CHAPTER

FIVE

THE FEEDING DETERRENT EFFECT OF GRAMINE AND HORDENINE ON ARMYWORMS

5.1 INTRODUCTION

In Chapter 4, it was found that gramine in combination with hordenine at the concentrations naturally occurring in barley leaves (cv.Lara) showed an adverse effect on the survival of *M. convecta* (ie. gramine 500 ppm+hordenine 60 ppm). This result suggested that gramine and hordenine were synergistic in reducing the survival of this insect and may also have delayed its development. Therefore, it is possible that gramine and hordenine are antibiotic substances which are toxic to *M. convecta*. However, alkaloids in plants may have toxic or repellent properties (Robinson 1979). Many researchers have demonstrated that gramine and hordenine may have feeding deterrent effects on sucking insects, such as aphids (Schoonhoven and Derksen-Koppers 1976, Argandona *et al.* 1987, Zuniga *et al.* 1988) and chewing insects, such as grasshoppers (Bernays 1990). Consequently, the possibility of a deterrent effect of gramine and/or hordenine on *M. convecta* was investigated in experiments described in this chapter. Barley leaf strips dipped in gramine and/or hordenine solutions were offered to armyworm larvae in choice and no-choice tests.

5.2 MATERIALS AND METHODS

5.2.1 The feeding deterrent effect of gramine and hordenine on early instar larvae

5.2.1.1 No-choice bioassay with second-instar larvae

There were four treatments in this bioassay. These treatments were based on the treatments of experiments presented in Chapter 4:

Treatment 1 control (no gramine and hordenine)

Treatment 2 gramine 500 ppm

Treatment 3 hordenine 60 ppm

and Treatment 4 gramine 500 ppm+hordenine 60 ppm

The bioassay was conducted under laboratory conditions (constant 25°C and 16 hours daylength) in the insectary. The design of this assay was a two-way factorial classification (2 chemicals with 2 concentrations) with 8 replications.

Gramine and hordenine were dissolved in distilled water to give 500 ppm and 60 ppm concentrations, respectively. Gramine could be dissolved in water in this experiment (whereas it could not be dissolved in the experiments of Chapter 4) because it was possible to use large volumes of water. Agral600® (ICI Australia Operations Pty Ltd.) 0.1% (v/v) was added as a sticker to each treatment solution, including the control. Barley cv.Schooner was used because of its naturally low concentrations of gramine and hordenine. The barley was grown in the glasshouse of the Department of Agronomy and Soil Science and was sown on 3rd May 1994. Leaves of 11 day old barley were cut into 1.5 centimetres lengths and each leaf strip was dipped in the treatment solution for 3-5 seconds. Excess liquid was removed by blotting with paper towel and leaf strips were air-dried.

The larvae used for this bioassay were larvae from egg masses of emerging moths of *M. convecta*, fed on a 5% sucrose solution via soaked dental wicks, reared in the insectary (constant 25°C and a 16 hours daylength). The methods of rearing the larvae were those described in Section 3.2.1, except that the pupae were left in the diet until adult emergence.

Four treated or four control leaf strips were fixed on a filter paper (No.1 Whatman) by staples (no.10) as shown in Figure 5.1. Treated and control leaf strips were placed in separate containers. The filter paper with attached leaf strips was photocopied before placing it in a plastic container (11 cm diameter and 5 cm high) which was covered with a lid having small holes for ventilation at the centre. Then four to five drops of water were added to moisten the filter paper in order to provide water to the leaf strips. Four early second-instar larvae were released into each container and allowed to feed for 24 hours. After feeding the moist filter papers with the leftover leaf strips were photocopied again (Figure 5.2).

Photocopied images, before and after larval feeding, were scanned into an Apple MacIntosh (SE/30) computer using a flat bed scanner (Apple Scanner). The areas of the leaf images were measured using the public domain NIH Image program (written by Wayne Rasband at the U.S. National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov). All leaf area measurements were corrected for the area occupied by the staple. It was found that leaf strips sometimes expanded

after 24 hours, so the initial leaf area measurements were corrected for leaf expansion. This correction factor was determined by keeping 16 leaf strips in containers as above and measuring image areas before and after 24 hours. It was found that this Leaf Expansion Factor was 1.0375 (Leaf Expansion Factor = Area of leaf strips after 24 hours/Initial leaf strip areas). The data collected in this bioassay were feeding proportion [(Initial - Final area)/Initial area] by larvae on treated and control leaf strips.

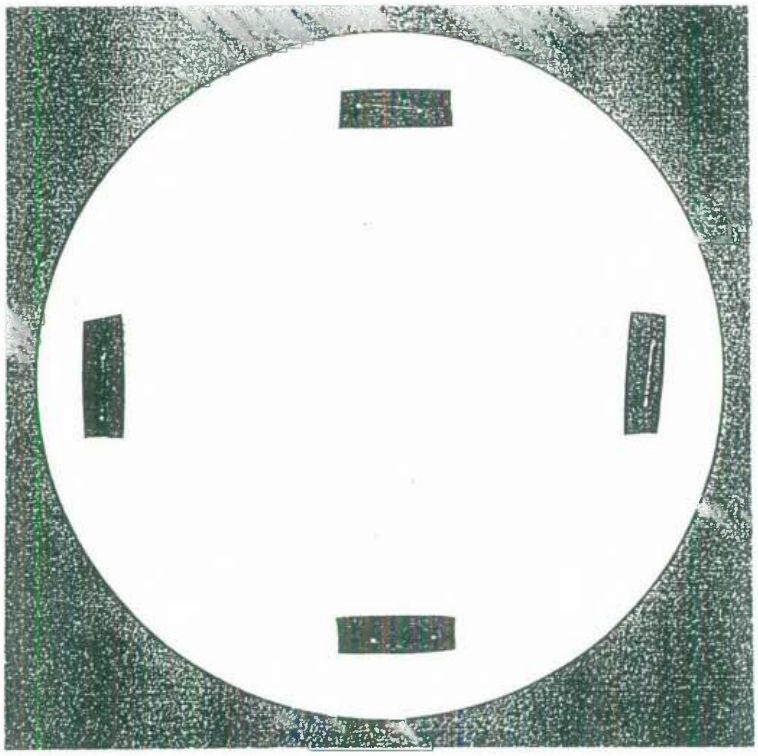

Figure 5.1. Photocopy of the filter paper with attached leaf strips, set up for a no-choice bioassay (before feeding).

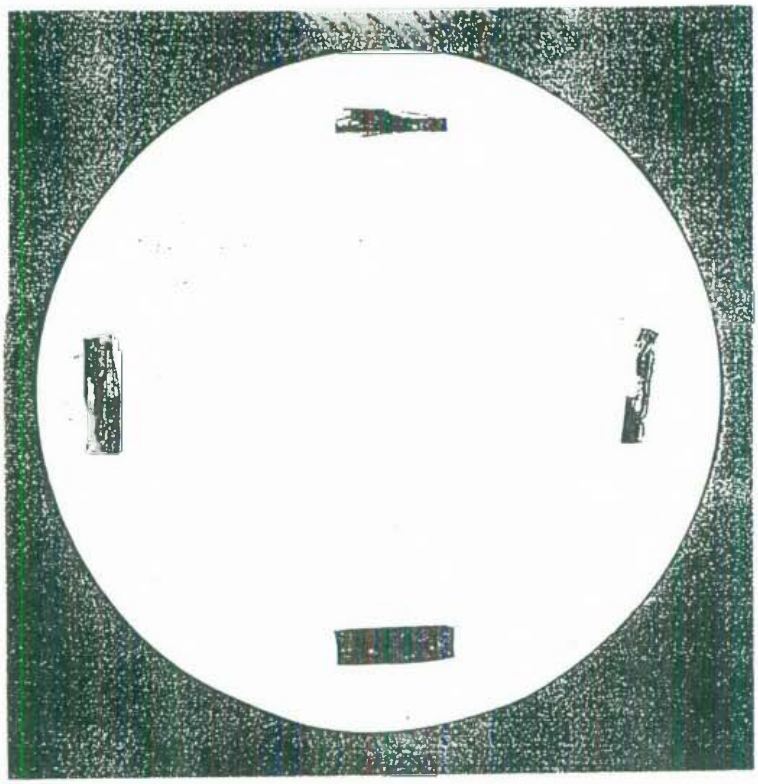

Figure 5.2. Photocopy of the filter paper with attached leaf strips, set up for a no-choice bioassay (after feeding).

5.2.1.2 Choice bioassay with second-instar larvae

The treatments and procedures of this assay were those described in Section 5.2.1.1 but two treated and two control leaf strips were fixed alternately on a filter paper (Figure 5.3). The filter paper was placed in the container with a lid. The barley leaf strips used were similar to those used in Section 5.2.1.1. Four early second-instar larvae from laboratory rearing (as described in Section 5.2.1.1) were released in the container. There were 8 replications per treatment and these treatments were placed in a completely randomised design. The feeding period and methods for leaf area measurement were those described in Section 5.2.1.1. Data collected were feeding proportion by larvae on treated and control leaf strips.

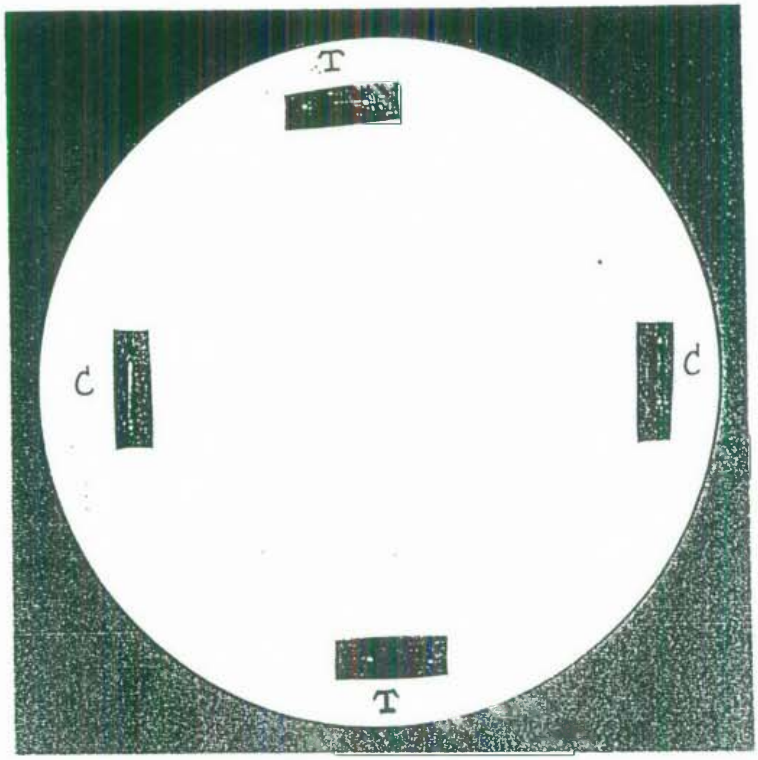

Figure 5.3. Photocopy of the filter paper with attached leaf strips, set up for a choice bioassay (before feeding). T = treated leaf strip, C = control

5.2.2 The feeding deterrent effect of gramine and hordenine on late instar larvae

5.2.2.1 No-choice bioassay with fourth-instar larvae

The same treatments and procedures in Section 5.2.1.1 were followed for this assay. However, barley cv.Schooner was sown in the glasshouse on 20th May 1994. Eight day old barley leaves were cut into 3 centimetre lengths. One early fourth-instar larva from laboratory rearing (as described in Section 5.2.1.1) was released into each container and allowed to feed for 24 hours. The larger leaf strips and smaller numbers of larvae used in this experiment compared to those in Section 5.2.1 were to accomodate the greater food consumption of fourth-instar larvae compared to second instars. Leaf area measurement procedures and data collected were the same as in Section 5.2.1.1. The design of this assay was a two-way factorial classification (2 chemicals with 2 concentrations) with 16 replications.

5.2.2.2 Choice bioassay with fou rth-instar larvae

The treatments and proced res of this assay were those described in Section 5.2.1.2 but the barley leaf strips use l were similar to those used in Section 5.2.2.1. One early fourth-instar larva from labor atory rearing (as described in Section 5.2.1.1) was released into each container. The fe ding period and methods for leaf area measurement were those described in Section 5.2.1.1. There were 16 replications per treatment and this assay was arranged in a comple ely randomised design. Data collected were feeding proportion by larvae on control and reated leaf strips.

5.2.3 Analysis of data

All data were analysed usi g the MINITAB statistical package (as in Section 3.2.6). For no-choice bioassay wit l second and fourth-instar larvae, data on feeding proportion of *M. convecta* were arc in transformed and analysed by two-way analysis of variance. For choice bioassay wit l second and fourth-instar larvae, data on feeding proportion of *M. convecta* were arc in transformed and were analysed by using Paired *t*-tests. Paired *t*-tests were used in ch ice bioassay because data on feeding proportion of larvae fed on control and treated le f strips were not independent (such leaf strips were fed on by the same larva/group of larvae in each replication). A one-way analysis of variance was used to compare feedi g proportion of control in all treatments.

5.3 RESULTS

5.3.1 The feeding deterrent effect of gramine and hordenine on early instar larvae

5.3.1.1 No-choice bioassay with second-instar larvae

In no-choice bioassay, feeding proportions by second-instar larvae offered leaf strips dipped in control, gramine 500 ppm, hordenine 60 ppm and gramine 500 ppm+hordenine 60 ppm solutions are shown in Table 5.1.

Table 5.1. Feeding proportion by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (no-choice bioassay: 2nd instar larvae).

Treatments	n	Proportion of feeding (Mean±se)
1. control	32	0.340±0.033
2. 500 ppm gramine	32	0.278±0.038
3. 60 ppm hordenine	32	0.345±0.043
4. 500 ppm gramine + 60 ppm hordenine	32	0.325±0.040

The two-way analysis of variance on feeding proportions (arcsin transformed) of second-instar larvae in this bioassay showed that there were no significant differences in feeding proportion on leaf strips dipped in gramine 500 ppm and control ($p>0.05$). Similarly, feeding proportions on leaf strips dipped in hordenine 60 ppm and control were not significantly different ($p>0.05$). There was also no interaction of gramine 500 ppm and hordenine 60 ppm compared to control ($p>0.05$) (Table 5.2).

Table 5.2. Two-way analysis of variance on feeding proportion (arcsin transformed) by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (no-choice bioassay: 2nd instar larvae).

SOURCE	DF	SS	MS	F	P
GRAMINE	1	0.067	0.067	1.012	>0.05
HORDENINE	1	0.037	0.037	0.557	>0.05
INTERACTION	1	0.009	0.009	0.130	>0.05
ERROR	124	8.207	0.066		
TOTAL	127	8.320			

5.3.1.2 Choice bioassay with second-instar larvae

In choice bioassay, feeding proportions by *M. convecta* second-instar larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control are shown in Table 5.3. Feeding proportion by larvae on treated leaf strips tended to be higher than those on control leaf strips. However, the paired *t*-tests showed that there were no significant differences between gramine 500 ppm and control (p = 0.51), hordenine 60 ppm and control (p = 0.25), and gramine 500 ppm+hordenine 60 ppm and control (p = 0.33) in feeding proportion (Table 5.4).

Table 5.3. Feeding proportion by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (choice bioassay: 2nd instar larvae)

Treatments	n	Proportion of feeding (Mean±se)	
		Treated	Control
1. 500 ppm gramine &control	8	0.393±0.067	0.317±0.053
2. 60 ppm hordenine & control	8	0.474±0.040	0.360±0.062
3. 500 ppm gramine + 60 ppm hordenine & control	8	0.429±0.034	0.351±0.041

Table 5.4. Paired *t*-tests on feeding proportion (arcsin transformed) by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (choice bioassay: 2nd instar larvae).

Treatments	n	df	*t*	p
1. 500 ppm gramine &control	8	7	-0.69	0.51
2. 60 ppm hordenine & control	8	7	-1.25	0.25
3. 500 ppm gramine + 60 ppm hordenine & control	8	7	-1.05	0.33

The one-way analysis of variance showed that there were no significant differences between treatments ($p = 0.829$) in feeding proportion on the control leaf strips (Table 5.5). This result, combined with the lack of significant differences between control and treated leaf strips within any one treatment, indicates that the overall feeding of second-instar larvae was not significantly affected by gramine or hordenine.

Table 5.5. Feeding proportion of control leaf strips by *Mythimna convecta* larvae (choice bioassay: 2nd instar larvae).

Treatments	n	Proportion of feeding (control leaf strips) (Mean±se)
1. 500 ppm gramine	8	0.317±0.053
2. 60 ppm hordenine	8	0.360±0.062
3. 500 ppm gramine + 60 ppm hordenine	8	0.351±0.041

non-significant ($p = 0.829$) by one-way analysis of variance

5.3.2 The feeding deterrent effect of gramine and hordenine on late instar larvae

5.3.2.1 No-choice bioassay with fourth-instar larvae

Feeding proportions by fourth-instar larvae offered leaf strips dipped in control, gramine 500 ppm, hordenine 60 ppm and gramine 500 ppm+hordenine 60 ppm solutions are shown in Table 5.6.

Table 5.6. Feeding proportion by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (no-choice bioassay: 4th instar larvae).

Treatments	n	Proportion of feeding (Mean±se)
1. control	64	0.334±0.053
2. 500 ppm gramine	64	0.367±0.054
3. 60 ppm hordenine	64	0.259±0.048
4. 500 ppm gramine + 60 ppm hordenine	64	0.382±0.054

The two-way analysis of variance on feeding proportion (arcsin transformed) showed that there were no significant differences in feeding proportion by larvae offered leaf strips dippd in gramine 500 ppm and control ($p>0.05$). There were also no significant differences in feeding proportion on leaf strips dipped in hordenine 60 ppm and control ($p>0.05$). Moreover, there was no interaction of gramine 500 ppm and hordenine 60 ppm compared to control ($p>0.05$) (Table 5.7).

Table 5.7. Two-way analysis of variance on feeding proportion (arcsin transformed) by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (no-choice bioassay: 4th instar larvae).

SOURCE	DF	SS	MS	F	P
GRAMINE	1	0.780	0.780	2.108	>0.05
HORDENINE	1	0.079	0.079	0.214	>0.05
INTERACTION	1	0.225	0.225	0.608	>0.05
ERROR	252	93.354	0.370		
TOTAL	255	94.438			

5.3.2.2 Choice bioassay with fourth-instar larvae

Feeding proportions by fourth-instar larvae offered leaf strips dipped in gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm solutions and control in choice bioassay are shown in Table 5.8. Larvae tended to feed on leaf strips dipped in hordenine 60 ppm and gramine 500 ppm+hordenine 60 ppm rather than control leaf strips. Paired *t*-tests, however, showed that these trends were not statistically significant: gramine 500 ppm and control ($p = 0.23$), hordenine 60 ppm and control ($p = 0.06$), and gramine 500 ppm+hordenine 60 ppm and control ($p = 0.09$) (Table 5.9).

Table 5.8. Feeding proportion by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (choice bioassay: 4th instar larvae).

Treatments	n	Proportion of feeding (Mean±se)	
		Treated	Control
1. 500 ppm gramine &control	8	0.319±0.082	0.437±0.043
2. 60 ppm hordenine & control	8	0.569±0.094	0.447±0.068
3. 500 ppm gramine + 60 ppm hordenine & control	8	0.401±0.092	0.285±0.064

Table 5.9. Paired *t*-tests on feeding proportion (arcsin transformed) by *Mythimna convecta* larvae on leaf strips treated with gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control (choice bioassay: 4th instar larvae).

Treatments	n	df	*t*	p
1. 500 ppm gramine &control	16	15	1.25	0.23
2. 60 ppm hordenine & control	16	15	-2.00	0.06
3. 500 ppm gramine + 60 ppm hordenine & control	16	15	-1.81	0.09

The one-way analysis of variance showed that there were no significant differences ($p = 0.126$) between treatments in feeding proportion on the control leaf strips (Table 5.10). As with second-instar larvae in choice bioassay, the result indicates that the overall feeding of fourth-instar larvae was not significantly affected by gramine or hordenine.

Table 5.10. Feeding proportion of control leaf strips by *Mythimna convecta* larvae (choice bioassay: 4th instar larvae).

Treatments	n	Proportion of feeding (control leaf strips) (Mean±se)
1. 500 ppm gramine	16	0.437±0.043
2. 60 ppm hordenine	16	0.447±0.068
3. 500 ppm gramine + 60 ppm hordenine	16	0.285±0.064

non-significant ( ) = 0.126) by one-way analysis of variance

5.4 DISCUSSION

5.4.1 The feeding deterrent effect of gramine and hordenine on early instar larvae

The results of no-choice bioassay with second-instar larvae (Section 5.3.1.1) showed that there were no significant differences in feeding proportion by larvae offered leaf strips dipped in gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control solutions (Table 5.2).

For choice bioassay with second-instar larvae (Section 5.3.1.2), larvae tended to feed more on treated leaf strips with all treatments, but there were no significant differences between treatments and the control in feeding proportion by larvae (Table 5.4).

From the results of these bioassays, it is likely that dipping in gramine 500 ppm, hordenine 60 ppm and gramine 500 ppm in combination with hordenine 60 ppm has no deterrent effect on *M. convecta* second-instar larvae.

5.4.2 The feeding deterrent effect of gramine and hordenine on late instar larvae

The results from no-choice bioassay with fourth-instar larvae (Section 5.3.2.1) showed no significant differences in feeding proportion by larvae offered leaf strips dipped in gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm and control solutions (Table 5.7).

For choice bioassay with fourth-instar larvae (Section 5.3.2.2), there were also no significant effects of gramine 500 ppm, hordenine 60 ppm, gramine 500 ppm+hordenine 60 ppm on feeding proportion by larvae (Table 5.9). The results from both no-choice and choice bioassays indicate that dipping leaves in gramine 500 ppm, hordenine 60 ppm and the combination of gramine 500 ppm and hordenine 60 ppm do not deter the insect.

5.4.3 Alternative techniques for bioassays

The methods of observing plant-insect interactions may be divided into three categories: field observations, seminatural condition observations and laboratory observations. Each method has its strengths and weaknesses which should be considered when designing a study (Opp and Prokopy 1986).

The results of these bioassays may be affected by some variation that could not be controlled. The thickness of leaf discs (strips) taken from different leaves and differential uptake (at the cut edge) of chemicals applied by dipping may affect the result (Antonious and Saito 1981 cited by Lewis and van Emden 1986). Glass fiber discs may be used instead of leaf strips to reduce the variable in thickness of leaf strips and chemicals may be applied with a pipette (Adams and Bernays 1978). However, the use of glass fiber discs requires a feeding stimulus in combination with test chemicals. Hence, this method may be not appropriate because the feeding stimulus may also have effects on feeding of insects and may interact with test chemicals. The use of pipetting has the advantage that the amount of test solution applied to each disc is constant (Cook 1976). Alternately, test chemicals may be applied to the leaf discs (strips) by painting or spraying (Jermy *et al.* 1981 cited by Lewis and van Emden 1986). All these methods may give different final concentrations and/or distributions of the chemicals on and in the leaves; it is difficult to relate any of them to natural leaves. Furthermore, Lewis and van Emden (1986) considered that chemicals obtained from commercial sources may be different in their behavioural effects from ones obtained from plants. Consequently, the use of chemicals extracted from plants may be more reliable for the study on insect behaviour.

The wide variation between individual insects, for instance, feeding and searching ability may also have affected the results. Even in the same brood of caterpillars, they do not all feed at the same time and at the same amount. Some caterpillars feed greedily whereas others are likely to be more rapidly satisfied. Some are more restless, spending much more time in roving around before they settle down to

feed (Cheesman 1932). It is common to find at least 5%-10% of insects which do not follow the general pattern (Adams and Bernays 1978).

Lewis and van Emden (1986) reviewed the conditions of these types of tests that may affect insect feeding behaviour They considered food and water deprivation, light and temperature, previous experience; stage and sexes of insect, the number of insects and cage size as possible influences Moreover, they also suggested that if all sources of variability in testing procedure are controlled, the variability in behavioural responses may be due to genetic differences.

5.4.4 The perception of gramine and hordenine by insects

In general, the feeding behaviour of insects consists of the following: locomotion (bringing the insect to its food), cessation of locomotion on arrival, biting (probing, sucking, etc.), continued feeding, and termination of feeding (satiation) (Dethier 1966). Plant allelochemicals and/or nutrients are considered to be important factors in insect feeding behaviour. In order to understand plant-insect interactions completely, it is necessary to know how these chemicals affect the sense organs of insects.

In the evolutionary point of view, the perception of secondary plant chemicals by insects is of interest. Insects perceive secondary plant chemicals by the chemical senses of olfaction and gustation which are divided into "specialized cells" and "generalized cells". Specialized cells are highly sensitive to one specific chemical whereas generalized cells respond to a wide range of chemicals. Olfactory sensilla are concentrated on the antennae while gustatory receptors are concentrated on the mouthparts (Chapman and Blaney 1979). Among herbivorous insects, grasshoppers have very large numbers of sensilla on the mouthparts which enables them to recognize many classes of stimulating chemicals (Chapman and Blaney 1979).

Deterrence of feeding by a food or chemical could be coded by stimulation of a 'deterrent' cell, inhibiting of an 'acceptance' cell and/or some 'complex code' involving many cells (Frazier and Hanson 1986). In caterpillars, deterrent chemicals are recognized by deterrent receptors and synergistic effects of chemicals may be due to the addition of impulse number at the receptor level (Adams and Bernays 1978).

The perception of gramine and/or hordenine by *M. convecta* larvae may be associated with gustatory receptors on the mouthparts. Blaney and Simmonds (1988) found that feeding behaviour of *S. littoralis*, *H. virescens* and *H. armigera* on sugars,

amino acids, sugar alcohols and allelochemicals was correlated with the electophysiological responses of maxillary styloconic sensilla in the larvae and proboscis styloconic sensilla in the adult. Moreover, the larvae showed much more sensitivity to allelochemicals than the adults. Hanson and Peterson (1990) demonstrated that in *Manduca sexta*, the medial styloconica (the medial "deterrent cells") is responsible for deterring feeding on unacceptable non-host plants such as the canna lilly, *Canna generalis*. From these studies, it is likely that the maxillarly styloconic sensilla is an important chemoreceptor of caterpillars.

5.4.5 The feeding deterrent effect of gramine and hordenine compared to their toxic effect

The results of Experiment 5 (Chapter 4) showed that gramine 500 ppm delayed the development of *M. convecta* reared on artificial diet. However, it did not show feeding deterrent effects on the insect (this chapter). These results indicate that gramine is a toxic substance but not a deterrent. Hordenine 60 ppm, by itself, did not have either toxic or deterrent effects.

The combination of gramine and hordenine at the concentrations found in barley leaves (cv.Lara) had a synergistic effect in reducing survival of *M. convecta* (Experiment 5 in Chapter 4). However, the results of bioassays in this chapter demonstrated that gramine in combination with hordenine at such concentrations had no effect in reducing feeding of the insect. These results indicate that the synergistic effect of gramine and hordenine is toxicity rather than deterrency.

Cottee *et al.* (1988) found that eight plant secondary compounds including allylisothiocyanate, azadirachtin, nicotin hydrogen tartrate, quinine chloride, salicin, sinigrin, tomatine and umbelliferone at natural concentrations deterred *L. migratoria*. Some of these compounds also deterred *S. gregaria,* especially azadirachtin. However, they demonstrated that the deterrency of these compounds was poorly related to oral toxicity. Similarly, Bernays (1990) demonstrated that six plant secondary compounds that are quite widespread in nature including amygdalin, caffeic acid, gramine, linamarin, sinigrin and vanillic acid had deterrent effects on *L. migratoria*. Nevertheless, these compounds did not show deleterious effects on the growth of fifth instar *L. migratoria*. Because of the poor correlation between deterrency and oral toxicity, from the evolutionary point of view, it appears that avoidance responses may frequently have developed under selective pressures unrelated to post-ingestional toxicity (Bernays 1990), and this may be the case with the allelochemicals of barley.

5.4.6 The potential role of gramine and hordenine on feeding behaviour of insects

Argandona *et al.* (1987) suggested that the effect of gramine on the aphid, *S. graminum*, might be a consequence of the deterrent properties of the compound because it was not present in the vascular bundles that were preferred feeding sites of the aphid. Moreover, the presence of gramine in epidermal tissues may protect barley against other insects and plant pathogens. Gramine has a deterrent effect, not only on sucking insects but also on some chewing insects. Bernays (1990) found that gramine at the concentration of 0.5 % (dry weight) had a significant deterrent effect on *L. migratoria*.

In the work described in this thesis, neither gramine nor hordenine showed feeding deterrent effects on *M. convecta* both early and late instar larvae and both choice and no-choice bioassays. Nevertheless, Schoonhoven and Derksen-Koppers (1976) found that hordenine had deterrent effects on a polyphagous aphid, *M. persicae*, suggesting that hordenine may also have similar effects on other insects.

Gramine in combination with hordenine at the concentrations naturally occurring in barley leaves (cv.Lara) did not reduce feeding of *M. convecta* larvae. However, further study on synergistic effect of deterrents may be valuable for breeding resistant cultivars, since even low concentrations of deterrents will be valuable if they have additive effects. Plants contain numerous secondary plant chemicals and it may be a better and more flexible defense strategy for the plant to possess mixtures of deterrents that have additive effects than to have an accumulation of any one chemical in larger amounts (Adams and Bernays 1978). Therefore, it is not always necessary to breed cultivars with very high concentration of an allelochemical. On the other hand, a cultivar that contains low concentrations of deterrents with additive effects may be useful. Moreover, a cultivar that contains very high concentrations of allelochemicals may be not needed if it is used in integrated pest management program. These considerations suggest that further studies of the feeding deterrent effects of gramine and hordenine in combination with other barley allelochemicals might be worthwhile.

The deterrent effect of chemicals on different insect species is variable. For locusts, Cottee *et al.* (1988) demonstrated that many compounds such as allylisothiocyanate, salicin, sinigrin, tomatine and umbelliferone at concentrations occurring naturally in the plants were more deterrent to *L. migratoria* than to *S. gregaria.* Although gramine in combination with hordenine at the concentrations naturally occurring in barley leaves (cv.Lara) did not show a significant feeding deterrent effect on *M. convecta*, further studies on the effect of such combinations on other insect pests of barley (such as *Agrotis* spp.) may also be of interest.

CHAPTER

SIX

CONCLUSIONS

Allelochemicals in plants play an important role in agroecosystems because of their ability to affect crop pests including weeds, insects and diseases. The evidence from many studies has shown that allelochemicals have potential as pesticides and are involved in resistance of plants to pests. Furthermore, a major advantage of the use of allelochemicals as pesticides is that they are easily biodegradable. Most allelochemicals are safer and environmentally cleaner than the synthetic pesticides in present use (Rizvi *et al.* 1992). Thus, allelochemicals may be an alternative pest control method that can be substituted for conventional chemical control in pest management. Nevertheless, some allelochemicals have adverse effects not only on plants, diseases and insects, but also on the health of domestic animals. Such allelochemicals may accumulate in the food chain and finally affect man (Rizvi *et al.* 1992). Hence, the use of allelochemicals in pest management needs information on effects of these allelochemicals not only on pests but also on animals and humans.

Gramine, an allelochemical in barley, has been found to have effects on the biology of insects including aphids (Zuniga *et al.* 1985, Zuniga *et al.*1988, Kanehisa *et al.* 1990, Rustamani *et al.* 1992) and grasshoppers (Bernays 1990, Westcott *et al.* 1992). In this thesis, gramine at the concentration naturally occurring in barley (cv.Lara) has been shown to have adverse effects on the growth and development of common armyworm, *Mythimna convecta*, a serious pest of barley. There are many studies which indicate that gramine is involved in the resistance of barley to aphids (Zuniga *et al.* 1985, Zuniga *et al.*1988, Kanehisa *et al.* 1990, Rustamani *et al.* 1992). Therefore, it is possible that gramine may be a resistance factor of barley to *M. convecta*. However, gramine incorporated in artificial diet at the concentration found in barley (cv.Lara) did not show significant adverse effects on the growth and development of the insect.

It seems likely that the growth and development of *M. convecta* fed on barley (cv.Lara) may be affected by not only gramine but also other allelochemicals in barley. Apart from gramine, hordenine is also found in roots (Leete *et al.* 1952, Leete and Marion 1953, Frank and Marion 1956) and in the work described in this thesis it was found in leaves of barley. There is some evidence that hordenine has significant negative effects on the biology of an aphid and the migratory grasshopper (Schoonhoven and Derksen-Koppers 1976, Westcott *et al.* 1992). Therefore, hordenine was considered to be an allelochemical that may have synergistic effects with gramine. Gramine, in

combination with hordenine, incorporated in artificial diet at the concentrations found in barley (cv.Lara) showed a synergistic effect in reducing survival of *M. convecta*. No significant effects of such concentrations of these allelochemicals, alone or in combination, were found for the survival of *Agrotis ipsilon* and *Helicoverpa punctigera*. However, there were technical problems with these experiments. Hordenine could not be recovered from the *Helicoverpa* diet, and it was shown that the ethanol used to dissolve gramine had adverse effects on the insect. In addition, the effects of these allelochemicals on the growth and development of *A. ipsilon* have not been clearly demonstrated because of the high mortality during the experiment which was probably due to the sub-optimal nature of the artificial diet. Hence, studies on the effect of gramine and/or hordenine on these insects need to be repeated.

There are many groups of enzymes which are involved in the detoxication system of insects (Brattsten 1979, Berenbaum and Zangerl 1988). Mixed-function oxidase enzymes were found in the midgut microsomes of many lepidopterous larvae such as fall armyworms, corn earworms and tobacco budworms (Yu and Ing 1984). Consequently, enzymes involve in detoxication system of *M. convecta*, *A. ipsilon* and *H. punctigera* are of interest.

Since alkaloids in plants may have toxic or repellent properties (Robinson 1979), gramine and/or hordenine may possess repellence as well as toxicity. Gramine and hordenine were found to have deterrent and/or toxic effects on aphids (Schoonhoven and Derksen-Koppers 1976, Zuniga *et al.* 1985, Argandona *et al.* 1987, Zuniga *et al.* 1988) and grasshoppers (Bernays 1990, Westcott *et al.* 1992). On the other hand, gramine, hordenine, and the combination of gramine and hordenine at the concentrations found in barley (cv.Lara) did not show significant feeding deterrent effects in this thesis.

Deterrency of some allelochemicals to grasshoppers has a poor correlation with oral toxicity (Cottee *et al.* 1988, Bernays 1990). From the evolutionary point of view, this indicates that avoidance responses may often have developed under selective pressures unrelated to post-ingestional toxicity. Similarly, gramine in combination with hordenine at the concentrations naturally occurring in barley (cv.Lara) has synergistic effects in reducing survival of *M. convecta* but not in reducing feeding of the insect. However, further studies on feeding deterrent effect of gramine and/or hordenine in combination with other allelochemicals, or on other insect pests such as *A. ipsilon* may be worthwhile.

Although gramine in combination with hordenine at the concentrations found in barley (cv.Lara) has synergistic effects in reducing survival of *M. convecta* under laboratory conditions, these effects should be further investigated in the field. Since gramine concentration in barley is affected by environmental conditions, particularly temperature (Hanson *et al.* 1981), the cultivars that show effects on insects in the glasshouse should be tested in the field to determine whether such cultivars will resist insects even when grown in variable environments.

Studies on synergistic effects of allelochemicals are valuable for breeding resistant cultivars. It is not necessary to breed a cultivar that has high concentrations of an allelochemical, if the plant contains a small number of different allelochemicals but these chemicals have additive effects in reducing survival, growth and development and feeding of insects. Plants that have been bred for high alkaloid content may be susceptible to insect attack while plants that contain a small number of different alkaloids with high individual variability may be more resistant to insects (Robinson 1979). The use of such resistant cultivars may be more effective if it is integrated with other pest control methods.

Moreover, it should be recognized that gramine has adverse effects on palatability of plants such as reed canarygrass to grazing animals (Marten *et al.* 1976, Marten *et al.* 1981). Therefore, breeding high gramine concentration cultivars may affect the palatability of barley to animals. Zuniga *et al.* (1985) suggested that the study of alternative resistance mechanisms of barley to insects should be undertaken before recommending the selection of cultivars with a low gramine concentration. This is because, while eliminating or decreasing gramine concentration of plants may be convenient for feeding livestock, it may also enhance the susceptibility of the crop to insects. In addition, the synergistic effect of gramine and hordenine may give an advantage to barley in both reducing pest populations and maintaining palatability of barley to grazing animals.