown (1974) reported that barley reduced dry weight of *Abutilon theophrasti* by 83% but, interestingly, the yield of barley was not reduced by the weed. These data suggest that germinating barley produces an inhibitor to neighbouring plants.

Working on competition between barley and several weed species, Scragg and McKelvie (1976) found that *Ageratum repens* was the least competitive weed compared to *S. media* and *Avena fatua*. This was, possibly, due to the early emergence of barley compared with *A. repens*.

Liu and Lovett (1990) investigated allelopathic effects of barley on white mustard (*Sinapis alba L.*) seedlings in the laboratory. They reported that radicle elongation of white mustard was increasingly inhibited as barley seed rate increased when the two species were sown at the same time. Interestingly, when barley had been germinating for two days or more before introduction of white mustard, the latter was inhibited at an even lower seed rate.

In a Petri dish bioassay, Liu and Lovett (1993b) demonstrated that germination of white mustard was delayed and the radicle lengths were significantly inhibited at a density of 0.5 barley seed/cm^2. Transmission electron microscopic examination of white mustard radicle tips, exposed to gramine and hordenine, indicated damage to cell walls, increase in both size and number of vacuoles, autophagy and disorganisation of organelles. The higher the concentrations of gramine and hordenine, the more severe was the damage.

2.11.2. Activity of barley allelochemicals against other organisms

Apart from its ability to suppress other plants' growth, barley showed self-defence ability against other organisms through the production of gramine and hordenine. Sepulveda and Corcuera (1990) tested the effects of gramine on the bacterium *Pseudomonas syringae*. They found that gramine decreased the size of the bacterial population and higher concentrations of gramine decreased respiration rate. In a barley leaf disk study, the bacteria caused 36% damage (necrotic area) on leaf disks with natural gramine while 80% damage occured on leaf disks without gramine. This work suggested that gramine may increase barley resistance to *P. syringae*.

Barley plants may be severely damaged by aphids, mainly because they may transmit viruses, remove essential nutrients and disrupt tissues (Corcuera 1993). Gramine, however, contributed to the resistance of barley seedlings to some insect species. Zuniga and Corcuera (1986) demonstrated that the population growth rate of the aphid *Rhopalosiphum padi* negatively correlated with gramine content in leaves of barley. The presence of gramine negatively affected the feeding behaviour and, subsequently, the performance of the aphids *R. padi* and *Schizaphis graminum* (O'Donovan, Varanda and Corcuera 1988; Zuniga *et al.* 1988) and the survival and reproduction *of Metopolophium dirhodum* and *R. maidis* (Corcuera, Argandona and Zuniga 1992).

When incorporated into artificial diets, gramine decreased survival and amount of diet ingested by *R. padi* and *S. graminum* (Zuniga and Corcuera 1986; Zuniga *et al.* 1988). The concentration of 2.9 mM was the LD_{50} of gramine, in artificial diets, for the aphids (Zuniga and Corcuera 1986). They found the concentrations of gramine in the artificial diets similar to those found in barley leaves. Therefore, it is suggested that gramine may play an important defensive role against aphid attacks (Zuniga and Corcuera 1986; Zuniga *et al.* 1988; and Corcuera 1993). Gramine and hordenine which were added into the diet significantly decreased survival and mean weight of nymphs of the migratory grasshopper, *Melanoplus sanguinipes* (F.) (Westcott *et al.* 1992). These workers identified that gramine and flavone showed detrimental effects among the secondary compounds tested.

Goelz, Rothenbacher, Wiggins, Kendall and Hershberger (1980) examined the effects of gramine and hordenine on a vertebrate species, meadow voles (*Microtus pennsylvanicus*). The workers found that gramine markedly reduced the vole survival and weight gains.

Lovett and Hoult (199[illegible]) reported that gramine and hordenine had a inhibitory effect on a fungal pathogen (*Drechslera teres*) and armyworm (*Mythimna convecta*). Gramine was effective at 10mM whereas hordenine greatly reduce the growth of the fungus at 5mM. Even though there was no consistent trend, both gramine and hordenine reduced the growth of armyworm larvae.

Bioassay of gramine on armyworm (*M. convecta*) larvae indicated that gramine had adverse effects on the growth and development but not on the survival of the larvae. The combination of gramine and hordenine showed synergistic effects in reducing the survival of *M. convecta*. However, both gramine and hordenine, alone or in combination, did not show any significant effects on the growth, survival and feeding intake of other insect species i.e. *Agrotis ipsilon* (Hufnagel) and *Helicoverpa punctigera* (Wallengren) (Kiannmatee 1995). Therefore, there is a possibility that both gramine and hordenine have the potential to contribute to barley self defence against other species.

2.11.3. Deficiencies in knowledge and need for further research

The literature review has shown that allelopathy of barley has been widely studied on different species such as weeds, fungi and insects (invertebrate). However, there is still a lack of evidence for barley allelopathy as a self defence agent against vertebrates.

In order to test the allelopathic properties of alkaloids gramine and hordenine in this context, an inexpensive and rapid method of detecting the effects is necessary. For instance, Rosenberg and Zoebisch (1952) reported that the chick assay was an efficient, reliable and time-saving technique by which toxicity of *Indigofera* spp. may be detected. From published reports it appeared that mouse, rabbit and chicken would meet the criteria of a rapid and inexpensive biological assay for toxicity. Therefore, the studies which follow have been designed to assess the allelopathic effects of gramine and hordenine on two different species of vertebrates, mouse and broiler chicken, as animal models.