CHAPTER
ONE
INTRODUCTION

Much attention is being paid at the present time to studies of pest control methods other than the use of pesticides. This is largely due to increasing recognition of the drawbacks of pesticides. These include: pollution of streams, lakes and underground water; damage to beneficial insects such as bees, damage to fish and wildlife populations, and effects on human health (Headley and Lewis 1967). The heavy use of nonselective neurotoxic insecticides, for example, induces resistance of insects to these chemicals, the resurgence of secondary insect pests, produces residues that are toxic to man, birds, and other nontarget organisms and increases the cost of production (Gliessman 1989). "Biorational" chemicals (biologically active chemicals that have modes of action more specific for insects) are therefore required for modern commercial insect control (Klocke and Barnby 1989). Consumers, nowadays, feel that both nutrition and safety are important when they select food. Food safety and the use of chemicals in production of food have had a great impact on the food industry in the past decade (Scroggins 1991).

A contemporary alternative to reliance on pesticides alone is the use of pest control methods in combination, called, "integrated pest management". It is generally better than any single control method because no single method can completely control pests. Methods used in integrated pest management may include plant quarantine, crop rotation, sanitation, biological control, chemical control and resistant cultivars. However, the use of resistant cultivars may be the cheapest and most effective method (Russell 1978). This method may decrease the use of pesticides and decrease the costs of production and environmental pollution (Hedin 1986).

The importance of allelopathy in both natural and agroecosystems has been well documented (Putnam and Duke 1978). The potential of allelochemicals for pest control, integrated pest management, crop rotation and agroforestry systems was described by Rizvi and Rizvi (1992). Studies of allelopathic interactions provide basic data for the science of allelopathy that can be applied to understanding the problems of plant-plant, plant-microbe and plant-insect interactions and to exploiting these in improving production from manipulated ecosystems (Rizvi et al. 1992). Allelopathy, when incorporated into natural and agricultural management systems, may decrease the use of herbicides, fungicides and insecticides, and cause less pollution. Allelochemicals produced by plants, microorganisms, other soil organisms and insects may afford new

strategies for maintaining and increasing agricultural production in the future. These allelopathic substances may provide novel chemistry for the synthesis of herbicides, insecticides and fungicides that are not derived from persistent petroleum-derived compounds (Chou and Waller 1989). Moreover, they may play an important role in breeding cultivars resistant to pests, as chemical factors are involved in the resistance of plants to pests (Plimmer 1985, Hedin 1986). It is possible that plants or microbes can be manipulated genetically to produce higher content of the desired allelochemicals (Putnam 1983).

Allelochemicals present in barley have effects on weeds (Overland 1966, Lovett *et al.* 1989, Liu and Lovett 1990), diseases (Sepulveda and Corcuera 1990), and insects (Zuniga *et al.* 1985, Zuniga *et a'.* 1988, Kanehisa *et al.* 1990, Barria *et al.* 1992, Rustamani *et al.* 1992). There are many resistant lines of barley that contain allelochemicals which have adverse effects on insects such as aphids (Kanehisa *et al.* 1990, Rustamani *et al.* 1992). However, the possibility of resistance to other insects in barley is also interesting and the study of biological effects of allelochemicals from barley on insects is necessary since there is inadequate knowledge of such effects. In addition, effects of allelochemicals on vertebrates and on beneficial insects are of interest.

The aims of this thesis were

-to study the effect of gramine (an allelochemical in barley leaves) on survival, growth and development of common armyworms [*Mythimna convecta* (Walker), also known as *Leucania convecta* (Walker)], an important pest of barley. In addition, the effect of gramine on survival, growth and development of a locust [*Locusta migratoria* (Linnaeus)] was also investigated to provide a comparison with *M. convecta*;

-to study the synergistic effects of gramine and hordenine (both found in barley) on survival, growth and development of various insect pest species include *M. convecta*, black cutworm [*Agrotis ipsilon* (Hufnagel)] and cotton budworm [*Helicoverpa punctigera* (Wallengren)].

-to study the deterrent effect of gramine and hordenine on *M. convecta*.

These studies were conducted both in the glasshouse and in the insectary of the University of New England, Armidale, New South Wales, Australia.

CHAPTER
TWO
LITERATURE REVIEW

2.1 ALLELOPATHY

2.1.1 Meaning of allelopathy

The term " allelopathy " was coined by Molisch in 1937 to describe biochemical interactions between all types of plants including microorganisms. He indicated that this term covered both inhibitory and stimulatory biochemical interactions (Molisch 1937, cited by Rice 1984). In contrast, according to Rice (1974) the term "allelopathy" was defined as any direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds that were released into the environment. However, ten years later, Rice (1984) believed that most organic compounds that were inhibitory at some concentrations were stimulatory to the same processes in very small concentrations. Therefore, he agreed with the definition established by Molisch and this definition has been followed by most researchers except for a relatively small number in North America.

Allelopathy is viewed as one of many stress factors which exist in natural and managed ecosystems. Moreover, it may be perceived as part of a complex of chemical communication between plants and other organisms which generally appear to give an element of self defense to the plant that exhibits allelopathic manifestations (Lovett 1989). The term "allelopathy" is different from the term "competition". The effect of allelopathy depends on a chemical compound being added to the environment while competition involves the reduction of some factor that is required by some other plant sharing the habitat, such as water, minerals, food and light, from the environment (Rice 1984).

It is evident from the literature that allelopathy has been observed and discussed by many botanists, farmers, and gardeners for over 2000 years. However, controlled scientific experiments on this phenomenon were not conducted until after the year 1900 (Rice 1984).

2.1.2 Role of allelopathy in a *j*roecosystems

For the past several decades agricultural production has come to depend on a wide range of chemical inputs which are often evaluated in terms of their efficacy in controlling pests, decreasing yield osses and increasing the output and profitability of the cropping system. However, during recent years, it is evident that agricultural sustainability is threated by the long-term and intensive use of these chemicals (Gliessman 1989).

Gliessman (1989) indicated hat the excessive use of agrochemicals is associated with the following problems:

-surface and ground water contamination;
-deleterious impacts on nontarget organisms;
-development of secondary pests;
-development of pesticide resistance and loss of effectiveness of chemicals on
 target organisms;
-residues on food;
-expanding evidence for links between agrochemicals and human health
 problems;
-hazard for the applicator and other farm workers;
-increasing cost of agrochemicals application;
-increasing regulation of chemical application.

He also noted the important criteria for ensuring that a particular agricultural system is sustainable:

-the agricultural system works with natural processes rather than against them;
-farming should be regenerative, with ecological principles driving
 agroecosystem re-establishment from the disturbances caused by
 management and harvest each season;
-the system cannot rely excessively on non-renewable resources;
-manipulation of the system cannot contaminate or degrade the
 environment, either on or off the farm;
-agriculture should be truly productive, rather than extractive;
-farm management should respect the basic right of all people to safe food, air
 and water;
-economic assessments of agriculture must include long-term impacts and
 externalities as well as short-term requirements and pressures;

-agriculture must provide the greatest benefit for as many people as possible from farmers to consumers, as well as those in between.

To meet these criteria for a sustainable agriculture, allelopathy and phytochemical ecology can play a very important role (Gliessman 1989). Allelopathy could offer the development of alternative pest control strategies where the impacts of allelochemicals in agroecosystems are positive.

2.2 ALLELOCHEMICALS

2.2.1 Introduction

According to Whittaker and Feeny (1971), the term "allelochemics" or "allelochemicals" describes secondary metabolites produced by an individual of one species and able to affect the growth, health, population biology or behaviour of another species. There are many types of allelochemicals such as attractants, repellents, allergenics and toxins (Rosenthal 1986). Similarly, Chou and Waller (1989) indicated that allelochemicals are secondary plant metabolites that play an important role in plant-plant, plant-microorganism and plant-insect interaction, both profitably and harmfully, and act as significant ecological factors which influence plant dominance, succession, climax vegetation and crop productivity.

Many plant secondary products show physiological activity against organisms other than the producer plant. Plants have adapted to the presence of toxic compounds by using a variety of physical, chemical, and biochemical mechanisms. For example, the producer plants avoid autotoxicity by the physical removal of noxious products from cellular sites and enzyme systems where antagonism might otherwise occur (Fowden and Lea 1979).

Plants produce and store large numbers of secondary metabolic products or allelochemicals such as alkaloids, essential oils, phenolic compounds, steroids, terpenoids, coumarins, flavonoids, cyanohydrins, polyacetylenes, etc., which vary in their biosynthetic pathways, concentration, and localization depending on species. These allelochemicals affect the growth and development of predators (insect or higher grazers), bacteria, algae, fungi and also the growth of other neighboring plants (Waller 1989).

The following are some significant sites and processes which are influenced by allelochemicals (Rice 1984, cited by Rizvi *et al.* 1992):

-cytology and ultrastructure;

-phytohormones and their balance;

-membrane and its permeability;

-germination of pollens/spores;

-mineral uptake;

-stomatal movement, pigment synthesis and photosynthesis;

-respiration;

-protein synthesis;

-leghaemoglobin synthesis and nitrogen fixation;

-specific enzyme activity;

-conducting tissue;

-water relations of plants;

-genetic material.

Metabolic substances that are involved in allelopathy are liberated from plants in several ways, as shown in Figure 2.1. Large amounts of organic and inorganic metabolites are leached from above-ground parts of plants by rain and dew (A). Volatile substances are released from plants such as carbon dioxide, ethylene and terpenes (B). Leaves and other plant parts fall to the ground and are decomposed by weathering and by soil microorganisms (C) and metabolites are released from roots into the surrounding rhizosphere (D) (Tukey 1969). In addition, these substances in plants may be eaten by herbivores such as insects (E).
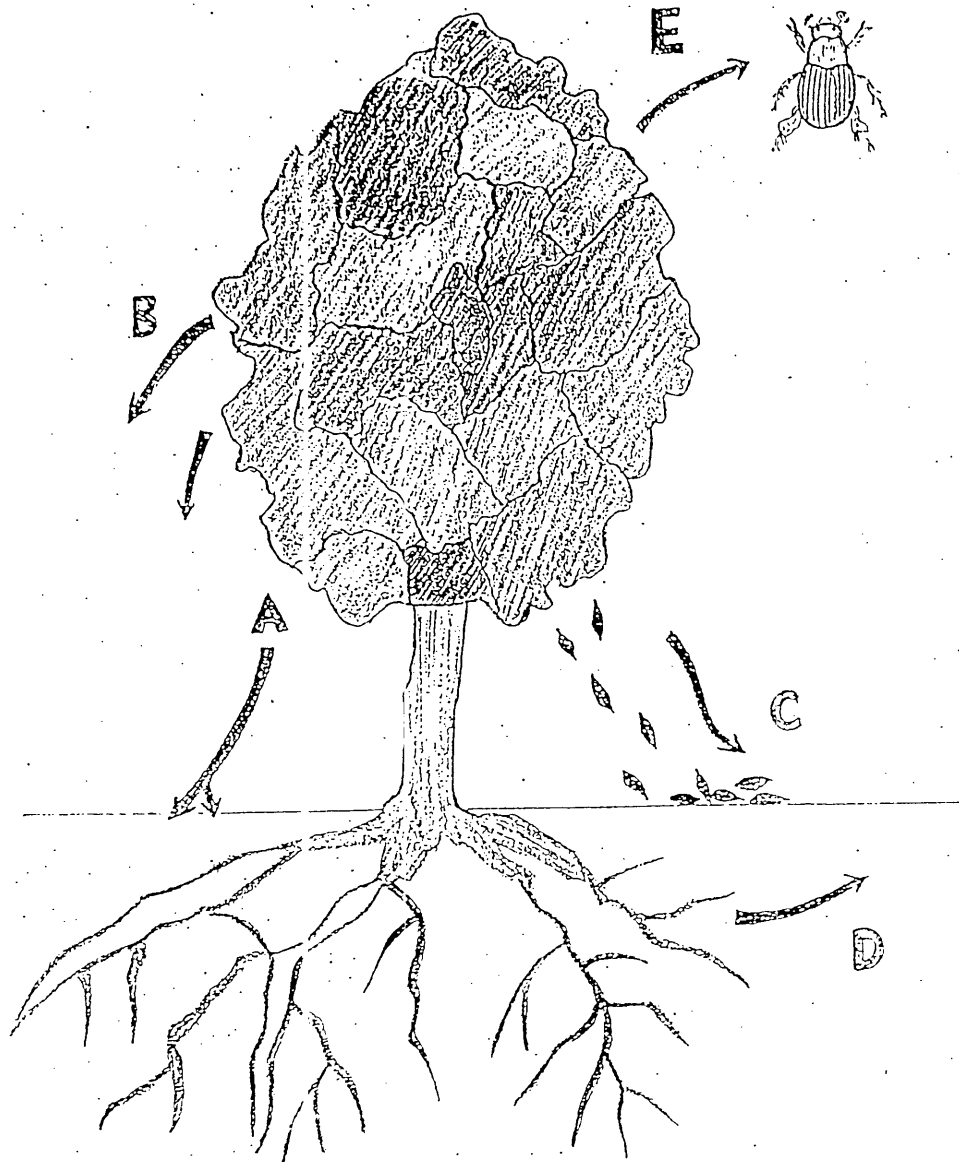
Figure 2.1. Allelochemicals removed from plants by several ways A.leaching by rain, dew, and mist; B.volatilization of plant metabolites; C.litter accumulation; D.root exudation; E.eaten by herbivores (modified after Tukey 1969).

2.2.2 Potential for allelochemicals in weed management

Hundreds of thousands of secondary compounds are produced by plants and also by microorganisms. Many of these compounds are phytotoxic and have potential as herbicides or as templates for new herbicide classes. According to Patterson (1986) 13 of the world's 18 "worst weeds" have been reported to produce allelochemicals which reduce crop growth and yield (Worsham 1989). However, only about 3% of a possible 400,000 secondary metabolites from plants and microorganisms have been isolated and identified (Einhellig and Leather 1988, cited by Worsham 1989). Allelochemicals from higher plants with potential phytotoxicity were classified into several classes such as alkaloids, benzoxazinones, cinnamic acid derivatives, coumarins, cyanogenic compounds, flavonoids, polyacetylenes, quinones and terpenes (Putnam 1988, cited by Worsham 1989).

Robinson (1974) considered that the inhibition of growth of several plants by barley plants may have real importance in plant competition. His consideration was based on the results of Overland (1966), who found that allelopathic substances produced by both the seeds and plants of barley (*Hordeum vulgare* L.), were able to inhibit growth of several plants in the greenhouse. Overland (1966) also found that an inhibitory substance was produced by germinating barley seeds which affected other species by reducing germination. Inhibitory effects were found in both living and dead barley roots, but a much stronger effect and a much higher concentration of substances was found in living root extract. Therefore, she indicated that the secretion of living plants was biologically active. In addition, her experiments demonstrated that the inhibitor of barley could function under the normally variable conditions of the field.

Lovett *et al.* (1989) found that seedlings of *Linum usitatissimum* L. (linseed) showed responses in radicle length to the allelochemical benzylamine. Similarly, *Sinapis alba* L. (white mustard) showed responses in radicle length to the alkaloids gramine and hordenine produced by barley during germination. The root tip cells of linseed and white mustard showed response to allelopathic stress. As the concentration of allelochemicals increased, the number and size of vacuoles increased, nuclei became less distinct and some mitochondria showed evidence of disorganization.

Liu and Lovett (1990) further investigated allelopathic effects of barley on white mustard seedlings in the laboratory. They found that gramine and hordenine were released from germinating barley at concentrations of 22 ppm gramine and 48 ppm hordenine four days after germination, significantly reducing the radicle length of white

mustard. These allelochemicals, wh en released from the roots in a hydroponic system, reduced radicle length of white mustard for at least 70 days after the commencement of barley germination. In addition, influences on white mustard by hordenine and gramine included reduction of radicle leng h and apparent reduction in health and vigour of radicle tips. They commented that these compounds could be an important factor in interference with weed species if si nilar results were maintained in the field.

Worsham (1989) suggest d techniques involving allelopathy for weed suppression which may take the from of:

-using natural allelochemica s or modified allelochemicals as herbicides;

-plant breeding or genetic engineering methods to transfer allelopathic traits into commercial crop cultiv irs;

-using allelopathic plants in :rop rotation, companion plantings or smother crops;

-using phytotoxic mulches a id cover crops;

-using allelochemicals or derivatives thereof to stimulate weed seed germination and, therefore, decrease the soil weed seed load.

2.2.3 Potential for allelochemicals in plant disease management

Allelochemicals do not affect only weed management but also plant disease management. A common method of biological control of plant diseases is the use of plant residues as organic amendments added to the soil for controlling soil-borne plant pathogens (Patrick 1986), for exa nple, the use of cabbage residues for controlling *Fusarium oxysporum* f.sp. *conglutinans* and the use of lettuce residues for controlling the foot and root rot disease of tom ito caused by *F. oxysporum* f.sp. *radicis-lycopersici* (Jarvis and Thorpe 1981, Villapuc ua-Ramirez and Munnecke 1985, cited by Patrick 1986).

There are many studies whi :h indicate that volatile compounds with phytotoxic as well as antifungal properties are obtained during decomposition of plants from the family Brassicaceae in soil (Lewis and Papavizas 1971, Papavizas and Lumsden 1980). Lewis and Papavizas (1971) found that vapors from several sulfur-containing compounds had adverse effects on various processes in the life cycle of pea root rot fungus, *Aphanomyces euteiches*. Vapors of isothiocyanates such as allylisothiocyanate (AIT) and methylisothiocyanate (MIT), were more effective than those of sulfides such as carbon disulfide (CS_2), methanethiol (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$), in inhibiting growth, zoospore forma ion, motility, and zoospore germination. However,

the vapors arising from the decomposition of cabbage tissue in soil (S-containing volatiles) were not as inhibitory to *A. euteiches* as were the vapors of authentic S-containing volatiles. Vapors of cabbage decomposing in soil showed adverse effects on morphology of the fungus, development of oospores (the sexual structures), and mycelial growth. In contrast, vapors arising from the decomposition of corn tissue had no effect on the fungus. Therefore, they concluded that these effects might be important in the reduction of *Aphanomyces* root rot by amendments of plants from the family Brassicaceae and they suggested the possibility of using materials found in such amendments as fumigants for control of the disease. Such fumigants could complement those that are already in use in other situations, such as CS_2 and MIT.

Pathogens and propagules that are adversely affected by allelopathic agents obtained from decomposition of crop residues or addition of organic amendments to the soil include *F. solani* f.sp. *phaseoli* and other *Fusarium* spp., *Cochliobolus sativus*, sclerotia of *Phymatotrichum omnivorum*, *Sclerotium rolfsii*, and *Verticillium* spp., chlamydospores of *Phytophthora* spp., conidia and chlamydospores of *Thielaviepsis basicola* and, possibly, many other pathogens (Patrick 1986).

Gramine, an indole alkaloid found in epidermis and mesophyll parenchyma cells of barley leaf, was suggested to decrease infection and damage of *Pseudomonas syringae* in barley leaves (Sepulveda and Corcuera 1990). The bacteria caused 80% damage (necrotic area) on leaf disks without gramine while they caused only 36% damage on leaf disks with natural gramine.

In addition, there are many groups of secondary metabolites which play a defensive role against pathogens. These include tannins, phytoalexins and phenolic acids. Tannins are a most important group of defensive secondary metabolites because of their ability to precipitate all proteins and inhibit most enzymes. Phytoalexins are another important group of defensive secondary metabolites which inhibit the development of a pathogen when it comes into contact with the host cells. Phenolic acids seem to be the most common potent antibiotics but there is little known about the quantitative or qualitative variations from organ to organ or the method by which they are exuded onto the surface of plants (Swain 1977).

Plant pathologists have, so far, paid only limited attention to the possible role of allelopathic mechanisms because of the variable and unpredictable results obtained in the field with organic amendments. However, the proper use of allelopathic mechanisms

may offer considerable potential for exploitation to successful biological control of plant diseases (Patrick 1986).

2.2.4 Role of allelochemicals in insect pest management

Plant chemistry, especially secondary chemicals, is an important factor in coevolution between insects and plants (Spencer 1988b). It can affect the processes of specialization and generalization in herbivores (Spencer 1988c). For example, plants in the genus *Passiflora* produce cyanogenic glycosides that can affect host specialization in Heliconiine herbivores through the release of the toxic compounds, hydrogen cyanide (HCN) and aglycone (Spencer 1988a). Similarly, Cottee *et al.* (1988) demonstrated that plant secondary compounds affected the host selection of grasshoppers. For instance, *L. migratoria* was attracted to grasses because of the avoidance of plant allelochemicals which exist mainly in the Dicotyledons. Although *L. migratoria* normally feeds on grasses and sedges, it also feeds on some dicots (Uvarov 1966).

Plant defensive substances do not affect all potential enemies uniformly. Generally, specialized herbivores with narrow host ranges are much more resistant to the effects of defensive substances in their host plants than are generalized herbivores. The distinct effects of plant secondary substances on animals are normally regarded as resulting from counteradaptation in the animals due to coevolution (Rhoades 1979).

Many models of plant and herbivore coevolution lead to the assumption that variation in plant defenses and herbivore counteradaptations is heritable. However, environmental factors are also involved in such variation. Gould (1988) suggested that a specific defense that decreased the density of a single herbivore species might not be selected for at all if herbivore communities were strongly structured by competition. This was because the reduction of the single herbivore's density would increase the density of other herbivore species. On the other hand, a specific defense could be expected if the defense against a single herbivore does not make a plant more susceptible to damage by the rest of the herbivore community.

Insects can affect the amount of allelochemicals in plants but finally the allelochemicals have negative effects on them. Plant allelochemicals can be induced by the feeding of herbivorous insects. Conversely, the induced allelochemicals reduce the growth, survival, and/or fecundity of insects resulting in decline in the insect population (Myers 1988). In addition, plant allelochemicals can play a role as enzyme inhibitors and a plant can produce much higher levels of such inhibitors after being attacked by

herbivores. This gives an advantage to the plant because it would not spend energy for synthesis of material until it is necessary (Hagen *et al.* 1984). For pest management, it may be possible that the plant is inoculated by a species that does not cause economic damage in order to induce resistance against the more damaging species (Karban 1991).

Wounding can induce proteinase inhibitors in leaves of tomato but the level of such inhibitors depends on the environmental conditions. Although wounded plants always have negative effects on growth rate of *Manduca sexta* (L.) larvae, this is not necessarily associated with high levels of induced proteinase inhibitors (Wolfson 1991). Coleman and Jones (1991) stated that not only biotic factors such as herbivores and pathogens can affect phytochemical induction in plants, but also plant genotype, phenology, ontogeny and abiotic environment. These factors may influence plant functions and subsequently the quality and suitability of plant tissues for herbivores.

Secondary plant chemicals are expected to be perceived by the chemical senses of olfaction and gustation in animals, including insects. Olfactory and gustatory receptor cells are divided into "specialized cells" and "generalized cells". Specialized cells are highly sensitive to one specific chemical and show little sensitivity to other chemicals while generalized cells respond to a wide range of chemicals of many different classes. According to much evidence, the latter is a majority of chemosensory cells in both insects and mammals. The ability to perceive and recognize secondary plant chemicals affects the evolution of both the herbivores and the plants (Chapman and Blaney 1979).

Foreign compounds or xenobiotics are chemicals that do not appear to provide basic structural-nutritional metabolism in organisms. Allelochemicals are good examples of xenobiotics and play a primary role in the ecologically interrelated systems of which all organisms are part. Herbivores frequently expose themselves to toxic allelochemicals in plants. However, they are able to defend themselves from toxicity via biochemical defense mechanisms which involve many groups of enzymes. Moreover, pesticides are also xenobiotics which can be detoxified by such mechanisms (Brattsten 1979).

In insects, there are many groups of enzymes which are involved in detoxication systems such as mixed-function oxidases (MFOs), epoxide hydrases, reductases, hydrolytic enzymes, and group transfer enzymes (e.g. glutathione S-transferases) (Brattsten 1979, Berenbaum and Zangerl 1988). These enzymes are also found in mammals and their functions were reviewed by Brattsten (1979).

The most important enzymes which are involved in the primary metabolism of lipophilic foreign compounds are mixed-function oxidases (MFOs) (Brattsten 1979). An example of mixed-function oxidase enzyme, hydroxylase, was found in the midgut microsomes of fall armyworm [*Spodoptera frugiperda* (J.E.Smith)] larvae, velvetbean caterpillar, corn earworm, tobacco budworm, mole cricket, american cockroach and honey bee (Yu and Ing 1984).

Since plant allelochemicals are able to induce metabolic defenses of herbivorous insects (Yu and Ing 1984, Brattsten 1988), this mechanism can help insects develop permanent resistance to insecticides. The induction assists susceptible insects with an unexpressed resistance mechanism to survive and reproduce. As a result, the frequency of the resistance mechanism increases in the next generation(s) (Brattsten 1988).

Some of the negative effects of various plant chemicals on physiological development of insects seem to be associated with ecdysone insufficiency. Insect hormones and certain of their pharmacobiological mimics are found in plants and these hormones can inhibit the development and reproduction of insect herbivores. Therefore, the use of these hormones can offer an alternative method for controlling populations of insect pests by means of nontoxic, selectively acting, and environmentally safer natural products. Further studies on these hormonally linked relationships between plants and herbivores will be valuable because they will be able to indicate methods for utilizing these chemicoecological interactions in integrated pest management programs (Slama 1979).

Co-occurring substances in plants may potentiate toxicants (synergism) or may reduce their efficacy (antagonism) (Stipanovic *et al.* 1986, Berenbaum and Zangerl 1988). Berenbaum and Neal (1985) found that myristicin, a methylenedioxyphenyl (MDP) that presents in the leaves of many plants in the family Umbelliferae, was a highly effective synergist of the co-occurring furanocoumarin xanthotoxin. Myristicin at the concentrations occurring naturally in the plants was not toxic to *Helicoverpa zea* (Boddie) but at very low concentrations was an effective synergist of xanthotoxin. However, they found that there was no increase in the deterrency of xanthotoxin in the presence of myristicin, and suggested that the mechanism of synergism was not behaviourial but rather biochemical, via MDP competitive inhibition of microsomal mixed function oxidases (MFOs). Adams and Bernays (1978) also demonstrated synergism of deterrent effects of different phenolic compounds from *Sorghum bicolor* at concentrations naturally occurring in plant, on the feeding behaviour of *L. migratoria*.

2.2.4.1 Effects of allelochemicals on insect pests

Pickett (1988) indicated that a promising method for controlling pests without damaging the environment and causing pesticide resistance was the use of semiochemicals (behaviour-controlling chemicals) such as pheromones, antifeedants and host recognition compounds. Included in these are:

1.allomones: compounds that are released by one organism and can evoke a reaction in an individual of a different species that is beneficial to the emitter but not to the receiver;

2.kairomones: compounds that are released by one organism which evoke a response beneficial to the receiver but not to the emitter;

3.synomones: compounds that are released by an organism which benefit both the emitter and the receiver;

4.antimones: substances released by an organism which evoke in the receiver a behavioural or physiologic al reaction that disadvantages both the emitter and the receiver (Whitman 1988).

Lukefahr (1982) stated that studies of host-plant resistance have been increased because of the environmental problems associated with chemical pesticide use. According to Painter (1951) resistant cultivars are classified into 3 main categories:

1.nonpreference, which refers to characteristics of the plant that make it unattractive to insect pests for oviposition, feeding, or shelter;

2.antibiosis, which refers to the adverse effects of the host plants to bionomics of the insect feeding on them;

3.tolerance, which refers to the ability of the host plant to show only slight injury when exposed to an insect population which would severely damage susceptible hosts (Pathak 1975).

New techniques for breeding resistant cultivars are available. Induced mutation by either physical means or by chemical mutagens can be applied to increase genetic variability. Several cytogenetic techniques can be used to introduce resistance genes from one genotype into others in breeding programmes more quickly and surely than by most other techniques (Russell 1978). For genetic engineering methods, *Agrobacterium* is a micro-organism that is used for transfering genes into plants (Lycett and Grierson 1990). Other gene transfer system including the use of a transposable element (the P element) from *Drosophila melanogaster* Meigen as the transfer vector, the "biolistic" method (the DNA is delivered inside the cells by utilizing high-velocity

microprojectiles), and "electroporation" (the use of an electric pulse to the same end) (Whitten and Oakeshott 1991).

The use of resistant cultivars is probably the cheapest and most effective method to control pests. It can be used in integrated pest management which is considered to be better than using any single pest control method alone. Hence, for breeding resistant cultivars, it is not always necessary to breed for a very high level of resistance because incomplete resistance has often given an adequate level of control in the field when it has been integrated with other pest control methods (Russell 1978). For examples, Taksdal (1992) showed that the partially resistant cultivar of swedes (*Brassica napus* L.spp.*rapifera* [Metz.] Sinsk.), cvs Melfort and Angus, gave satisfactory control of the turnip root fly (*Delia floralis* Fallen) at the lowest level of attack. At higher levels of attack, Melfort and Angus were adequately protected by a half dose of chlorfenvinphos. Johnson and Gould (1992) demonstrated that in tobacco, *Bacillus thuringiensis* endotoxin-mediated partial resistance and natural enemies [*Campoletis sonorensis* (Cameron) and *Cardiochiles nigriceps* Viereck] had synergistic effects in reducing populations of the tobacco budworm [*Heliothis virescens* (Fabricius)] during the first stadium.

The effects of plant allelochemicals on immediate behavioural responses of insects have been known for many years but only recently have the more chronic effects on growth and development been studied extensively (Reese and Holyoke 1987). Reese (1983) implied that the growth of insects was slowed if the plant contained the essential nutrients for the insect but utilization of these nutrients was blocked in some way by allelochemicals or by too much or too little water. He also supported the hypothesis that even the most susceptible plants were notably well defended against insect invasion when compared to an artificial diet containing low concentrations of defensive compounds and no morphological means of defense.

Reese and Field (1986) found that larvae of black cutworm, *A. ipsilon*, grew more slowly on susceptible corn seedlings (Pioneer 3368A and G4507A) than on artificial diet (pinto bean diet). This result confirmed that even susceptible plants have defenses. In addition, they found that the acetone extract, one of many solvent extracts of 'Pioneer 3368A' incorporated into artificial diet, was the most inhibitory fraction. Other solvent extracts of ' Pioneer 3368A', such as benzene and methanol, also reduced growth. Similarly, Butler (1976) found that larvae of bollworm (*H. zea*) developed more rapidly when reared on a semisynthetic diet than on corn and cotton at any constant temperature.

According to Klun *et al.* (1970), DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) content in corn was used for grading for resistance to the corn borer because the higher the DIMBOA level in the plant leaf, the greater resistance to the corn borer (Pathak 1975). DIMBOA is a member of a class of allelochemicals known as hydroxamic acids. There is some evidence to indicate that hydroxamic acids are involved in resistance of wheat to aphids. Argandona *et al.* (1987) found that in tissues of wheat leaves, hydroxamic acids were present in the parenchyma cells of the mesophyll and in the vascular bundles but not in the epidermis. Aphids generally feed in vascular bundles and are unlikely to ingest substances in the epidermis. This suggests that hydroxamic acids are antibiotic allelochemicals rather than feeding deterrents. Leszczynski *et al.* (1989) also demonstrated that plant secondary substances found in winter wheat, especially hydroxamc acids and total phenols, had antibiotic effects on the grain aphid [*Sitobion avenae* (Fabricius)].

Copaja *et al.* (1991) investigated the hydroxamic acid content of perennial Triticeae. They found that the levels of DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) were higher than of DIMBOA, especially in species of *Critesion*, *Elymus* and *Pseudoroegneria*. In addition, the genera *Hordeum*, *Psathyrostachys* and *Secale* did not contain DIMBOA. The information from this study may be useful for producing wheat with high levels of hydroxamic acids, through wide hybridization. Niemeyer *et al.* (1992) suggested that hydroxamic acids may be used as chemotaxonomic markers for breeding wheat resistant to aphids.

In barley, DIBOA was found in seedlings of wild *Hordeum* species but not of cultivated barley. DIBOA mixed into artificial diets was toxic to the aphid *Rhopalosiphum padi* (L.). Moreover, DIBOA levels correlated negatively with performance of *R. padi* on *Hordeum* seedlings (Barria *et al.* 1992). Although hydroxamic acids have not been found in cultivated barley, indole alkaloids presented in these plants, such as gramine, appeared to be related to aphid resistance (Zuniga *et al.* 1988). Therefore, biological effects of allelochemicals may be a possible alternative for insect control in barley.

Schoonhoven and Derksen-Koppers (1976) demonstrated that most of 24 secondary plant substances which occur in acceptable food plants of the green peach aphid [*Myzus persicae* (Sulzer)] when incorporated in artificial diet render the diet less acceptable to the insect due to their feeding deterrent character. Furthermore, some of these allelochemicals such as atropine, azadirachtin, caffeine, conessine, hordenine, phlorizin, quinine and tannic acid showed significant effects on survival of the aphid. Dreyer and Jones (1981) also found that most flavonoids which occur in wheat showed

strong feeding deterrency against *S:hizaphis graminum* (Rondani) and *M. persicae*. In addition, *M. persicae* was found slightly more tolerant than *S. graminum*. They suggested that this may be becat se *M. persicae* has a wider host range than *S. graminum*, so *M. persicae* must (ope with a greater variety of allelochemicals. In sorghum, *p*-hydroxybenzaldehyde, Jhurrin and procyanidin isolated from leaves were the major feeding deterrents against *S. graminum* (Dreyer *et al.* 1981).

Three plant allelochemicals, triacontanol, tricin and tetramethyl-N,N-bis(2,6-dimethylphenyl)cyclobutane-1,3-dii nine, were extracted from *Arundo donax* L. (Gramineae) and showed significant antifeedant activity against the boll weevil (*Anthonomus grandis* Boheman) v'hich is one of the most destructive cotton pests in the United States (Miles *et al.* 1993).

Gossypol, the phenolic sesqJiterpenoid aldehyde dimer, has been isolated from subepidermal pigment glands of cotton (*Gossypium* sp.) and found to have negative effects on larval development of *H. virescens*. This prolonged development time provides advantages to high glanded cotton over glandless or low glanded plants due to prolonged period of exposure of larvae to adverse environmental effects, disease, parasites and predators, which ca1 reduce the survival to adulthood and limit the number of generations throughout the growing season (Stipanovic *et al.* 1986). Similarly, Kay *et al.* (1979) demoilstrated that gossypol incorporated in artificial diet had adverse effect on weight of larvae, pupae and adults, and increased the larval development period of *H. punctigera* and *Helicoverpa armigera* (Hubner). High gossypol concentrations also caused high mortality in both species. Furthermore, plant phenolics such as resorcinol and gallic acid found in most of the cotton cultivars had additive effects to the activity of δ-endotoxin of *B. thuringiensis* var.*galleriae* on *H. armigera* (Sivamani *et al.* 1992).

α and β-4, 8, 13-duvatrieile-1, 3-diols (DVT diols) and α and β-4, 8, 13 duvatrienols (DVT ols), the secondary compounds from leaf surface of tobacco (*Nicotiana tabacum* L.), were fourd to be ovipositional stimulators for *H. virescens*. However, the mixture of high levels of DVT diols and sucrose esters (also chemicals found in tobacco) suppressed larval development. Resistant cultivars such as TI 1112 contained traces of α and β-DVT diols while the susceptible cultivar, NC 2326, contained high levels of α and β-DVT diols (Cutler *et al.* 1986).

Zhang *et al.* (1993) derionstrated that chaparrin and chaparrinone, the compounds that were isolatec from chaparro amargo (*Castela tortuosa,*

Simaroubaceae), have detrimental effects on *H. virescens* and the beet armyworm [*Spodoptera exigua* (Hubner)]. As the concentration of these compounds in the diet increased, larval development was slower. However, only chaparrinone caused high mortality of these insects. Furthermore, as the concentration of these compounds in the diet increased, food consumption of *H. virescens* larvae decreased. Therefore, they suggested that the growth disruption may be caused by antifeeding activity. Chou and Mullin (1993) also found that antifeedant activity for western corn rootworm (*Diabrotica virgifera virgifera* LeConte) was positively correlated with the concentration of sesquiterpene lactones, particularly argophyllin A and its isomer argophyllin B, isolated from cultivated sunflower (*Helianthus annuus* L.). They proposed that argophyllins were a chemical defense against insect herbivory.

Byers *et al.* (1986) found that 3-Nitropropionic acid and two glucose esters (cibarian and karakin) which were isolated from leaves, flowers, and stems of crownvetch (*Coronilla varia* L.) significantly increased mortality of larvae and reduced pupal weight of sparganothis fruitworms [*Sparganothis sulfureana* (Clemens)]. These compounds are also toxic to non-ruminant animals (chickens and pigs) and to the cabbage looper (*Trichoplusia ni* Hubner).

L-Canavanine, a non-protein amino acid found in higher plants, is an allelochemical that has adverse effects on survival and the reproductive potential of *M. sexta* (Dahlman and Berge 1986). This allelochemical metabolite, canaline, inhibited the essential enzyme 'ornithine-oxo-acid amino transferase', causing the accumulation of ornithine in the hemolymp of *M. sexta*. The larvae developed edema and finally died.

Griffiths *et al.* (1988) demonstrated that ajugarin I, a compound found in the labiate plant *Ajuga remota*, was active against feeding of the mustard beetle (*Phaedon cochleariae*) at the concentration of 0.00001%. Moreover, this compound was also active against feeding of the diamondback moth [*Plutella xylostella* (Linnaeus)] at the concentration of 0.01%. However, ajugarin I at the concentration of 1.0% did not show any activity against an aphid (*M. persicae*). This study showed that ajugarin I was a highly active antifeedant against coleopteran and lepidopteran pests, but not homopterans.

Many polyhydroxyalkaloids which have been found in a variety of organisms, including higher plants, are potent inhibitors of glycosidase activity in insects, mammals and microorganisms. However, some of these compounds are less inhibitory to

mammalian glycosidases than those of other organisms. Hence, such compounds may be applied in crop protection formulations (Fellows *et al.* 1986).

Secondary plant compounds also have effects on seed-eating insects. For instance, various secondary plant compounds have detrimental effects on the number of emerging adults of the bruchid beetle or the southern cowpea weevil (*Callosobruchus maculatus*). Generally, the most toxic compounds were alkaloids, and non-protein amino acids were more toxic than protein amino acids (Janzen *et al.* 1977).

Nowadays, several plant allelochemicals have potential for use in commercial insect control. For example, methyl ketones are used as tomato host plant resistance factors to tobacco hornworm (*M. sexta*), salannin is used as an antifeedant to Colorado potato beetle [*Leptinotarsa decemlineata* (Say)], and azadirachtin (a limonoid found in seeds of the neem, *Azadirachta inaica*) is used as an antifeedant to fall armyworm (*S. frugiperda*) and as a moulting inhibitor to *H. virescens* (Klocke and Barnby 1989). Moreover, other allelochemicals found in the neem tree also cause diverse behavioural and physiological effects on insects such as repellency, feeding and oviposition deterrency, reproduction and growth inhibition, and other physiological disorders. The use of neem products to control insect pests would be environmentally desirable since they are highly biodegradable and most of the activity is lost within two weeks (Koul 1992). Furthermore, it seems likely that neem allelochemicals have potential for use as a pesticide since neem preparations have adverse effects on 123 species of insects, 3 species of mites and 5 species of nematodes (Jacobson 1986).

2.2.4.2 Effects of allelochemicals on beneficial insects

The third trophic level (beneficial insects) has been ignored by most plant breeders in their studies of host plant resistance. However, plant breeding can be used to improve qualities of plants which may assist beneficial insects (Nordlund *et al.* 1988).

The secondary compounds of plants used as food by a herbivorous insect not only affect the physiology and behaviour of the herbivore but also the quality of the herbivore as food for beneficial insects (Williams *et al.* 1988). Allelochemicals produced by plants can stimulate host/prey selection behaviour of beneficial insects (synomones) and allelochemicals (kairomones) released by hosts or prey can be utilized by beneficial insects for host/prey location (Nordlund *et al.* 1988). Chemicals from plants and herbivores used by natural enemies in searching their victims are called "infochemicals" (information-conveying chemicals which constitute a subcategory of

semiochemicals) (Vet and Dicke 1992). Many studies demonstrate the importance of plant allelochemicals in the host-habitat and host-finding behaviour of parasitoids. Plant allelochemicals can enhance parasitoid search and oviposition behaviour, and as a result, increase host mortality.

Natural enemies are known to be able to use pheromones of their host or prey in host-prey location (Wood 1982, Noldus and van Lenteran 1985). Noldus and van Lenteren (1985) found that the females of the egg parasite *Trichogramma evanescens* Westwood were attracted by a volatile substance(s) released by virgin females of the cabbage white butterfly (*Pieris brassicae* L.). Also, calling virgin cabbage moths (*Mamestra brassicae* L.) release sex pheromone that attracts *T. evanescens*. Noldus (1988) showed that the sex pheromone released by calling *H. zea* moths serves as a kairomone for the egg parasitoid *Trichogramma pretiosum* Riley. Scolytidae (bark beetles) also release pheromones that attract both predators and parasites (Wood 1982).

Plant allelochemicals ingested by herbivores, on the other hand, can have negative effects on survival, growth and development, and behaviour of beneficial insects (Barbosa 1988, Jones *et al* 1988, Williams *et al.* 1988). The effect of plant allelochemicals on insect parasitoids mostly depends on the effects of those chemicals on their herbivore hosts. For example, if the effects of a given allelochemical are more severe on specialist than on generalist herbivores, similar effects on their parasitoids by the allelochemical would be expected (Barbosa 1988).

Williams *et al.* (1988) found that small quantities of gossypol, an allelochemical produced by glanded cotton, stimulated *H. virescens* growth while larger dosages produced a negative sigmoidal curve and this effect was carried over to parasitoids reared from them. Similarly, Barbosa *et al.* (1991) reported that the effects of three plant allelochemicals, nicotine; rutin; and hordenine, on the parasitoid, *Cotesia congregata* (Say), generally paralleled those on the unparasitized tobacco hornworm (*M. sexta*).

Many phytophagous insects can also defend themselves against predators by using chemicals ,which can be obtained in two ways. First, insects may produce the compounds themselves (autogenous chemicals). Second, insects may store compounds from the diet, with or without chemical modification (sequestered chemicals) (Jones *et al.* 1988).

Most Chrysomelidae are highly specialized feeders, some of them on toxic plants. In several cases, chemical defense in this group is strongly influenced by host

plants. For example, larvae of several chrysomeline species secrete salicylaldehyde, the product that is produced from plant phenolglucoside and salicin found in the leaves and bark of many willows and poplars (Salicaceae)(Pasteels *et al.* 1988). Salicylaldehyde was found to be a highly repellent o various ant species (Pasteels *et al.* 1983, cited by Pasteels *et al.* 1988).

Diet breadth is considered by Jones *et al.* (1988) to be an important factor that can initiate and constrain the evo ution of chemical defenses. They found that diet breadth affected autogenous and sequestered chemicals in the lubber grasshopper (*Romalea guttata* Houttyun) (a gereralist insect). The grasshopper, when restricted to single-species diets, had a reduction in the production of autogenous compounds that were used for defense against a rumber of predators, while plant compounds were sequestered to defend against specific predators. They assumed that this was due to concentration of compounds ingested by the insect.

Turlings and Tumlinson (1992) found that injured corn released volatiles (terpenoids) which attracted the parasitoid (*Cotesia marginiventris*). The releasing of volatiles was not limited to the sites of damage, but occurred throughout the plant. They concluded that plant volatiles that were induced by herbivore-injury could serve as a signal with benefits to the plant but disadvantages to the herbivores.

2.2.5 Factors affecting amounts of allelochemicals

The amount of plant allelochemicals can be affected by environmental factors, such as light, soil fertility, age of plant, temperature, pathogens and predators. However, genetics must also play a significant role in determining amounts of allelochemicals produced by a given plant (Rice 1984).

Rice (1984) stated that plants growing in glasshouses do not produce as large amounts of allelopathic compounds as the same kinds of plants growing out-of-doors. He suggested that light quality had in important effect on the production of allelopathic compounds. '

Marten *et al.* (1974) reported the influence of environmental factors such as soil fertility on alkaloid concentration They indicated that concentrations of alkaloids (gramine, N,N-dimethyltryptamine. 5-methoxy-N,N-dimethyltryptamine) in clones of reed canarygrass (*Phalaris arundinacea* L.) were doubled when the clones were grown in an infertile peat compared to a fertile mineral soil. Alkaloid concentration of grass

growing in the soil, provided N levels did not exceed 240 kg/ha, was significantly reduced by supplying the deficient nutrients (especially P and K) to an infertile peat. They also found that alkaloid concentrations occurring in reed canarygrass grown in soil supplied with ammonium sources of N (NH_4CL and urea) were greater than in reed canarygrass grown in soil supplied with a nitrate source ($NaNO_3$). They implied that this result might be due to a more direct use of NH_4^+ for alkaloid formation, whereas NO_3^- was more functional for overall plant growth. In addition, grass supplied with both NH_4^+ and NO_3^- sources of N (NH_4NO_3) had an intermediate alkaloid concentration.

Moreover, they concluded that alkaloid levels in reed canarygrass were likely to be increased by N fertilization in situations where alkaloids were already high. On the other hand, alkaloid problems for grazing animals would probably not be initiated if practical levels of N (up to at least 200 kg/ha) were used on those strains inherently low in alkaloids. Although P, Mn, or Cu individually did not have an effect on alkaloid concentrations, fertilization of infertile soils with deficient nutrients (other than N) might reduce alkaloid concentration of reed canarygrass. However, the types of alkaloids present in reed canarygrass were not changed by fertilizer elements, indicating that this variable was genetically controlled.

Marten *et al.* (1973) found that alkaloid type and relative concentration among plants of reed canarygrass were not greatly affected by the environment associated with diverse geographic locations in temperate and sub-arctic areas, but concentration of alkaloids normally declined as maturity increased. However, absolute alkaloid concentration may be affected by climatic variables as well as maturity.

Hanson *et al.* (1983) studied the effects of temperature on growth and gramine concentration of three barley cultivars, Arimar; CI12020; and Proctor. They found that all cultivars grew best at 21°C/16°C (day temperature/night temperature); 30°C/25°C was somewhat supraoptimal but heat injury symptoms (chlorosis of the expanding leaves) appeared only at 38°C/33°C. Temperature affected gramine concentration of Arimar and CI12020 in the same way: gramine concentration was low at suboptimal and optimal growth temperatures, then increased markedly when moderately supraoptimal, falling again as the heat stress was severe. Proctor contained no indole alkaloids at any growth temperature. This result indicated that temperature affected gramine concentration in gramine-accumulating cultivars such as Arimar and CI12020 and that these cultivars might suffer autotoxic effects at high leaf temperatures.

Woods and Clark (1971) stu died the seasonal variation of both the gramine and the tryptamine group in reed canarygrass under two conditions of management, regularly clipped and unclipped. They show ed that gramine quality was significantly higher in regularly clipped than in unclipped reed canarygrass. A maximum value of gramine content under regular clipping was 605 µg/g fresh weight while that under unclipped conditions was 80.5 µg/g fresh weight. In addition, gramine content under regular clipping increased rapidly throughout the season while under unclipped conditions it increased slowly and decreased late in the season. Tryptamine content varied during the season in a similar fashion to gramine. These results indicated that rapid vegetative growth appeared to be the criterion for high alkaloid production.

Woods *et al.* (1979) showed that concentrations of alkaloids in reed canarygrass (gramine and hordenine) were much higher in regrowth forage than in first growth. First growth forage generally contained more hordenine than gramine while regrowth forage contained more gramine than hordenine. They also indicated that leaf blades contained higher concentrations of gramine than did leaf sheaths. Conversely, leaf sheaths contained higher hordenine concentrations than did leaf blades. The total herbage concentrations of hordenine and gramine found in the upper one-half of the plant canopy were 48% and 69%, respectively.

There is evidence indicating that marked increases in concentrations of phenolics and other types of allelochemicals in plants can be caused by infection by many pathogens (Rice 1984). In addition, plants sometimes respond to herbivores by turning on inducible defenses (Strong *et al.* 1984). For example, cotton plants that were previously exposed to mites had lower mite populations than unexposed plants. This demonstrates that such insects can induce resistance, that may involve allelochemicals, to subsequent herbivores (Karban and Carey 1984).

2.3 ALKALOIDS IN PLANTS

2.3.1 Classification and function

Most alkaloids contain nitrogen as part of a heterocyclic ring. However, not all plant compounds which possess a basic nitrogen atom in a heterocyclic ring system are regarded as alkaloids (Petterson *et al.* 1991). Alkaloids can be classed according to the type of ring system present in their structures. These classes of alkaloids including pyrrolidines, piperidines, pyridines, tropanes, pyrrolizidines, quinolizidines, quinuclidines, indoles, quinolines, purines, benzylisoquinoline, β-carbolines,

aporphines, protoberberines, benzophenanthridines, morphinans, erythrinans and steroids (Robinson 1979).

Petterson *et al.* (1991) classified alkaloids into three major groups:

1.True alkaloids, with few exceptions, are basic and carry nitrogen in a heterocyclic ring; they are derivatives of amino acids, e.g. nicotine and atropine;
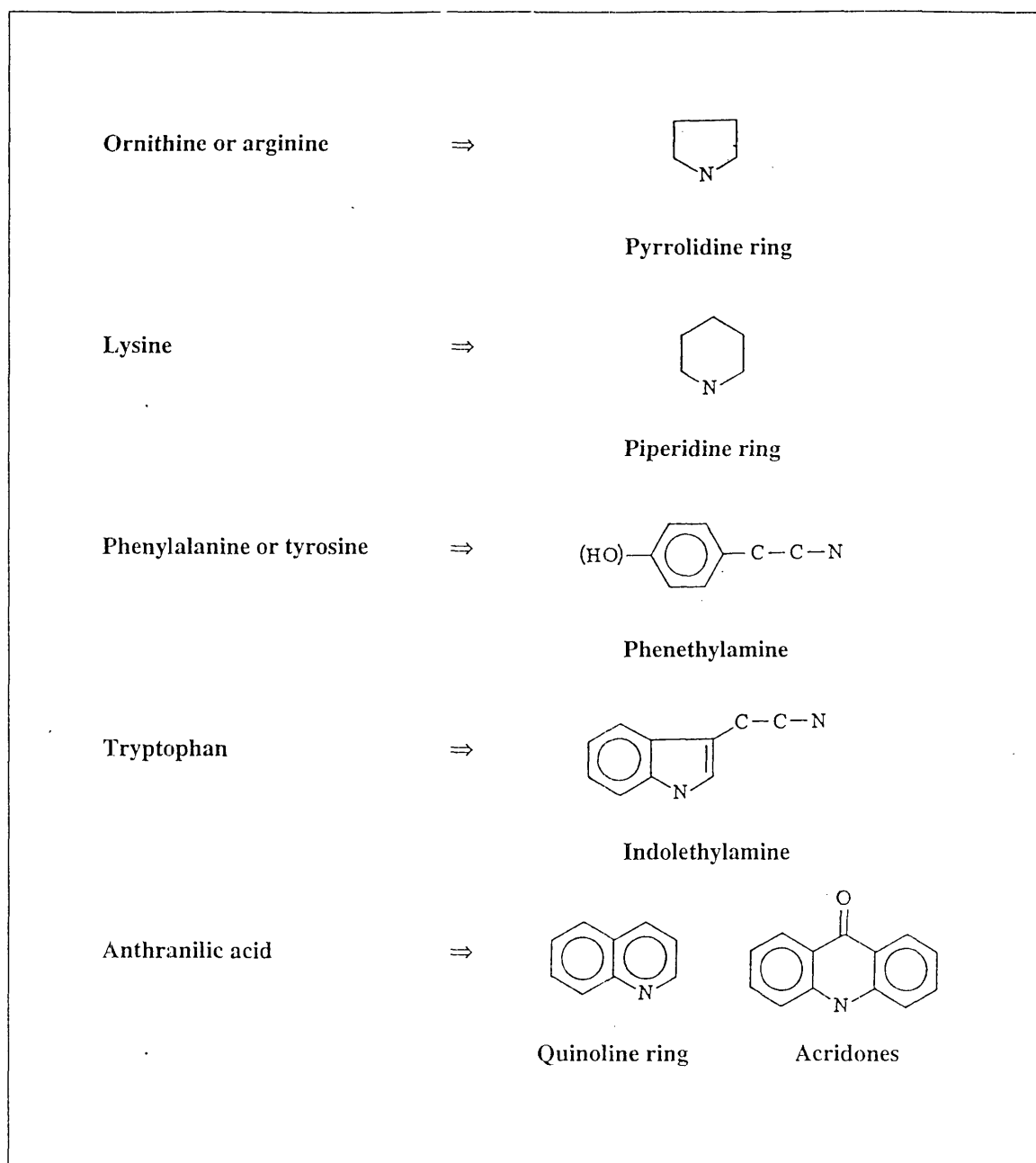
2.Pseudoalkaloids are generally basic nitrogenous compounds, but they are not derivatives of amino acids, e.g. solanidine and caffeine;

3.Protoalkaloids are basic amines that are derivatives of amino acids, but their nitrogen is not part of a heterocyclic ring, e.g. mescaline and ephedrine.

The majority of alkaloids are derived from amino acids and the most significant pathways from amino acid to alkaloids are illustrated in Figure 2.2. Robinson (1974) considered that alkaloids were not inert end products but that total concentration and rate of turnover fluctuated. Variations in the content of alkaloids often correlate with developmental stages.

Figure 2.2. Amino acid precursors of some common structural elements of alkaloids (Robinson 1979).

Ornithine or arginine	⇒	Pyrrolidine ring
Lysine	⇒	Piperidine ring
Phenylalanine or tyrosine	⇒	(HO)—C—C—N Phenethylamine
Tryptophan	⇒	C—C—N Indolethylamine
Anthranilic acid	⇒	Quinoline ring Acridones

Alkaloids are secondary sub stances that are offshoots from metabolic pathways with important functions in prima y metabolism (Figure 2.3). Whittaker and Feeny (1971) implied that, with few exceptions, secondary chemicals can be classified on biosynthetic grounds into five majc r groups: phenylpropanes, acetogenins, terpenoids, steroids, and alkaloids. They considered that alkaloids found primarily in the higher plants were grouped together large y because they hold basic nitrogen, not because of shared metabolic origin. Among sec ondary plant compounds, alkaloids are found widely in nature (Petterson *et al.* 1991).
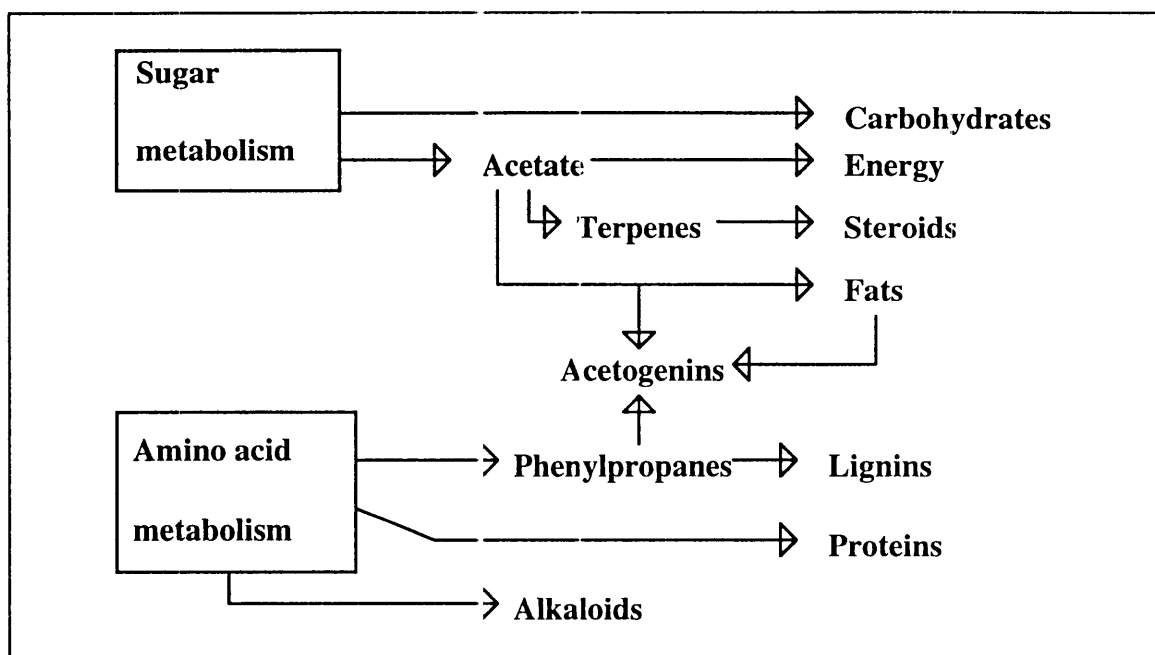
Figure 2.3. Metabolic relationship of the major groups of secondary compounds to primary metabolism (Whittaker and Feeny 1971).

The function of alkaloids ir plants is of the greatest general interest. The most widespread belief is that they are poisons or repellents toward insects. For example, the steroidal alkaloids of potato are repellent to potato beetles (Robinson 1974); nicotine is the most important of the natural alkaloids used as insecticides, and veratrine and ryanodine are also used (Balandrin *et al.* 1985). In contrast to the toxic effects on insects, alkaloids may act as stimulatory substances. For example, the invasion of aphids on broom (*Sarothamnus scoparius*) was stimulated by the presence of the alkaloid sparteine (Robinson 1974). Furthermore, they seem to be chemical defenses against microorganisms, phytophagous animals, and other competing plant species (Petterson *et al.* 1991,Wink and Twardowski 19⁵ 2).

2.3.2 Effects of alkaloids on palatability of plants

From an agricultural point of view the important disadvantage of alkaloids present in plants is that they have effects on the palatability of plants used for feeding animals (Marten *et al.* 1976, Marten *et al.* 1981). There is much evidence that alkaloids in reed canarygrass have adverse effects on palatability of the grass to ruminant animals.

Marten *et al.* (1976) found that total alkaloid concentration of reed canarygrass had significant adverse effects or average daily weight gains of lambs and steers.
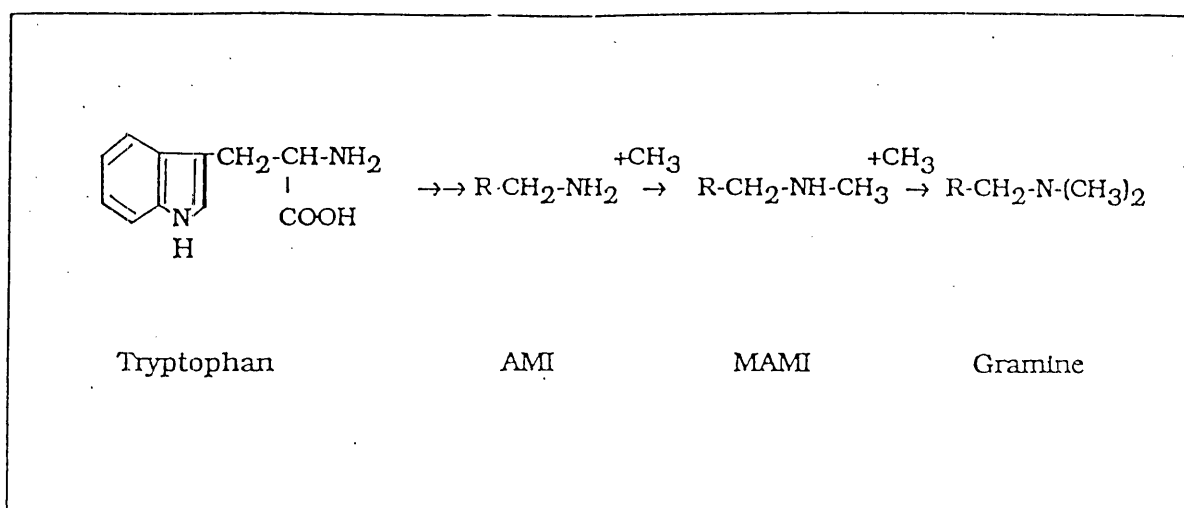
Moreover, they indicated that high alkaloid plants and tryptamine-carboline containing plants caused more diarrhea in grazing animals than gramine-containing plants. The effect of alkaloid concentration and type in two commercial cultivars of reed canarygrass, cvs.Rise and Vantage, and in a low-alkaloid tryptamine-carboline-free strain, MN-76, on performance and health of grazing lambs was investigated. Rise contained an average of 0.28 to 0.32% dry weight of a mixture of gramine and tryptamine-carbolines; Vantage contained 0.20 to 0.33% gramine, and MN-76 contained 0.09 to 0.12% gramine. The indole alkaloid concentration threshold in reed canarygrass at which lambs would show reduced gain was suggested to be 0.20% dry weight, and MN-76 was considered to be a biologically important grass breeding advance (Marten *et al.* 1981). Because of the adverse effect of alkaloids on palatability, low alkaloid cultivars may be desired for breeding plants that are used for feeding animals.

2.4 GRAMINE IN BARLEY

2.4.1 Introduction

Gramine, an indole alkaloid, is known to be toxic to animals and might play a defensive role in plants. Gramine has been suggested to be a feeding deterrent and/or a toxic substance for herbivores. It is found in plants such as reed canarygrass and barley and its relatives (*Hordeum* spp.) (Marum *et al.* 1979, Hanson *et al.* 1981, Lovett and Hoult 1992).

Gramine (or N,N-dimethyl-3-aminomethylindole) is a derivative compound of tryptophan (Bowden and Marion 1951a). For young barley shoots, the gramine biosynthesis pathway is shown in Figure 2.4. The indole ring, the methylene side chain and probably the amino N are derived from tryptophan. AMI (3-aminomethylindole) is the first stable intermediate identified after tryptophan, which is methylated stepwise to MAMI (N-methyl-3-aminomethylindole) and then to gramine (Hanson *et al.* 1983). Massicot and Marion (1957) found that gramine isolated from the shoots of sprouting barley was biochemically stable.

CH$_2$-CH-NH$_2$ +CH$_3$ +CH$_3$

| COOH   →→ R·CH$_2$-NH$_2$ → R-CH$_2$-NH-CH$_3$ → R-CH$_2$-N-(CH$_3$)$_2$

Tryptophan   AMI   MAMI   Gramine

Figure 2.4. Gramine biosynthesis pathway established for young barley shoots (Hanson *et al.* 1983).

Marum *et al.* (1979) indicated that in reed canarygrass the ability to synthesize gramine was controlled by the double recessive mm tt genotype. This makes selection for this type easy because no progeny testing is essential to verify the genotype. A single dominant allele T at one locus controlled synthesis of indole alkaloids classified in group T, including N-methyltryptamine (NMT), N,N-dimethyltryptamine (DMT), and 2-methyl-1,2,3,4-tetrahydro-β-carboline (MTHC). A single dominant allele M at a second locus controlled synthesis of indole alkaloids in group MeO, including 5-methoxy-N-methyltryptamine (5-MeO-NMT), 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) and 2-methyl-6-methoxy-1,2,3,4,-tetrahydro β-carboline (6-MeO-THC). Duynisveld *et al.* (1990) detected gramine in five cultivars of reed canarygrass, cvs.Rival, Castor, Vantage, Palaton and Venture. They found that cv.Rival had higher concentrations of gramine than cv.Venture, with the remaining cultivars being intermediate.

In barley (*H. vulgare*), gramine level was affected by environmental conditions and high levels of gramine were found in genotypes originating in warm regions. This suggests that the accumulation of gramine might be an adaptive metabolic response to high temperatures (Hanson *et al.* 1981). Gramine accumulation in plants depends on the capacity to synthesize and the capacity to degrade the compound. Cultivars that lack gramine may lack the capacity to synthesize it or have a capacity to degrade it faster than it is made. The balance between net gramine synthesis and net degradation is highly sensitive to the environment in established barley plants (Hanson *et al.* 1981)

Gramine was not present in seeds of barley (Zuniga *et al.* 1985) but was present in leaves. Argandona *et al.* (1987) indicated that higher concentrations of gramine were always present in younger leaves of barley than in older leaves. In leaves of barley, gramine was not present in the vascular bundles but in the epidermal tissues and the parenchyma cells of the mesophyll.

Bowden and Marion (1951b) indicated that gramine was formed in the leaves at least up to the 11th day of growth. According to Brandt *et al.* (1935) the total amount of gramine in the leaves remains constant during this period and the gramine disappears from the leaf after one month (Bowden and Marion 1951b).

In contrast, Hanson *et al.* (1981) showed that 24-day-old Arimar and Maraini (cultivated barley cultivars) were still actively synthesizing gramine, so these cultivars could maintain their high gramine concentration well beyond the four or five-leaf stage, especially when grown at high temperatures. In addition, they surveyed 24 genotypes of barley plants and found that some cultivars of cultivated barley including cvs.Proctor, Morex, Coho, Larker and Bowers, contained little or no gramine. Certain *H. vulgare* cultivars, such as cvs.Arimar and Maraini, and *Hordeum spontaneum* Koch (wild barley) races, grown in the field or in controlled environments contained gramine at concentrations that could be expected to have adverse effects upon the performance of ruminants such as sheep. For breeding low gramine cultivars, if *H. spontaneum* lines including PI282572, PI227301, PI211041 and cv.Rosh Pinna which have high or very high gramine levels, are used as an exotic parent in crosses with low-gramine breeding lines, there is some risk that undesirable high-alkaloid characteristics could be introduced into breeding populations (Hanson *et al.* 1981).

Apart from *H. spontaneum* lines, *H. agriocrithon* lines and the landraces (barley lines selected by farmers) were found to produce high levels of gramine. Some cultivars of Australian-grown lines including cvs.Forest, Windich, Schooner and Galleon, have small but significant quantities of gramine. This suggested that a gene(s) for gramine production might be possessed by these cultivars but that the expression of this gene was almost totally suppressed (Lovett and Hoult 1992).

2.4.2 Effect of gramine on insects

Gramine could be an advantage in pest management because of its feeding deterrence for herbivores. Thus, cultivars that contain high levels of gramine may be desirable for breeding cultivars which are resistant to insects. Zuniga *et al.* (1985) reported that population growth rate of the greenbug (*S. graminum*) was negatively correlated with gramine content of leaves of several barley cultivars. The cultivars that lacked gramine were susceptible while the cultivars with higher levels of gramine were more resistant to the aphids. Therefore, they indicated that gramine played a role in the resistance of barley seedlings to *S. graminum*. Moreover, because gramine content in barley leaves decreased with age, the susceptibility of barley to aphids increased in older plants.

Although gramine was not present in the vascular bundles that were preferred feeding sites of *S. graminum*, its effects on this aphid might be a consequence of the deterrent properties of the compound during penetration of the leaf by the stylet and subsequent probing before reaching the phloem. In addition, the presence of gramine in epidermal tissues was considered to possibly protect barley against other insects and plant pathogens (Argandona *et al* 1987). Zuniga *et al.* (1988) confirmed that the feeding behaviour of aphids such as *S. graminum* and *R. padi* in barley might be affected by gramine content and its location.

Other studies that show the importance of gramine as a resistance factor of barley to aphids are those of Kaneh sa *et al.* (1990) and of Rustamani *et al.* (1992). Both studies indicated that resistant lines had large amounts of gramine and gave lower population densities of aphids while susceptible lines had small amounts of gramine and gave higher aphid population densities. The most strongly resistant lines were two lines of *H. spontaneum* (No.2558 and Nc.4969).

Salas *et al.* (1990) found that nitrate fertilization such as KNO_3 caused changes in gramine concentration in leaves which might affect aphid performance on barley seedlings. For example, barley seedlings watered with nutrient solutions with a higher NO_3^- content had lower population growth rate of *S. graminum*. They also indicated that the concentration of gramine increased in the second leaf (youngest), and decreased in the first one (oldest) with increasing NO_3^- in the nutrient solution.

It is interesting that gramine not only affects sap-sucking aphids, including specialists such as *S. graminum*, but also grasshoppers (generalists and foliage feeders).

Bernays (1990) found that gramine 0.5% dry weight had a deterrent effect on *L. migratoria* but there was no post-ingestional toxicity on the insect. Westcott *et al.* (1992) also demonstrated that gramine at concentrations similar to those occurring in barley had significantly negative effects on survival and mean weight of the migratory grasshopper [*Melanoplus sanguinipes* (Fabricius)]. Since gramine shows adverse effects on the biology of both specialists and generalists, and both sucking and chewing insects, it may have a potential for controlling a wide range of insect pest species.

These studies indicate that it is likely to be possible to breed barley cultivars that contain high levels of gramine, in order to obtain cultivars resistant to aphids, grasshoppers and possibly other insects which could be affected by gramine. However, the appropriate amount of gramine in barley plants that can both reduce insect populations and increase the palatability of plants to the animals should be investigated.

2.5 HORDENINE IN BARLEY

Hordenine (N-dimethyltyramine) is a protoalkaloid (Robinson 1979) found in barley roots. It is also found in certain cultivars of reed canarygrass such as cvs.Rival, Castor, Vantage, Palaton and Venture (Duynisveld *et al.* 1990). There is evidence to suggest that hordenine may have detrimental effects on insects and animals.

N-methyltyramine and hordenine are synthesized by the methylation of tyramine through a mechanism analogous to that known to exist in certain molds (the synthesis of choline from ethanolamine by the mold *Neurospora crassa*) and in animals (Kirkwood and Marion 1950). Kirkwood and Marion (1951) found that choline and hordenine were isolated from barley roots when they fed sprouting barley with potassium formate labeled with C^{14}. Therefore, they suggested that the N-methyl groups of choline and hordenine originated from formate ions.

Tyramine methyltransferase is an enzyme involved in the hordenine biosynthesis pathway. It has been isolated from the roots of germinating barley and it seems likely that the primary function of this enzyme is tyramine methylation. Moreover, the enzyme can also methylate a number of tyramine analogues (Mann and Mudd 1963). Mann *et al.* (1963) found that the occurrence of tyramine methyltransferase activity in roots of germinating barley was strikingly similar in time to the accumulation of its product, N-methyltyramine. It increased rapidly during germination; maintained high levels for several days and then the level began to fall by the end of the first week. Moreover, they showed that puromycin, analogues of lysine, valine, phenylalanine and sodium arsenate

inhibited the formation of the enzyme during the germination of barley. On the other hand, the formation of the enzyme by embryos could be stimulated by casein hydrolysate or by certain individual amino acids. In addition, kinetin caused a delayed and prolonged increase in the enzyme levels.

Leete *et al.* (1952) found that there was a higher content of hordenine than of N-methyltyramine in roots of germinating barley and no trace of tyramine was detected. They also demonstrated that no trace of such alkaloids was detected in barley seeds. In addition, they suggested that hordenine might be converted to other substances because of a rather high activity of the roots. Derivative salts and degradation products of hordenine such as hordenine picrate, hordenine hydrochloride, hordenine platinichloride, hordenine methiodide, o-methylhordenine methiodide, trimethylamine platinichloride, homoanisaldehyde oxime and *p*-acetoxybenzoic acid were obtained from the plant.

Leete and Marion (1953) demonstrated that tyrosine was a precursor of N-methyltyramine and hordenine as it was the source of tyramine in the roots of barley. In addition, methionine methyl and formate served as precursors of hordenine methyl but the former was more efficient. In contrast, choline methyl and bicarbonate did not serve as precursors of hordenine methyl in barley plants (Matchett *et al.* 1953). Massicot and Marion (1957) also showed that phenylalanine was a precursor of hordenine in barley as it was transformed into tyrosine. Tyrosine was decarboxylated to tyramine which was methylated by methionine to N-methyltyramine and to hordenine (Leete *et al.* 1952, Leete and Marion 1953, Matchett *et al.* 1953). Hordenine, on the other hand, partly reverted to tyramine but did not revert to tyrosine (Frank and Marion 1956).

Hordenine was found to have adverse effects on insects such as an aphid and the migratory grasshopper, and also animals such as rats. Hordenine (0.01 M) incorporated into the diet was found to have deterrent effects on a polyphagous aphid (*M. persicae*) after 24 hours of feeding. At the same concentration, hordenine had significantly negative effects on survival of the insect after feeding on the diet for 2 days, and no insect survived after 4 days of feeding (Schoonhoven and Derksen-Koppers 1976). As with gramine, Westcott *et al.* (1992) found that hordenine at the levels occurring naturally in plants gave significantly negative effects on survival and mean weight of the migratory grasshopper (*M. sanguinipes*). Barwell *et al.* (1989) demonstrated that hordenine acted as an inhibitor of noradrenaline uptake in isolated vasa deferentia of the rat. Therefore, they suggested that hordenine is likely to be able to exhibit adverse pharmacological effects upon the sympathetic nervous system of the rat. However, the

effect of hordenine on ruminant animals needs further investigation (Duynisveld *et al.* 1990).

2.6 INSECT PESTS OF BARLEY

In the USA, the greenbug (*S. graminum*) is a key pest of barley, and common aphids on barley in North America including English grain aphid (*S. avenae*), corn leaf aphid [*R. maidis* (Fitch)], oat bird chery aphid (*R. padi*), and yellow sugarcane aphid [*Sipha flava* (Forbes)]. Other sap-feeding pests include chinch bug, leafhoppers and planthoppers, mites, and hessian fly. Chewing insects including cereal leaf beetle, armyworms, cutworms, grasshoppers and thrips. Borers include barley jointworm, wheat stem maggot, and sawflies. Soil insects include wireworms and false wireworms, and white grubs (Starks and Webster 1985). In Australia, foliage feeders, seedling pests and stored products pests are more important than aphids. Foliage feeders and seedling pests include armyworms, cutworms, blue oat mite, red-legged earth mite and lucerne flea (Sparrow and Doolette 1987). Stored products pests, such as rust-red flour beetle and confused flour beetle, saw-toothed grain beetle, flat grain beetles, and cadelle beetle, are also important (Greening and Gellatley 1987). Other pests that occur on barley and other winter cereals include white curl grubs, false wireworms, wireworms, Curculionid larvae (weevils), field crickets, brown wheat mite, Australian plague locust and aphids (Goodyer and Sykes 1991). Aphids, particularly the oat aphids, occasionally damage crops by direct feeding. However, they are generally of much greater concern as possible vectors of barley yellow dwarf virus (Goodyer and Sykes 1991). In addition, the Russian wheat aphid [*Diuraphis noxia* (Mordwilko)] is considered likely to arrive in Australia in the near future. This aphid arrived and spread rapidly within South Africa in 1978, Mexico in 1980 and the USA in 1985, resulting in major crop losses of both wheat and barley (Evans *et al.* 1989).

2.6.1 *Mythimna convecta* (Walker)

In Australia, armyworms are known as pests of barley and other cereal crops such as wheats and oats, and also pastures. They are classified in Order Lepidoptera, Family Noctuidae.

Armyworms cause their most serious damage by feeding on and cutting off the seedheads of ripening crops. They may also defoliate or eat out large areas of establishing crops in some years. During establishment, severe defoliation significantly decreases the yield. Infestations are frequently associated with heavy rainfall or

flooding. Armyworms can be found feeding near the top of plants at night or on overclouded days, or on the surface of the ground under clods or shaded debris and on the lower stems and leaves of the plants during warm, sunny days (Goodyer and Sykes 1991).

Of the several species of armyworms, the common armyworm, *Mythimna convecta* (Walker) (= *Leucania convecta* (Walker)) is a serious pest throughout Australia (Broadley 1979, cited by Smith 1984). McDonald and Smith (1986) indicated that *M. convecta* was the most abundant species in cereals including barley, wheat and oats. It was found generally in the warmer months of spring and summer in south-eastern Australia. *Persectania ewingii* (Westwood) was the most predominant armyworm species in pastures. The least abundant species of armyworms, *Persectania dyscrita* Common, occured mostly in the drier inland areas ,especially in the west. *M. convecta* was the most common armyworm species in north-eastern New South Wales while other species, such as *P. ewingii* and *Mythimna loreyimima* (Rungs) were recorded in low densities (Del Socorro 1991).

In northern New South Wales, *M. convecta* moths were collected in tower-mounted light traps at Point Lookout and Mt. Dowe. Catches varied between sites and years. At Point Lookout, significant numbers were caught in spring and autumn in 1985/86 and 1986/87, in late summer and autumn in 1987/88 and 1988/89, and in spring in 1989/90. At Mt. Dowe, the only season that significant numbers of moths were caught was in autumn (1986/87). It is believed that *M. convecta* moths caught were in process of long-distance migration (Gregg *et al.* 1993). Coombs *et al.* (1993) found that *M. convecta* females trapped at Point Lookout were predominantly (97.1%) unmated. They suggested that migration of the insect is chiefly undertaken prior to the beginning of reproduction.

In pasture grasses, *M. convecta* had maximum growth rates on barley grass (*Hordeum leporinum* Link), performed well on ryegrass (*Lolium perenne* L.) and kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov), and had significantly slower growth rates on paspalum (*Paspalum dilatum* L.) and Mitchell grass (*Astrebla pectinata* L.) (McDonald 1991). In addition, the greatest incidence of *M. convecta* was found in the pastures and crops which grew up through a stubble or dried grass, or those with very dense foliage. This is due to the preference for ovipositing on dry material on the part of adult females (McDonald 1988).

The life cycle of the common armyworm is egg, larva, pupa and adult. Adults live for 2 to 4 weeks and each female may lay several hundred eggs. Eggs generally hatch in 1 to 3 weeks. The larval period varies from 3 to 6 weeks during warm weather to more than 3 months in winter. The pupal period is 2 to 4 weeks under favourable conditions while it can be delayed by continued cold or hot weather (more than 8 weeks in either late winter or mid summer). Common armyworm may have three or four overlapping generations between spring and late autumn (Goodyer 1983). Smith (1984) found that larvae of the common armyworm had 6 or 7 instars when reared on artificial diet at 25°C and head capsule widths could be used for separating instars. Developmental periods of eggs, larvae and pupae were affected by temperature. In addition, the developmental rates of females were slightly faster than males.

2.6.2 *Agrotis ipsilon* (Hufnagel)

Three important species of cutworms are found in New South Wales: common cutworm [*Agrotis infusa* (Boisduval)], black cutworm or greasy cutworm (*A. ipsilon*), and brown cutworm or pink cutworm (*Agrotis munda* Walker). Common cutworm and black cutworm are widespread but the latter are most common on the North Coast. Common cutworms occur during winter and early spring, black cutworms occur during late summer and autumn while brown cutworms are active during mid-spring, summer and early autumn (Goodyer 1978). They normally feed on seedling plants, cutting through their stems near ground level, and may also feed on the foliage of older plants. They generally attack weeds, field crops, vegetables and ornamental plants. Weeds attacked include fat hen, dandelion, pigweed, common sowthistle, dock, caltrop, shepherd's purse and morning glory Field crops attacked include lucerne, wheat, barley, oats, maize, sweet corn, grain sorghum, linseed, sunflower, safflower, lupins, rapeseed, soybeans and tobacco. Vegetables include tomatoes, cabbages, peas, lettuce, carrots, onions, potatoes, sweet potatoes and watermelons. Ornamentals attacked are gladiolus, dahlia, aster, carnation and geranium (Goodyer 1978; 1985).

Moths of cutworms usually live for 4 to 6 weeks and each female may lay several hundred eggs. In warm weather, eggs hatch in 3 to 7 days whereas they take several weeks to hatch in cold weather. The larvae normally develop through six stages and the larval period is from 3 weeks to more than 12 weeks depending on the weather. The pupal period is only 2 to 4 weeks in warm weather while it can take more than 8 weeks in cold weather (Goodyer 1985).

Cutworms are migratory insects. Gregg *et al.* (1994) found that *A. infusa* was the most common species caught in light traps at Point Lookout and Mt. Dowe in northern New South Wales. According to Common (1954), it migrates from south-eastern New South Wales and Victoria in northerly or north-westerly direction in late summer and autumn (Gregg *et al.* 1994). However, some moths can be found in northern New South Wales in mid summer suggesting that outbreaks of the insect on winter cereals may not be necessarily the result from the invasion from the south (Gregg *et al.* 1994).

In the USA, Showers *et al.* (1989) demonstrated that *A. ipsilon* migrated great distances in a very short time (two to four nights) by using variation of the synoptic weather systems. The nocturnal w nd maximum (called the low-level jet) affords the rapid transport of the insect over large distances. The low-level jet is a layer of relatively strong winds which normally forms during the evening (300-900-m elevation) and it occurs in various regions of central North America.

The black cutworm (*A. ipsilon*) is a cosmopolitan insect. Larvae can survive to pupation on a variety of crop plants and weeds found relatively abundant in Indiana. The highest survival was found on b uegrass, curled dock and wheat while no larvae survived to pupation on giant foxtail or debris. It also could survive on lambsquarters, oats, soyabeans, yellow rocket, alfalfa, rough pigweed, corn, velvetleaf, jimsonweed and annual morning glory. Time to pupation of the insect varied from 24.6 days on wheat to 47.0 days on annual morning glory (Busching and Turpin 1977). In addition, it can have three or four overlapping generations each year (Goodyer 1985).

Sherrod *et al.* (1979) found that weeds, especially winter annual weeds, in Illinois fields attracted ovipositing black cutworm moths during late winter and spring. These weeds including common chickweed, mouse-eared chickweed, purslane speedwell, bitter cress, shepherd's purse, yellow rocket and pepper grass. In addition, the potential for presence of black cutworm was increased by agronomic practices that support the establishment of winter annual or perennial weeds.

Gholson and Showers (1979) demonstrated that under greenhouse conditions late instar *A. ipsilon* larvae cut more seedling corn plants and fed on more organic baits under dark conditions than in light. They also preferred wet baits to dry baits which indicated that wet baits could reduce the damage of corn seedling by *A. ipsilon* larvae. Moreover, apple-grape, grape and bran pellets were the most attractive baits of the larvae.

Although cutworms may be controlled by natural factors including weather, predatory birds, parasitic insects and bacterial diseases, these factors do not help much in crop protection since crops are severely damaged by the larvae before these natural control agents become effective (Goodyer 1985).

2.7 *LOCUSTA MIGRATORIA* (LINNAEUS)

L. migratoria is not a pest of barley in Australia. It has a tropical and sub-tropical distribution (Hill 1983), while barley is grown mainly in temperate areas. However, it is a convenient laboratory model for studies on insect physiology (Wigglesworth 1972) and has frequently been used for testing the effects of allelochemicals. Its responses to gramine have been studied previously (Bernays 1990) and it was used in this thesis to provide a comparison with armyworms.

L. migratoria is classified in Order Orthoptera, Family Acrididae. There are many subspecies of *L. migratoria* such as *L. m. migratoria*, *L. m. rossica*, *L. m. gallica*, *L. m. manilensis* (Uvarov 1966). *L. migratoria* has the largest distribution area of any locust and has been reported as feeding on a wide range of crops and wild plants (Uvarov 1977). According to Kozhanchikov (1950, cited by Uvarov, 1966), *L. m. migratoria* can complete its life-cycle on Gramineae and Cyperaceae. Although its development is completed on Compositae, Cruciferae, Plantaginaceae, Leguminosae and Urticaceae, adults do not mature sexually (Uvarov 1966).

Its life cycle is varied according to local ecological conditions. For example, the oriental *L. m. manilensis* has no egg diapause and produces one to two generations per year in northern China but three to four in the southern provinces. *L. m. migratoria* normally has egg diapause when confined to cold winter areas but hatching without a diapause has been observed in years with exceptionally warm autumns (Uvarov 1977).

Locusta is primarily graminivorous and adapted to feeding on narrow leaves and stalks. It nibbles the straw of cereal crops, resulting in dropping of the ears which causes serious crop losses. It also bites the leaf-stalks of plants that are not very acceptable, resulting in defoliation of plants without the leaves being eaten (Uvarov 1977).

Migration patterns of *L. migratoria* are different between subspecies and the factors affecting the occurence and the patterns of their migrations such as weather conditions and food supplies (Uvarov 1977).

2.8 *HELICOVERPA* SPP.

Among *Helicoverpa* species, *Helicoverpa armigera* (Hubner), *Helicoverpa zea* (Boddie) and *Helicoverpa punctigera* (Wallengren) are the three major pest species which can be the key pests of several agricultural and horticultural crops (Fitt 1989). In Australia, *H. punctigera* and *H. armigera* are the two economic important pests of field crops. *H. punctigera* is widespread in Australia while *H. armigera* is more common in eastern coastal Australia and up to 100 miles inland (Zalucki *et al.* 1986).

Host plants of *Helicoverpa/Heliothis* species in many countries are maize, sorghum, oats, barley, pearl millet, chickpea, pigeonpea, cowpea, peas, various beans, cotton, sunflower, safflower, tobacco, tomato, brinjal, cucurbits, sweet potato, groundnut, flax, citrus, lucerne, sunnhemp, cape gooseberry, potato, etc. In South Africa, there are various wild host plants of *Helicoverpa/Heliothis* species. The most important host plants are *Acalypha segatalis* (Euphorbiaceae), *Amaranthus thunbergii* (Amaranthaceae), *Malvastrum tr cuspadatum* (Malvaceae), *Nicandra physaloides* (Solanaceae), *Sonchus oleraceus* and *Xanthium pungens* (Compositae) (Jayaraj 1982).

H. punctigera and *H. armigera* attack a wide variety of crops. In Australia, *H. punctigera* attacks tomatoes, linseed, lucerne, canning and field peas, cabbages, deciduous fruits and tobacco whereas *H. armigera* attacks maize, tomatoes, cotton, lucerne, tobacco and vegetables (Common 1953). Moreover, they also attack sunflower, groundnuts and soyabeans. Wheat and sorghum are important hosts of *H. armigera* during early and late-season, respectively (Wardhaugh *et al.* 1980). Although Common (1953) postulated that *H. punctigera* did not regularly attack cotton, Wardhaugh *et al.* (1980) stated that *H. punctigera* was a major pest of cotton during the early and middle parts of the season. *H. punctigera* is called 'native budworm' and it is found feeding upon the terminal leaf buds and squares (flower buds) of cotton while *H. armigera* is called 'cotton bollworm' since it attacks squares and boll (Wilson and Waite 1982). Furthermore, medics and weeds are also available to *H. punctigera* in the spring (Wardhaugh *et al.* 1980). Zalucki *e al.* (1986) noted that *H. punctigera* was likely to be dominant on dicotyledons while *H. armigera* attacked on both di- and monocotyledons. In the inland of Australia (during winter and spring), Zalucki *et al.* (1994) collected *H. punctigera* larvae from 47 plant species in 8 families and *H. armigera* larvae from 28 plant species in 10 families. Most of these plant species were new host for both moth species and were found predominantly in the Asteraceae and Fabaceae.

Light-trap and crop survey data from the Namoi Valley of New South Wales suggested that there were four generations of *H. punctigera* and *H. armigera* a year (Wardhaugh *et al.* 1980). A single large peak of *H. punctigera* was found between late November and early January each year while *H. armigera* was dominant from January-February onwards. Gregg *et al.* (1993) showed that *H. punctigera* was mostly caught in tower-mounted light traps in northern New South Wales (at Point Lookout and Mt. Dowe) in spring and early summer. In contrast, *H. armigera* was caught throughout the season.

Fitt (1989) stated that the control of *Helicoverpa* spp. is still heavily reliant on the use of insecticides in most crops. The efficacy of chemical applications is frequently reduced by insecticide resistance. As a result, more frequent application of harder chemicals is necessary to achieve control. Natural enemies are often destroyed by such frequent use of insecticides.

Gunning *et al.* (1984) demonstrated that resistance of *H. armigera* to pyrethroids occurred in eastern Australia, especially at Emerald in central Queensland where pyrethroids were extensively applied in irrigated crops, particularly cotton, sorghum and soyabeans. In contrast, Gunning and Easton (1994) showed that *H. punctigera* was not resistant to DDT, endosulfan and pyrethroids. They suggested that the difference in resistance to insecticide of *H. armigera* and *H. punctigera* may be due to significant differences in genetic, ecological or behavioural factors between the species. Forrester *et al.* (1993) also found that pyrethroid resistance was discovered in field populations of *H. punctigera* at low frequencies and endosulfan resistance was not detected. In response to field pyrethroid failures against *H. armigera*, they introduced an insecticide resistance management (IRM) strategy for controlling the insect in summer crops in eastern Australia. This strategy was based on the rotation of unrelated chemical groups on a per generation basis, integrated with the use of ovicidal mixtures and other non-chemical control methods (biological and cultural control).

Farrow and Daly (1987) proposed that pest status of *Helicoverpa* species was, in part, a result of their ability to move between crops in a local region and *H. punctigera* was more migratory than *H. armigera*. Gregg (1994) stated that most long-distance migration in these species is pre-reproductive and migration allows them to escape from deteriorating habitats and colonise new ones. Among the Australian *Helicoverpa* species, *H. punctigera* is the most migratory and its large scale migration from sources in inland Australia take place normally in early spring. Farrow and Daly (1987) classified *Helicoverpa* movements into 3 levels:

1. short-range movement wi:hin or near the crop canopy;

2. long-range movement abc ve the canopy within the biological boundary layer;

3. long-range movement in the upper air above the biological boundary layer.

The migration of the insect is affected by wind systems and layering of the migration was found during the day as well as the night (Drake *et al.* 1981). Erratic rainfall seems to have an influence on migration of *H. punctigera* due to its effect on availability of hosts of the insect (Gregg 1994). Moth migrations (including *Helicoverpa* species) are generally associated with disturbed weather patterns. Catches of moths in light traps seemed to be obtained when there were depressions or troughs, wind shifts, upper wind convergence, high relative humidity and rainfall (Gregg *et al.* 1994). In addition, the weather (such as temperature, rainfall and photoperiod) encountered during development as well as the weather during the period when the adult is physiologically capable of flight can influenced all stages of migration (Drake 1994).

Fitt (1989) argued that four physiological, behavioural and ecological characteristics of *Helicoverpa* spp.: polyphagy, high mobility, high fecundity and a facultative diapause, enable them to survive in variable habitats and to successfully colonize and exploit agricultural systems. He suggested that future cotton pest management (e.g. pest management of *Helicoverpa* species) must involve a mix of control techniques which are likely to include host plant resistance, cultural control, conservation and augmentation of natural enemies, and behavioural manipulation (e.g. trap cropping, pheromones for mating disruption) (Fitt 1994). Moreover, the use of transgenic cotton will be the major change in future pest management.

As with *L. migratoria*, *H. punctigera* is not a pest of barley. However, it was used in this thesis to provide a comparison with *M. convecta*. Both are noctuid moths, with comparable life histories. However, *H. punctigera* does not normally feed on monocotyledons whereas these are the primary hosts of *M. convecta*. Comparing the effects of barley allelochemicals on these two species therefore provides an opportunity to compare responses of a species which has co-evolved with these and similar substances, with one which presumably has not.