CHAPTER

# THREE

# EFFECT OF GRAMINE ON INSECTS

## 3.1 INTRODUCTION

Allelochemicals are secondary substances that show important effects on weeds, plant diseases and insects. Gramine, an indole alkaloid, is present in several barley cultivars and has shown biological effects on weeds such as white mustard, diseases such as *Pseudomonas syringae* and insects such as aphids. There are many studies which indicate that gramine has adverse effects on aphids and might have effects on other insects.

The common armyworm, *M. convecta*, is an important pest of barley throughout Australia. Thus, the biological effects of allelochemicals from barley on *M. convecta* are of interest. The objective of this chapter is to study the effects of two barley cultivars which differ in their gramine content on *M. convecta*, and also the effects of gramine on *M. convecta* when incorporated in artificial diet. In addition, the effects of the same cultivars of barley on *L. migratoria* are investigated.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Experiment 1 - Preliminary study

The experiment was conducted in a glasshouse maintained at 20-30°C at the University of New England from 4th-26th May 1992. Two barley cultivars, Lara and Schooner (supplied by New South Wales Agriculture and Fisheries, Agricultural Research Centre, Tamworth), were sown densely (>100 seedlings/pot) in 15 cm diameter pots on 4th May 1992. The pots were placed in a completely randomised design with three replicate pots for each cultivar. When the plants were about 10-15 cm height, barley leaves of each pot were cut for gramine analysis. Then twenty neonate larvae were placed in each pot. Most were placed on the plants on 13th May 1992, but some were placed on the next day, because of the limited number of larvae. Larvae were confined to the pots by clear plastic cylinders, 14 cm diameter x 45 cm height, placed over the barley plants and fitted with nylon stocking on top (Figure 3.1). The pots were placed in a water tray every 3 days to avoid disturbance and damage to the larvae from surface watering.

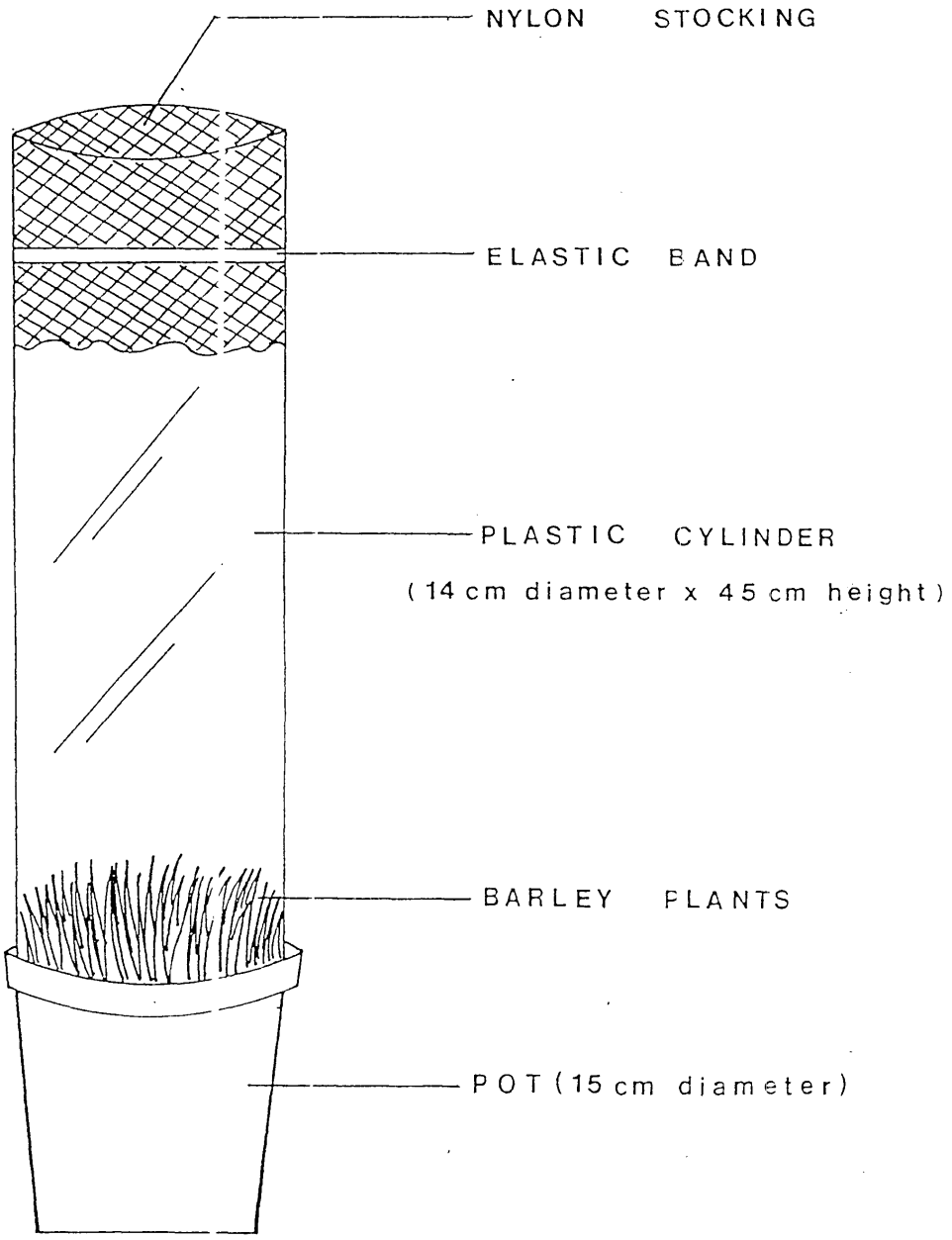



**Figure 3.1.** Experimental set up showing restriction cages.

Larvae used in the experiment were from egg masses laid by *M. convecta* fed on a 5% sucrose solution via soaked dental wicks and reared in the insectary of the Department of Agronomy and Soil Science, University of New England at the optimum temperature (25°C) and optimum day length (16 hours per day). The stock culture was maintained on armyworm artificial diet (Appendix A, Griffith and Smith 1977), provided *ad libitum* in clear 35-ml plastic cups with holes ventilated in the lids. Freeze-dried ground barley leaves were added in the diet as dried natural food. Larvae were reared in the insectary (25°C and 16 hours day length). When they pupated, they were placed individually into clear 35-ml plastic cups and covered with a 20 mm layer of moistened vermiculite.

Temperature throughout the experiment was recorded using a temperature recorder (Grant Model DB9-7U2L; Grant Instruments, Cambridge, UK), set at hourly intervals. Two probes were used for measuring temperatures. One was placed among the leaves of the barley plants inside the plastic cylinder to detect the temperatures experienced by larvae on the host plants. Another was placed outside the plastic cylinder to monitor the temperature in the glasshouse.

The water tray and stocking were examined regularly for escaping larvae and none were found. The pots were searched for larvae after 13 days. All larvae found were individually weighed to the nearest milligram. Smith (1984) demonstrated that instars could be clearly separated on the basis of head capsule widths. Head capsule widths were measured to the nearest 0.05 mm for estimating instar category. Barley leaves of each pot were also cut for gramine analysis.

### 3.2.2 Experiment 2 - Effect of barley cultivars on armyworms

This experiment was conducted in the same glasshouse as the preliminary study. The experiment commenced with the sowing of barley on 15th May 1992. The methods and barley cultivars used were the same as in the preliminary study. The pots were placed in a completely randomised design. There were five replications per cultivar.

Larvae used in this experiment were also neonates from egg masses of emerging moths of *M. convecta*, fed on a 5% sucrose solution via soaked dental wicks, reared in the insectary as described in section 3.2.1. Such moths were the adults of larvae reared on artificial diet using the same method as in the preliminary study. The temperature and day length in the insectary were those used in the first experiment (25°C, 16 hours a day).

Barley leaves were kept from each pot for gramine analysis when plants were about 10-15 cm height, and twenty neonate larvae were placed on barley plants of each pot on 23rd May 1992. Temperature in the glasshouse was recorded throughout the experiment as in the first experiment.

Larvae in each pot were collected and weighed after 14 days. The head capsule widths were measured for estimating instar category (Smith 1984). Barley leaves were kept for gramine analysis.

### 3.2.3 Experiment 3 - Effect of barley cultivars on locusts

This experiment was conducted in an insectary which was maintained at the optimum temperature (38°C day/ 32°C night) and day length (15 hours). Two barley cultivars, cvs.Lara and Schooner, were sown in 15 cm diameter pots on 4th May 1992 in the same glasshouse as in the first experiment.

*L. migratoria* used in the experiment were early third instars which had all hatched on the same day and had been fed on prairie grass (*Bromus catharticus* Vahl.) in the insectary until the experiment started. Then fifty *L. migratoria* were put in each of two cages, one used for feeding locusts with Lara barley, another used for feeding locusts with Schooner barley. In each cage was a light bulb for maintaining the temperature within the cage at 38°C. Barley plants were replaced every 2 days with fresh pots brought from the glasshouse. There was only one replication for each cultivar. The positions of the cages were rotated every 2 days to reduce the error from variation in environmental factors such as temperature.

Nymphs of locusts were placed in the cages with barley plants on 23rd May 1992. Barley leaves of each cultivar were kept for gramine analysis on this occasion and, subsequently, every time the barley plants were changed. Locusts fed on each cultivar were weighed, and their instar category and sex determined at the final stage of the experiment.

### 3.2.4 Experiment 4 - Effect of gramine on armyworms reared on artificial diets

The experiment was conducted in the insectary from 21st September 1992 to 15th November 1992. The insectary was maintained at 25°C with day length 16 hours per day.

Three concentrations of gramine, 0, 500 and 1000 ppm, were mixed into the armyworm artificial diet (Appendix A). Fifteen ml of ethanol was used to dissolve gramine in each treatment. The same volume of ethanol, without gramine, was added to the control diet. The ethanol/gramine solutions were mixed in the artificial diet when it was still liquid, but after the temperature fell below 50°C in order to avoid the degradation of gramine from high temperature.

Neonate larvae were placed individually in clear 35-ml plastic cups containing the artificial diet of each treatment, supplied in excess of consumption. There were forty larvae per treatment (twenty larvae per treatment were placed on the diet on each day). They were placed in a completely randomised design.

Diets were changed every week and kept for gramine analysis (both new and old diets) until the larvae became pupae. The date of pupation of each larva was recorded. Pupae were individually weighed and sexed within two or three days of pupation. Then they were placed individually into clear 35-ml plastic cups and covered with a 20 mm layer of moistened vermiculite to maintain moisture. The dates of adult emergences were also recorded individually.

### 3.2.5 Gramine analysis

Gramine concentrations in barley leaves and diets of all experiments were detected by High Performance Liquid Chromatography (HPLC). The method for the extraction and quantification of gramine was that used by Hoult and Lovett (1993). Barley leaves or diet (weighed quantities of about 1.5g fresh weight) were extracted with 0.01% acetic acid for 24 hours at room temperature. The extracts were then filtered through glasswool. The filtered extract was adjusted to pH 9.15 and centrifuged at 3000 rpm for 5 minutes before purification and concentration using Sep-pak $C_{18}$ cartridges (Waters Assoc.). The procedure for purification was as follows:

acetonitrile (ACN) 2 ml
|
0.001 M $KH_2PO_4$ pH 7 2ml
|
aliquot applied (sample)
|
0.05 M $KH_2PO_4$ pH 9.5 / isopropanol (70:30) 2 ml
|
0.05 M $KH_2PO_4$ pH 9.5 / isopropanol (95:5) 2 ml
|
0.05 M $KH_2PO_4$ pH 2.3 / isopropanol (70:30) 1.5 ml

Acetonitrile and 0.001 M $KH_2PO_4$ were used for preparing Sep-pak cartridges before using sample. The final eluates (from 0.05 M $KH_2PO_4$ pH 2.3 / isopropanol) were evaporated to dryness under nitrogen at 40°C and taken up in 1 or 10 ml mobile phase (0.025 M $KH_2PO_4$+0.1% Triethylamine (TEA) pH 7.15/ACN 60/40) depending on barley cultivars (cv.Lara 10 ml and cv.Schooner 1 ml) and the concentrations of gramine in the diets (control 1ml, 500 ppm 10 ml and 1000 ppm 10 ml). Ten µl samples were injected into a Waters HPLC system comprising M40 pump, flowrate 2 ml/min, U6K injector, Water's µBondapak Phenyl column 10 µ (3.9 mm x 300 mm) and UV spectrophotometer, at a wavelength of 219 nm. Elution was isocratic (the mobile phase). Identification of gramine in samples was by retention time relative to the authentic compound and confirmed by co-elution of representative samples with the authentic compound. Quantification was by peak areas using a Waters 745 data module. The use of HPLC for identification of gramine and hordenine has been described by Liu and Lovett (1990). Figure 3.1(b) shows typical HPLC traces for authentic gramine and for an extract from barley leaf.

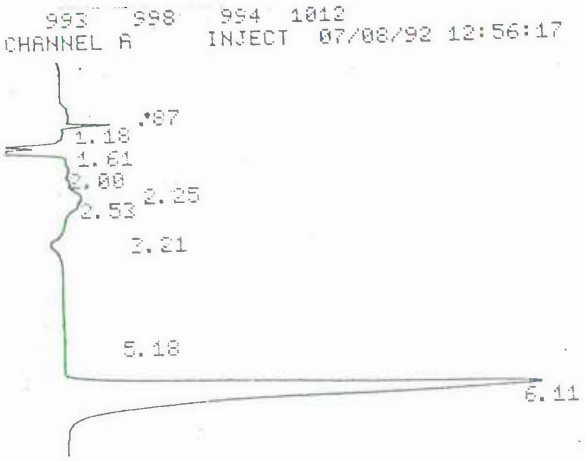
### 3.2.6 Analysis of data

The data were analysed using the MINITAB statistical package (Ryan *et al.* 1992). For Experiment 1 and 2, data on surviving numbers, head width and body weight of larvae of *M. convecta* were analysed by one-way analysis of variance. Data on instar category of *M. convecta* were analysed by $\chi^2$ tests.

For Experiment 3, data on the survival number and instar category of *L. migratoria* were analysed by $\chi^2$ tests. Data on body weight of *L. migratoria* were analysed by the general linear model (GLM procedure in MINITAB) and one-way analysis of variance. The general linear model (GLM) was used when the data were unbalanced.

For Experiment 4, data on surviving numbers of *M. convecta* were analysed by $\chi^2$ tests. Data on time to pupation, body weight of pupae, time to adult emergence, duration of pupal stage and sex, and weight of pupae and sex were analysed by one-way analysis of variance. Correlation between duration of pupal stage and weight of pupae of *M. convecta* was done and linear regression was used for further analysis.

(a)

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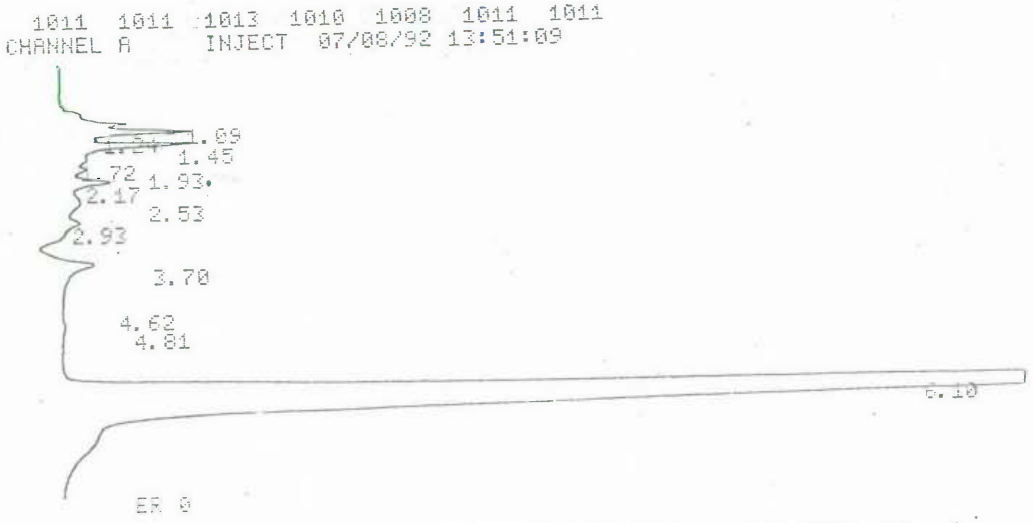
FILE 1. METHOD 0. RUN 22 INDEX 22

| PEAK# | AREA% | RT | AREA BC |
|---|---|---|---|
| 8 | 100. | 6.11 | 542693 01 |
| TOTAL | 100. | | 542693 |

(b)

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FILE 1. METHOD 0. RUN 26 INDEX 26

| PEAK# | AREA% | RT | AREA BC |
|---|---|---|---|
| 11 | 100. | 6.1 | 2248119 01 |
| TOTAL | 100. | | 2248119 |

**Figure 3.1(b)** HPLC traces for (a) Gramine standard (4.8 ppm), and (b) extract from leaves of barley, cv. Lara, prepared as described in the text.

# 3.3 RESULTS

## 3.3.1 Experiment 1 - Preliminary study

This experiment was terminated at 22 days after planting because poor temperature control (minimum temperature at 13±2°C and maximum temperature at 27 ±7°C) in the glasshouse led to excessive humidity, causing fungal diseases in the barley.

### 3.3.1.1 Survival

Data from Table 3.1 show that the survival of *M. convecta* fed on both cultivars of barley was high and there were no significant differences between cultivars.

**Table 3.1.** Mean and % survival of *Mythimna convecta* fed on 2 barley cultivars in the glasshouse ( Experiment 1). Twenty larvae were placed on each of 3 replicate pots.

| Cultivars | Survival (Mean ± se) | Survival (%) |
|---|---|---|
| Lara | 17.0 ± 1.7 | 85 |
| Schooner | 17.3 ±1.5 | 86.5 |

non significant (p = 0.89) by one-way analysis of variance

### 3.3.1.2 Head width

There were marginally but non-significant differences between cultivars in head widths of larvae. The head width of larvae fed on cv.Schooner was a little higher than the head width of larvae fed on cv.Lara, as shown in Table 3.2.

**Table 3.2.** Mean head width (mm) of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 1).

| Cultivars | n | Head width (Mean±se) |
|---|---|---|
| Lara | 50 | 2.017 ± 0.058 |
| Schooner | 52 | 2.161 ± 0.047 |

non significant (p = 0.06) by one-way analysis of variance

Most larvae found on both cultivars had head widths between 1.8 to 2.6 mm (Instar V). The head widths of larvae fed on cv.Lara varied from 0.8 to 2.5 mm (Instar III-V). The head widths of larvae fed on cv.Schooner varied from 1.3 to 2.6 mm (Instar IV-V). The number of larvae that had head widths between 1.8 to 2.6 mm was higher in cv.Schooner than in cv.Lara (Figure 3.2). On cv.Lara, there were 3 groups of instar (instar III, IV and V) were found but there was only 1 third instar larva. For statistical analysis it was included in instar IV. The proportions of instar IV and V of larvae fed on cv.Lara and cv.Schooner were not significantly different (Table 3.3). On cv.Lara, the percentage of larvae grouped in instar IV and V were 24 and 76, respectively. On cv.Schooner, the percentages were 13.5 and 86.5, respectively.

**Table 3.3.** The instar category of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 1).

| Cultivars | Instar IV | Instar V | Total |
|---|---|---|---|
| Lara | 12 | 38 | 50 |
| Schooner | 7 | 45 | 52 |

non significant ( $\chi^2 = 1.87$, df = 1, $p > 0.1$)

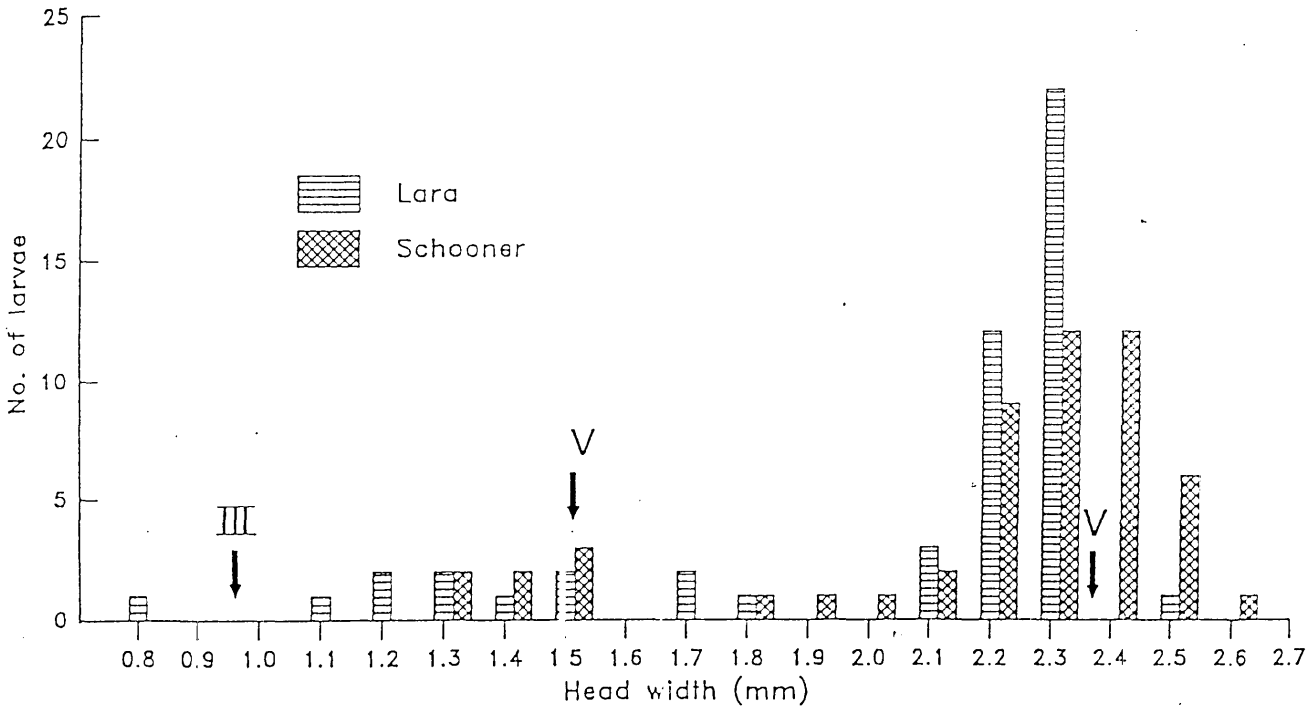


**Figure 3.2.** Head widths and instar categories of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 1). Roman numerals represent mean head widths for instar categories based on the data of Smith (1984).

### 3.3.1.3 Body weight

The body weights of larvae fed on cv.Schooner were marginally higher than those of larvae fed on cv.Lara but the differences were not statistically significant (Table 3.4).

**Table 3.4.** Mean body weight (mg) of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 1).

| Cultivars | n | Body weight (Mean±se) |
|---|---|---|
| Lara | 50 | 105.34 ± 8.99 |
| Schooner | 52 | 118.33 ± 8.61 |

non significant (p = 0.30) by one-way analysis of variance

### 3.3.1.4 Gramine concentration in leaves of 2 barley cultivars

Gramine concentration in cv.Lara leaves varied from 435.31 to 541.14 ppm and 394.32 to 549.43 ppm at 9 and 22 days after planting, respectively. Gramine concentration in cv.Schooner leaves varied from 0.00 to 4.15 ppm and 0.05 to 9.07 ppm at 9 and 22 days after planting, respectively (Table 3.5).

**Table 3.5.** Gramine concentration (ppm fresh weight) in leaves of 2 barley cultivars grown in the glasshouse (Experiment 1).

| Days after planting | Rep | Lara | Schooner |
|---|---|---|---|
| 9 | 1 | 435.31 | 4.15 |
| | 2 | 538.48 | 0.00 |
| | 3 | 541.14 | 1.40 |
| Mean ± se | | 504.98 ± 34.84 | 1.85 ± 1.22 |
| 22 | 1 | 549.43 | 0.05 |
| | 2 | 394.32 | 0.39 |
| | 3 | 445.27 | 9.07 |
| Mean ± se | | 463.01 ± 45.65 | 2.51 ± 2.06 |

## 3.3.2 Experiment 2 - Effect of barley cultivars on armyworms

This experiment was terminated at 22 days after planting because high temperatures (minimum temperature at 12±3°C and maximum temperature at 37±6°C) in the glasshouse reduced barley growth, and in some replications the barley was completely eaten out by larvae.

### 3.3.2.1 Survival

There were no significant differences in the numbers of larvae surviving between cultivars. The percentage survival of larvae fed on cv.Lara was marginally higher than that of larvae fed on cv.Schooner. Survival on both cultivars was high (Table 3.6).

**Table 3.6.** Mean and % survival of *Mythimna convecta* fed on 2 barley cultivars in the glasshouse (Experiment 2). Twenty larvae were placed on each of 5 replicate pots.

| Cultivars | Survival (Mean±se) | Survival (%) |
|---|---|---|
| Lara | 16.0 ± 1.3 | 80 |
| Schooner | 13.4 ± 0.7 | 67 |

non significant (p = 0.12) by one-way analysis of variance

### 3.3.2.2 Head width

The head width of larvae fed on cv.Schooner was higher than that of larvae fed on cv.Lara (Table 3.7).

**Table 3.7.** Mean head width (mm) of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 2).

| Cultivars | n | Head width (Mean±se) |
|---|---|---|
| Lara | 80 | 2.977 ± 0.069 |
| Schooner | 67 | 3.258 ± 0.054 |

The analysis of variance or head widths of larvae showed a highly significant difference (p = 0.002) in the head width of larvae fed on both cultivars (Table 3.8).

**Table 3.8.** Analysis of variance on head widths of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 2).

| SOURCE | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| CULTIVARS | 1 | 2.877 | 2.877 | 9.82 | 0.002 |
| ERROR | 145 | 42.499 | 0.293 | | |
| TOTAL | 146 | 45.376 | | | |

Most larvae found on both cultivars had head widths between 2.9 and 3.6 mm (Instar VI). On cv.Lara, the head widths of larvae varied from 1.5 to 3.6 mm (Instar IV-VI). On cv.Schooner, the head widths of larvae varied from 2.3 to 3.6 mm (Instar V-VI). The number of larvae that had head width between 2.9 to 3.6 mm was higher in cv.Schooner than in cv.Lara (Figure 3.3). Most larvae found in cv.Lara and cv.Schooner were VI instar larvae. The proportions of instar V and VI of larvae fed on cv.Lara and cv.Schooner were significantly different ($p<0.025$) (Table 3.9). On cv.Lara, the percentage of larvae grouped in instar V and VI were 36.3 and 60, respectively. On cv.Schooner, the percentages were 19.4 and 80.6, respectively.

**Table 3.9.** The instar category of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 2).

| Cultivars | Instar IV | Instar V | Instar VI | Total |
|---|---|---|---|---|
| Lara | 3 | 29 | 48 | 80 |
| Schooner | 0 | 13 | 54 | 67 |

significant difference ($\chi^2 = 8.36$, df = 2, $p < 0.025$)

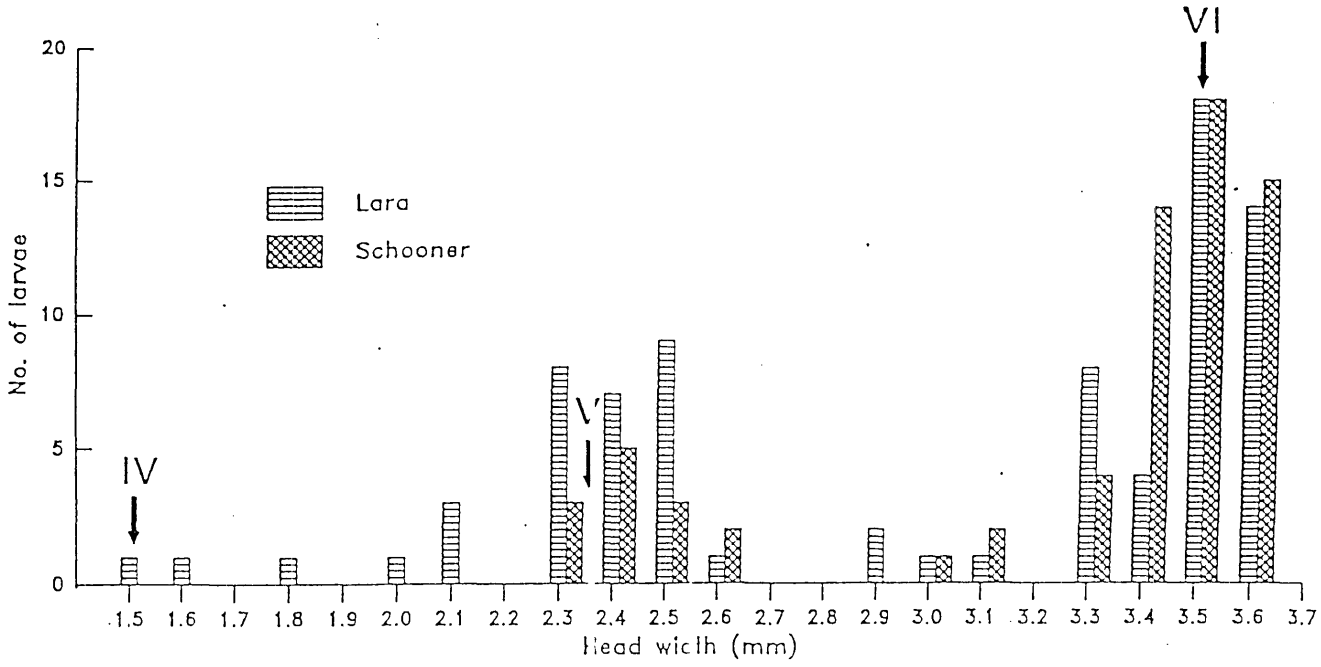


**Figure 3.3.** Head widths and instar categories of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 2). Roman numerals represent mean head widths for instar categories based on the data of Smith (1984).

### 3.3.2.3 Body weight

The body weight of larvae fed on cv.Schooner was higher than that of larvae fed on cv.Lara (Table 3.10).

**Table 3.10.** Mean body weight (mg) of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 2).

| Cultivars | n | Body weight (Mean±se ) |
|---|---|---|
| Lara | 80 | 159.8 ± 12.5 |
| Schooner | 67 | 227.8 ±14.6 |

The analysis of variance on the body weight of larvae showed that there was a highly significant difference (p = 0.001) in the body weight of larvae fed on different cultivars (Table 3.11).

**Table 3.11.** Analysis of variance on body weight of *Mythimna convecta* larvae fed on 2 barley cultivars in the glasshouse (Experiment 2).

| SOURCE | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| CULTIVARS | 1 | 168435 | 168435 | 12.66 | 0.001 |
| ERROR | 145 | 929669 | 13308 | | |
| TOTAL | 146 | 2098103 | | | |

### 3.3.2.4 Gramine concentration in barley leaves

The gramine concentration in leaves of cv.Lara varied from 13.08 to 372.72 ppm at 8 days after planting and from 1699.10 to 2837.60 ppm at 23 days after planting. For cv.Schooner, gramine concentration in leaves varied from 0.22 to 5.23 ppm at 8 days after planting and from 88.94 to 157.17 ppm at 23 days after planting (Table 3.12).

**Table 3.12.** Gramine concentration (ppm fresh weight) in leaves of 2 barley cultivars grown in the glasshouse (Experiment 2).

| Days after planting | Rep | Lara | Schooner |
|---|---|---|---|
| 8 | 1 | 156.43 | 1.39 |
| | 2 | 324.34 | 1.70 |
| | 3 | 13.08 | 0.22 |
| | 4 | 59.10 | 1.64 |
| | 5 | 372.72 | 5.23 |
| Mean ± se | | 185.13 ± 71.02 | 2.04 ± 0.84 |
| 23 | 1 | 2209.63 | - |
| | 2 | 2837.60 | 148.73 |
| | 3 | 1699.10 | 88.94 |
| | 4 | 2330.13 | - |
| | 5 | 2753.15 | 157.17 |
| Mean ± se | | 2365.92 ± 205.28 | 131.61 ± 21.47 |

## 3.3.3 Experiment 3 - Effect of barley cultivars on locusts

### 3.3.3.1 Development rate and survival

There were no significant differences between development rates of *L. migratoria* fed on cv.Lara and cv.Schooner. The proportions of different instars and the sex ratio were not significantly different between cv.Lara and cv.Schooner. The only 2 locusts which reached the adult stage were on cv.Schooner (Table 3.13).

**Table 3.13.** Instar category of *Locusta migratoria* fed on 2 barley cultivars.

| Cultivars | Males | Females | Males | Total |
|---|---|---|---|---|
| | v instar | v instar | Adult | |
| Lara | 11 | 13 | 0 | 24 |
| Schooner | 13 | 17 | 2 | 32 |

non significant ($\chi^2 = 1.59$, df = 2, $0.25 < p < 0.5$)

Percentage survival of *L. migratoria* fed on cv.Schooner was higher than that on cv.Lara, but the difference was not statistically significant (Table 3.14).

**Table 3.14.** % survival of *Locusta migratoria* fed on 2 barley cultivars.

| Cultivars | Survived | Died | % Survival |
|---|---|---|---|
| Lara | 24 | 26 | 48 |
| Schooner | 32 | 18 | 64 |

non significant ($\chi^2 = 2.60$, df = 1, $0.1 < p < 0.25$)

### 3.3.3.2 Body weight

The general linear model (GLM) showed that there were no significant effects of cultivar on body weight of locusts fed on cv.Lara and cv.Schooner ($p = 0.41$) and there were no significant effects of sex ($p = 0.46$). However, there was a significant interaction between cultivar and the sexes of locusts ($p = 0.02$) (Table 3.15). The body weight of male and female locusts fed on cv.Lara and cv.Schooner are shown in Tables 3.16 and 3.18.

**Table 3.15.** GLM analysis of body weight (mg) of *Locusta migratoria* fed on 2 barley cultivars.

| SOURCE | DF | SeqSS | AdjSS | AdjMS | F | P |
|---|---|---|---|---|---|---|
| CULTIVARS | 1 | 15730 | 16693 | 16693 | 0.70 | 0.41 |
| SEX | 1 | 37970 | 13557 | 13557 | 0.57 | 0.46 |
| CULTIVARS*SEX | 1 | 132437 | 132437 | 132437 | 5.55 | 0.02 |
| ERROR | 36 | 859368 | 859368 | 23871 | | |
| TOTAL | 39 | 1045505 | | | | |

Body weight of male locusts fed on cv.Schooner was higher than that of male locusts fed on cv.Lara (Table 3.16)

**Table 3.16.** Mean of body weight (mg) of *Locusta migratoria* fed on 2 barley cultivars (males only).

| Cultivars | n | Body weight (Mean±se) |
|---|---|---|
| Lara | 9 | 481.8 ± 47.4 |
| Schooner | 11 | 641.9 ± 24.8 |

A one-way analysis of variance on the body weight of male locusts showed that there was a highly significant effect ($p = 0.006$) of the cultivar on which the locusts fed (Table 3.17).

**Table 3.17.** Analysis of variance on body weight of *Locusta migratoria* fed on 2 barley cultivars (males only).

| SOURCE | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| CULTIVARS | 1 | 126928 | 126928 | 9.91 | 0.006 |
| ERROR | 18 | 230592 | 12811 | | |
| TOTAL | 19 | 357521 | | | |

For females only, the body weights of locusts fed on both cultivars were not significantly different (Table 3.18).

**Table 3.18.** Mean body weight (mg) of *Locusta migratoria* fed on 2 barley cultivars (females only).

| Cultivars | n | Body weight (Mean±se) |
|---|---|---|
| Lara | 7 | 562.1 ± 81.2 |
| Schooner | 13 | 485.9 ± 47.5 |

non significant ( p = 0.40) by one-way analysis of variance

### 3.3.3.3 Gramine concentration in barley leaves

Data shown in Table 3.19 indicate that gramine concentrations in leaves of cv.Lara fed to the locusts were much higher than in leaves of cv.Schooner. Gramine concentration in cv.Lara varied from 287.32 to 481.94 ppm while gramine concentration in cv.Schooner varied from 3.32 to 8.83 ppm.

**Table 3.19.** Gramine concentration (ppm fresh weight) in leaves of 2 barley cultivars used for feeding *Locusta migratoria*.

| Date of changing barley plant | Lara (ppm) | Schooner (ppm) |
|---|---|---|
| 23/5/92 | 444.72 | 4.07 |
| 25/5/92 | 481.94 | 3.32 |
| 27/5/92 | 397.42 | 4.58 |
| 29/5/92 | 391.12 | 4.32 |
| 30/5/92 | 287.32 | 3.86 |
| 2/6/92 | 314.98 | 3.50 |
| 4/6/92 | 408.14 | 7.77 |
| 6/6/92 | 378.44 | 6.98 |
| 7/6/92 | 322.15 | 8.83 |

## 3.3.4 Experiment 4 - Effect of gramine on armyworms reared on artificial diets

### 3.3.4.1 Survival to the pupal stage

There were highly significant differences ($p<0.005$) in survival of *M. convecta* pupae when larvae were reared on artificial diets containing different gramine concentrations. The lowest survival was that of pupae from larvae reared on diets containing 500 ppm gramine (Table 3.20).

**Table 3.20.** Survival of *Mythimna convecta* reared on artificial diets to the pupal stage.

| Treatments | Survived | Died |
|---|---|---|
| Control | 26 | 14 |
| 500 ppm | 10 | 30 |
| 1000 ppm | 25 | 15 |

highly significant ($\chi^2 = 16.07$, df = 2, $p < 0.005$)

### 3.3.4.2 Survival to the adult stage

There were also highly significant differences ($p<0.005$) in survival to the adult stage when larvae were reared on artificial diets containing different gramine concentrations. The lowest survival was found in diet containing 500 ppm gramine (Table 3.21).

**Table 3.21.** Survival of *Mythimna convecta* reared on artificial diets to the adult stage.

| Treatments | Survived | Died |
|---|---|---|
| Control | 23 | 17 |
| 500 ppm | 9 | 31 |
| 1000 ppm | 23 | 17 |

highly significant ($\chi^2 = 11.15$, df = 2, $p < 0.005$)

### 3.3.4.3 Time to pupation

There were no significant differences in time to pupation of *M. convecta* reared on artificial diets with 0 ppm gramine (control), 500 ppm gramine and 1000 ppm gramine (Table 3.22).

**Table 3.22.** Time to pupation (days) of *Mythimna convecta* reared on artificial diets.

| Treatments | n | Time to pupation ( Mean±se) |
|---|---|---|
| Control | 26 | 34.92 ± 0.29 |
| 500 ppm | 10 | 34.80 ± 0.83 |
| 1000 ppm | 25 | 36.12 ± 0.65 |

non significant (p = 0.18) by one-way analysis of variance

### 3.3.4.4 Weight of pupae

Data shown in Table 3.23 indicate that weights of pupae of *M. convecta* reared on artificial diets with 0 ppm gramine, 500 ppm gramine and 1000 ppm gramine were not significantly different.

**Table 3.23.** Weight of pupae of *Mythimna convecta* (mg) reared on artificial diets.

| Treatments | n | Weight of pupae ( Mean±se) |
|---|---|---|
| Control | 26 | 479.31 ± 8.13 |
| 500 ppm | 10 | 458.90 ± 16.69 |
| 1000 ppm | 25 | 462.24 ± 8.07 |

non significant ( p = 0.27) by one-way analysis of variance

### 3.3.4.5 Time to adult emergence

There were no significant differences between times to adult emergence of *M. convecta* reared on artificial diets with 0 ppm gramine, 500 ppm gramine and 1000 ppm gramine (Table 3.24).

**Table 3.24.** Time to adult emergence (days) of *Mythimna convecta* reared on artificial diets.

| Treatments | n | Time of adult emergence (Mean±se) |
|---|---|---|
| Control | 23 | 48.96 ± 0.28 |
| 500 ppm | 9 | 48.56 ± 0.99 |
| 1000 ppm | 23 | 50.17 ± 0.70 |

non significant (p = 0.18) by one-way analysis of variance

### 3.3.4.6 The correlation between duration of pupal stage and weight of pupae

Across all treatments and sexes, there was a correlation between duration of pupal stage and weight of pupae (r = 0.292). The larger pupae of *M. convecta* took longer to complete the pupal stage than the smaller ones and the regression equation was: duration of pupal stage = 11.4 + 0.0055 weight ($r^2$ = 0.085 and p = 0.031). Although the correlation was statistically significant, the proportion of variance explained by it ($r^2$) was low.

### 3.3.4.7 Duration of pupal stage and sex

There were significant differences between duration of the pupal stage of males and females of *M. convecta* reared on artificial diets. Males had a longer duration of pupal stage than females (Table 3.25).

**Table 3.25.** Duration of pupal stage of male and female *Mythimna convecta* reared on artificial diets.

| Sexes | n | Duration of pupal stage (Mean±se) |
|---|---|---|
| Males | 33 | 14.21 ± 0.14 |
| Females | 22 | 13.73 ± 0.18 |

The analysis of variance on duration of pupal stage showed that there was a significant difference (p = 0.03) in duration of pupal stage between the sexes of *M. convecta* (Table 3.26).

**Table 3.26.** Analysis of variance on duration of pupal stage of male and female *Mythimna convecta* reared on artificial diets.

| SOURCE | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| SEXES | 1 | 3.103 | 3.103 | 4.85 | 0.03 |
| ERROR | 53 | 33.879 | 0.639 | | |
| TOTAL | 54 | 36.982 | | | |

### 3.3.4.8 Weight of pupae and sex

There was no significant difference between weight of pupae of male and female *M. convecta* when reared on artificial diets (Table 3.27).

**Table 3.27.** Weight of pupae of male and female *Mythimna convecta* reared on artificial diets.

| Sexes | n | Weight of pupae (Mean±se) |
|---|---|---|
| Males | 33 | 468.52 ± 9.03 |
| Females | 22 | 474.59 ± 6.13 |

non significant (p = 0.62) by one-way analysis of variance

Weight of pupae was found to be positively correlated with duration of pupal stage though the correlation was relatively low (Section 3.3.4.6). The data from Tables 3.25 and 3.26 show that male pupae spent slightly longer in the pupal stage than female pupae although the weights of pupae were not significantly different between the sexes (Table 3.27). It is likely that both sex and weight of pupae affect the duration of the pupal stage of *M. convecta*. However, much of the variance in this experiment could not be accounted for by either factor.

### 3.3.4.9 Gramine concentration in artificial diets

Gramine concentration in new diets (the diets before feeding by larvae) of control, 500 ppm gramine and 1000 ppm gramine varied from 0.0 to 12.7, 218.9 to 545.2 and 348.9 to 1019.1 ppm fresh weight, respectively. In old diets (the diets left from larval feeding), gramine concentration of control, 500 ppm gramine and 1000 ppm gramine varied from 2.8 to 13.6, 279.3 to 632.0 and 474.5 to 1043.8 ppm fresh weight, respectively (Table 3.28).

For the first batch of artificial diets, average gramine concentrations in the old diets (at 12th day) of every treatment were higher than in the new diets (at the first day) of the same treatment. For the second batch of artificial diets, average gramine concentrations in the old diets (at 30th day) of every treatment were similar to those in the new diets (at 12th day) of the same treatment (Figure 3.4).

**Table 3.28.** Gramine concentration (ppm fresh weight) in artificial diet used for feeding *Mythimna convecta.*

| Time (days) | New/Old diets | Treatments (ppm) | Gramine content (ppm) | | | |
|---|---|---|---|---|---|---|
| | | | Rep I | Rep II | Rep III | Mean±se |
| 1 | New | Control | 0.00 | 4.2 | 6.9 | 3.72 ± 2.0 |
| | | 500 | 309.6 | 497.9 | 304.3 | 370.6 ± 63.6 |
| | | 1000 | 607.9 | 445.2 | 354.1 | 469.1 ± 74.2 |
| 12 | Old | Control | 2.8 | 13.5 | 13.6 | 9.97 ± 3.5 |
| | | 500 | 440.4 | 632.0 | 349.8 | 474.1 ± 83.2 |
| | | 1000 | 759.7 | 1043.8 | 690.2 | 831.3 ± 108.2 |
| | New | Control | 6.3 | 5.2 | 10.0 | 7.1 ± 1.5 |
| | | 500 | 371.8 | 545.2 | 242.1 | 386.4 ± 87.8 |
| | | 1000 | 744.7 | 1019.1 | 348.9 | 704.3 ± 194.5 |
| 30 | Old | Control | 3.6 | 4.3 | 12.6 | 6.8 ± 2.9 |
| | | 500 | 499.5 | 279.3 | 395.7 | 391.5 ± 63.6 |
| | | 1000 | 955.7 | 474.5 | 707.7 | 712.6 ± 138.9 |
| | New | Control | 5.4 | 7.3 | 12.7 | 8.5 ± 2.2 |
| | | 500 | 455.4 | 289.3 | 218.9 | 321.2 ± 70.1 |
| | | 1000 | 666.0 | 492.1 | 387.2 | 515.1 ± 81.3 |

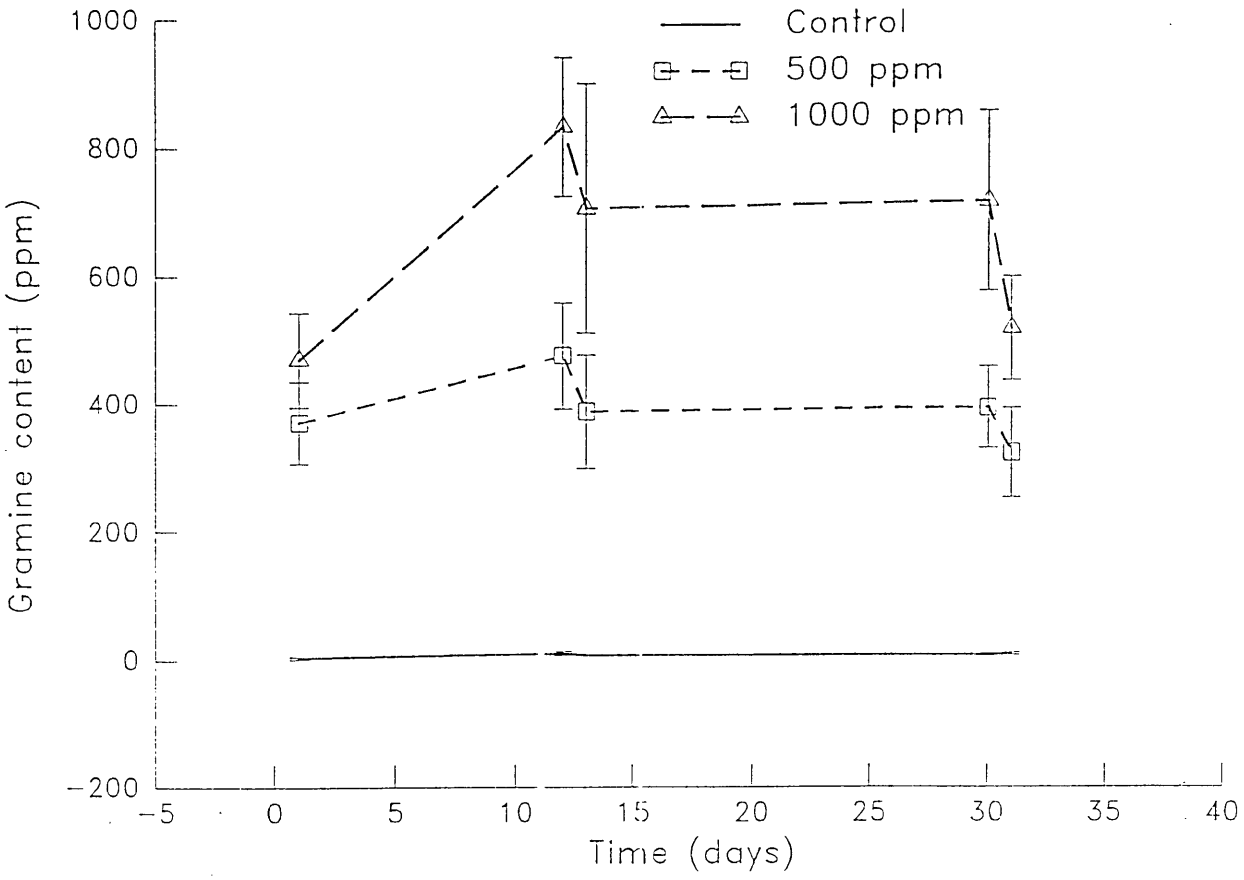


**Figure 3.4.** Gramine concentration in artificial diets used for feeding *Mythimna convecta.*

# 3.4 DISCUSSION

## 3.4.1 Effect of barley cultivars on insect survival

The results showed that there were no significant differences in survival of *M. convecta* fed on cv.Lara and cv.Schooner in Experiments 1 (Table 3.1) and 2 (Table 3.6). The survival of *L. migratoria* fed on cv.Lara and cv.Schooner in Experiment 3 was not significantly different (Table 3.14). Gramine concentrations found in leaves of cv.Lara were much higher than in leaves of cv.Schooner in all experiments (Tables 3.5, 3.12 and 3.19). It might have been expected that the barley cultivar containing the high level of gramine would have reduced survival of both insects, but the results did not support this hypothesis. It is possible that cv.Schooner, which contains low gramine levels, may also contain high levels of other allelochemicals which affect survival of these insects. It is not only chemical factors that affect survival of insects but also physical factors and the cultivars may also differ in this respect.

## 3.4.2 Effect of barley cultivars on insect development

There were no statistically significant differences in head width (Table 3.2), and body weight (Table 3.4) of *M. convecta* fed on cv.Lara and cv.Schooner in Experiment1. However, mean head width and mean body weight of *M. convecta* fed on cv.Schooner were marginally higher than those of larvae fed on cv.Lara. The instar categories of larvae fed on both cultivars were not significantly different, but the percentage of larvae grouped in instar V was marginally higher in cv.Schooner than in cv.Lara (Table 3.3 and Figure 3.2).

The effect of the two cultivars on the development of *M. convecta* was further investigated in Experiment 2. There were highly significant effects on the head width and body weight of *M. convecta* larvae. Head width and body weight of larvae fed on cv.Schooner were significantly higher than those on cv.Lara (Tables 3.7, 3.8, 3.10 and 3.11). In addition, the instar categories of larvae fed on both cultivars were significantly different and the percentage of larvae grouped in instar VI was much higher in cv.Schooner than in cv.Lara (Table 3.9 and Figure 3.3). Thus, the trends in size and development rate observed in Experiment 2 (which were statistically significant) were similar to those in Experiment 1 (which were not statistically significant). Experiment 2 had more replications than Experiment 1, and was continued for slightly longer. Overall, cv.Schooner (which has low gramine concentrations) is a more favourable cultivar for

the growth and development of *M. convecta* than cv.Lara (which has high gramine concentrations).

The effect of the two cultivars on development of *L. migratoria* was studied in Experiment 3. Although the results showed that there were no significant differences between development rate of *L. migratoria* fed on cv.Lara and cv.Schooner, the only adult locusts found were on cv.Schooner (Table 3.13). It is possible that the barley cultivar containing the high gramine level (cv.Lara) may have adverse effects on the development of *L. migratoria* in Experiment 3, but these effects could not be clearly revealed by this study. *L. migratoria* at the beginning of experiment was in the 3rd instar and the feeding period was not long enough. This study had to be abandoned after 17 days because the barley supply ran out. It is interesting that on cv.Lara, male locusts weighed less than females, while on cv.Schooner the reverse occurred (compare Tables 3.16 and 3.18). In *L. migratoria*, there is normally a difference in size between males and females, with the latter being larger. However, this dimorphism is affected by environmental conditions such as temperature, humidity and the degree of crowding (Uvarov 1966). The results of this experiment indicate that it may also be affected by the type of food. In particular, males fed on cv.Lara were unusally small. This result is consistent with the effects of cv.Lara on the size of *M. convecta*, but why it appeared only in males is not known.

These results might be interpreted as indicating that gramine influences the growth and development of armyworms and locusts. However, similar caution must be applied as in the case of results for survival (Section 3.4.1). The two cultivars may differ in respects other than gramine concentration.

### 3.4.3 Gramine concentrations in barley leaves

It was found that gramine concentrations in leaves of cv.Lara were much higher than in leaves of cv.Schooner in Experiment 1 (Table 3.5), Experiment 2 (Table 3.12), and Experiment 3 (Table 3.19).

For Experiment 2, gramine concentration in leaves of barley increased rapidly at 23 days after planting in both cv.Lara and cv.Schooner (Table 3.12). Bowden and Marion (1951b) showed that gramine was formed in the leaves at least up to the 11th day of growth and the total amount of gramine remained constant during this period. After one month it disappears from the leaf (Brandt *et al.*, cited by Bowden and Marion 1951b). The results of this study cannot be directly compared with those of Bowden and

Marion because gramine concentrations in leaves at the 11th day of growth were not measured. The increase in gramine concentration in this experiment may be because high temperatures in the glasshouse caused the leaves of the barley to dry out, so gramine concentration may have increased simply because of the declining percentage of water. Alternatively, high temperature may have increased gramine concentration directly (Hanson *et al.* 1983). Another possibility is that feeding of *M. convecta* on barley may increase gramine concentration in leaves, since insect feeding may induce an increase in allelopathic compounds in some plants (Kogan and Paxton 1983). It would have been desirable to calculate gramine concentration on a dry weight as well as a fresh weight basis in order to distinguish between these possibilities, but unfortunately this was not done.

Gramine concentrations in the leaves of cv.Lara at 8 days after planting were extremely variable, possibly due to the effect of shading on the production of allelopathic compounds (Rice 1984) as the pots were placed one behind the other in the glasshouse.

Gramine concentration in the leaves of cv.Lara used for feeding locusts in Experiment 3 were much higher than those of cv.Schooner throughout the experiment (Table 3.19). This result confirms that found in Experiment 1 (Table 3.5) and 2 (Table 3.12). All the barley used for this experiment was planted on the same day (4th May 1992), so the values in Table 3.19 show gramine content of barley aged 19 to 34 days. There were no consistent effects of age although the highest levels tended to be in the young plants. This result is quite different from those of Bowden and Marion (1951b) because gramine concentrations remained constant after the 11th day of growth and even at the 34th day of growth.

### 3.4.4 Problems with the barley experiments

Experiment 1 had to be abandoned after 22 days of barley growth, when the larvae were mostly in the fifth instar, because poor temperature control in the glasshouse led to excessive humidity and caused fungal diseases in barley. In Experiment 2, larvae had reached the sixth instar after 22 days of growth when it had to be abandoned after the larvae in some replicates had eaten all the barley. Although larvae in Experiment 1 were fed on barley as long as in Experiment 2, larvae in Experiment 2 tended to grow quicker than those of Experiment 1. This may be because larvae in Experiment 2 had eaten more barley than those in Experiment 1. The difficulties of continuing these experiments show that temperature control in the glasshouse was important and the optimum temperature for both larvae and barley should be considered in experiments

such as these. Getting the temperature right is difficult because the optimum temperature for barley growth is lower (15°C, Briggs 1978) than it is for armyworm larvae (25°C, Smith 1984).

Another problem in experiments like these is judging the food consumption of the insects. Noctuid larvae such as *M. convecta* consume 97% of their lifetime food requirements in instars V and VI, and 86% in instar VI alone (Goodyer 1978). Thus, extremely rapid depletion of the food supply, as occurred in Experiment 2, must be expected.

### 3.4.5 Effect of gramine in barley compared to gramine in artificial diets

Corcuera (1984) investigated effects of indole alkaloids from Gramineae including $N_{\alpha}$-methyltryptamine, 5-methoxy-N, N-dimethyl-tryptamine, 3-N,N-dimethylaminomethylindole (gramine) and 5-methoxy-tryptamine on aphids by incorporated these compounds in diets. He found that deleterious effects of gramine and related compounds when incorporated in diets were observed at concentrations similar to those found in plants.

The effects of gramine on *M. convecta* reared on artificial diets which contain gramine concentrations similar to those found in barley were investigated in Experiment 4. The results showed no significant differences between time to pupation (Table 3.22), weight of pupae (Table 3.23) or time to adult emergence (Table 3.24) of *M. convecta* reared on artificial diets incorporated with gramine at concentration 0 ppm, 500 ppm and even 1000 ppm. This suggests that gramine had no effects on the growth and development of *M. convecta.* The results of this study were different from those of Corcuera (1984) because the concentration of gramine present in barley cultivars that had significantly different effects on the growth and development of *M. convecta* (Experiment 2) showed no significant effects on the growth and development of *M. convecta* when incorporated in artificial diet. This inconsistency between the experiments with barley and those with artificial diet means that the deleterious effects on insects of cv.Lara cannot be unequivocally ascribed to gramine. It could be that other characteristics of cv.Lara are responsible. On the other hand, it might be the experiments with artificial diet which are misleading. Perhaps gramine only becomes toxic in the presence of another factor, which is found in barley plants but not in artificial diet.

Although there were no significant effects of gramine in diet on the growth and development, there was an effect on survival (Tables 3.20 and 3.21). The pattern was unusual with higher mortality in the 500 ppm treatment than in either the 1000 ppm treatment or the 0 ppm.

A possible factor that may affect survival, growth and development of *M. convecta* in artificial diets is the amount of diet ingested. Zuniga and Corcuera (1986) found that survival of aphids, *R. padi*, was highest at the lowest and highest concentrations of gramine incorporated in diets while the lowest survival was observed at intermediate gramine concentrations. They explained that the higher survival of aphids occurring at the highest gramine concentration in diet was probably due to non-feeding. When the diet was ingested a high mortality was observed. They suggested that gramine caused toxic effects and also feeding deterrency. A similar mortality pattern occurred in this study: survival of *M. convecta* was highest at the lowest and highest gramine concentrations and lowest at intermediate concentration. Unfortunately, the amount of diet consumed in each treatment was not measured. However, the reason described by Zuniga and Corcuera is unlikely to explain the results of this study, because small larvae of *M. convecta* which did not feed at all would have died well before the experiment ended, unlike the aphids where survival was measured over a short period (7 hours). Moreover, the data on size and development rate (Tables 3.22-3.24) give little indication of any deterrent effect of gramine at 1000 ppm.

There may have been marginally significant differences in the development rate of *M. convecta* when larvae were fed on diets containing different gramine concentrations. The time to pupation and adult emergence tended to be longer in the diets with 1000 ppm gramine than in the diets with 500 ppm gramine and control (Tables 3.22 and 3.24). The times to pupation and adult emergence of *M. convecta* in this experiment were longer than those recorded by Smith (1984). This may be because the temperature in the insectary was sometimes lower than the optimum temperature for *M. convecta* (25°C), or it may be an effect of the ethanol used to dissolve gramine for incorporation into artificial diets. Smith (1984) showed that temperatures lower than 25°C gave longer development periods of *M. convecta*. Conversely, temperatures higher than 25°C gave shorter development periods. At 25°C, the development periods of larvae and pupae of *M. convecta* were 25.4 and 14 days, respectively, compared to about 35 and 14 days in this study (Tables 3.22 and 3.24). The reason that ethanol was used to dissolve gramine in this study was because gramine is not very water soluble. However, the volume of ethanol was small (15 ml) and was expected to have no effect on the growth and development of *M. convecta*. Therefore, it seems more likely that

temperature was the important factor that delayed growth and development of *M. convecta.*

All the gramine incorporated in the diets was not recovered in the analysis (Table 3.28). This may be due to the effect of high temperature when the gramine was incorporated into artificial diets, which may degrade gramine. It was necessary to use high temperatures because the artificial diet is based on agar, which must be kept at high temperature to remain liquid during its preparation. Another reason may be the loss of gramine during the drying process for gramine analysis. Thus, a better technique to incorporate gramine in artificial diet is required and the drying operation during gramine analysis requires care. Despite these difficulties, the artificial diets clearly contained 3 distinct levels of gramine (Figure 3.4). The control diet contained a small amount of gramine, comparable to that found in Schooner barley (Tables 3.12, 3.19 and 3.28). This was probably because a small amount of freeze dried, ground barley (of unknown cultivar) was included in the diet (Appendix A). The 500 ppm diet contained gramine levels similar to those found in Lara barley (Tables 3.12, 3.19 and 3.28), while the 1000 ppm diet contained higher levels.

For the first batch of the diet, the diets that were left from larvae feeding (the old diets at 12th day) of all treatments had higher gramine concentration than the diets before larvae feeding (the new diets at 1st day) (Table 3.28 and Figure 3.4). This may be due to the old diets becoming dry.

## 3.4.6 Potential role for gramine in development of resistant cultivars

The effect of gramine on insects depends on the interaction between gramine levels in plants and insect feeding behaviour. For example, gramine is not present in the vascular bundles of barley leaves (Argandona *et al.* 1987) which are the preferred feeding site of most aphids, but it has effects on population growth rate of *S. graminum* (Zuniga *et al.* 1985, Argandona *et al.* 1987, Zuniga *et al.* 1988, Kanehisa *et al.* 1990, Salas *et al.* 1990, Rustamani *et al.* 1992) and *R. padi* (Zuniga *et al.* 1988). Therefore, Argandona *et al.* (1987) considered that its effect on aphids might reflect properties of gramine as a feeding deterrent. They also suggested that the presence of gramine in epidermal tissues may be relevant to the protection of barley against other insects rather than aphids. Considering the feeding behaviour of *M. convecta* larvae, it is likely that any effect of gramine on the growth and development of *M. convecta* larvae may be a consequence of gramine in epidermal tissues and parenchyma cells of the mesophyll of barley leaves, which are ingested by the larvae.

The results from the current work in comparison with other studies on the effects of gramine in barley on aphids (Kanehisa *et al.* 1990, Rustamani *et al.* 1992) suggest that the effects of gramine in barley on the growth and development of *M. convecta* and *L. migratoria* are not as strong as those found with aphids. This may be because *M. convecta* and *L. migratoria* have a wide range of food plants, giving them the ability to detoxify a wide range of allelochemicals. Generally, plant defensive chemicals are considered to be allocated mostly to tissues of external structures and are only present at low levels in vascular tissues (McKey 1979, cited by Mullin 1985). Thus, higher loadings of plant toxicants are expected to be consumed by chewing herbivores compared to sucking herbivores (Mullin 1985). As a result, metabolic adaptations to toxic chemicals are thought to be better developed in chewing herbivores such as lepidopteran larvae than in sucking herbivores such as aphids (Mullin 1985).

Because gramine in barley shows biological effects on insects, it is likely that this allelochemical may be an important resistance factor of barley to certain insects, particularly aphids (Kanehisa *et al.* 1990, Rustamani *et al.* 1992). Although there is no evidence to show that strongly resistant lines of barley to *M. convecta* exist, this study suggests that cv.Lara should have some resistance relative to cv.Schooner if the results of the laboratory studies described in this chapter are reflected in the field.

Although cv.Lara had no effect on survival of *M. convecta* and *L. migratoria*, the results of this study show that it could slow the development rate of these insects. Therefore, it may give a useful level of pest control when it is integrated with other methods such as biological control and chemical control (Russell 1978). For example, it might slow the population growth rate of the insects, allowing a longer time for natural enemies to increase their populations to overcome the pest populations.

Longer development times might also prolong the exposure of pest populations to their natural enemies, leading to higher rates of parasitism and predation. In the case of pesticides, chemical applications might be reduced because the time required for insect populations to reach an economic threshold of infestation might be longer. For these reasons, even relatively low levels of resistance can be useful if they are incorporated into an integrated pest management scheme.

The results of these experiments suggested adverse effects, perhaps due to gramine, in one barley cultivar on the growth and development of *M. convecta,* but not of gramine in artificial diet. Therefore, the investigation of the effect of gramine when incorporated in artificial diet on *M. convecta* needs to be repeated, preferably in conjunction with studies on the effects of other barley allelochemicals. Chapter 4 of this

thesis describes further experiments of this nature. The effect of other barley cultivars that have intermediate to high levels of gramine on *M. convecta* should be investigated both in the glasshouse and in the field as the knowledge gained may be useful for breeding cultivars resistant to this insect. As *H. spontaneum* was found to have high concentrations of gramine (Hanson *et al.* 1981, Kanehisa *et al.* 1990, Lovett and Hoult 1992, Rustamani *et al.* 1992), the effect of this species on *M. convecta* should be investigated. In addition, the biological effects of gramine in barley on other insects should be studied.

High gramine concentration may be undesirable in barley used for feeding animals because it has negative effects on the palatability of plants such as reed canarygrass (Marten *et al.* 1976,1981). Thus, cultivars that contain lower gramine concentration but are still resistant to insects are desirable for dual purpose crops. The cultivars which have relatively high gramine concentrations, but are still lower than *H. spontaneum* include *H. agriocrithon*, Landraces, Middle Eastern cultivars (Lovett and Hoult 1992) and Lara. Studies of the resistance of these cultivars to *M. convecta* and their palatability to animals should be undertaken.