

Table 4.37. Duration of pupal stage (days) of *Helicoverpa punctigera* reared on artificial diets containing gramine and hordenine.

Treatments	n	Duration of pupal stage (Mean±se)
1. 500 ppm gramine	17	14.53±0.29
2. 60 ppm hordenine	17	14.53±0.17
3. 500 ppm gramine + 60 ppm hordenine	18	14.22±0.19
4. 500 ppm gramine + 500 ppm hordenine	17	14.77±0.34
5. control (with ethanol)	15	14.87±0.27
6. control (without ethanol)	21	14.67±0.42

The treatments had no significant differences ($p = 0.754$) by one-way analysis of variance.

There was a highly significant difference ($p < 0.001$) in duration of pupal stage of male and female *H. punctigera* reared on artificial diets when data from all treatments were combined. Males had significantly longer duration of pupal stage than females (Tables 4.38 and 4.39).

Table 4.38. Duration of pupal stage (days) of male and female *Helicoverpa punctigera* reared on artificial diets (all treatments combined).

Sexes	n	Duration of pupal stage (Mean±se)
Males	54	15.20±0.17
Females	51	13.94±0.14

Table 4.39. Analysis of variance on duration of pupal stage of male and female *Helicoverpa punctigera* reared on artificial diets (all treatments combined).

SOURCE	DF	SS	MS	F	P
SEXES	1	41.81	41.81	34.29	<0.001
ERROR	103	125.58	1.22		
TOTAL	104	167.39			

The general linear model showed that there was a highly significant difference ($p < 0.001$) between the sexes in duration of pupal stage. However, there were no significant differences between treatments ($p = 0.359$) and there was no interaction effect between treatment and the sex ($p = 0.151$) (Table 4.40).

Table 4.40. GLM analysis of duration of pupal stage of *Helicoverpa punctigera* reared on artificial diets containing gramine and hordenine.

SOURCE	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	5	4.35	6.58	1.32	1.11	0.359
SEX	1	43.23	38.22	38.22	32.33	<0.001
TREAT*SEX	5	9.85	9.85	1.97	1.67	0.151
ERROR	93	109.96	109.96	1.18		
TOTAL	104	167.39				

4.3.3.7 Gramine and hordenine concentration in artificial diets

For treatment 1 (gramine 500 ppm), gramine concentration in both new diets (the diets before feeding by larvae) and old diets (the diets left from larvae feeding) varied from 513.6 to 589.8 ppm fresh weight (2603.1 to 2646.5 ppm dry weight). Hordenine was not found in both new and old diets.

For treatment 2 (hordenine 60 ppm), gramine was not found in both new and old diets. Hordenine concentration in both new and old diets varied from 0.0 to 0.9 ppm fresh weight (0.0 to 4.9 ppm dry weight).

For treatment 3 (gramine 500 ppm+hordenine 60 ppm), gramine concentration in both new and old diets varied from 526.3 to 608.4 ppm fresh weight (2617.2 to 2646.1 ppm dry weight). Hordenine was not found in both new and old diets.

For treatment 4 (gramine 500 ppm+hordenine 500 ppm), gramine concentration in both new and old diets varied from 466.7 to 538.6 ppm fresh weight (2346.6 to 2576.0 ppm dry weight). Hordenine concentration in both new and old diets varied from 0.0 to 34.7 ppm fresh weight (0.0 to 175.0 ppm dry weight).

For treatment 5 (control with ethanol) and treatment 6 (control without ethanol), no gramine and hordenine were found in both new and old diets (Figure 4.4 and Appendix L).

The old diets from the second batch of each treatment could not be kept for gramine and hordenine analysis because the larvae pupated and the pupae were left in the diets until adult emergence.

Figure 4.4. Gramine and hordenine concentration (ppm) in artificial diet used for feeding *Helicoverpa punctigera* (The concentrations of gramine and hordenine at the 1st, 7th and 21st days were the concentrations of the first batch/new diet, the first batch/old diet and the second batch/new diet, respectively). — fresh weight ----- dry weight

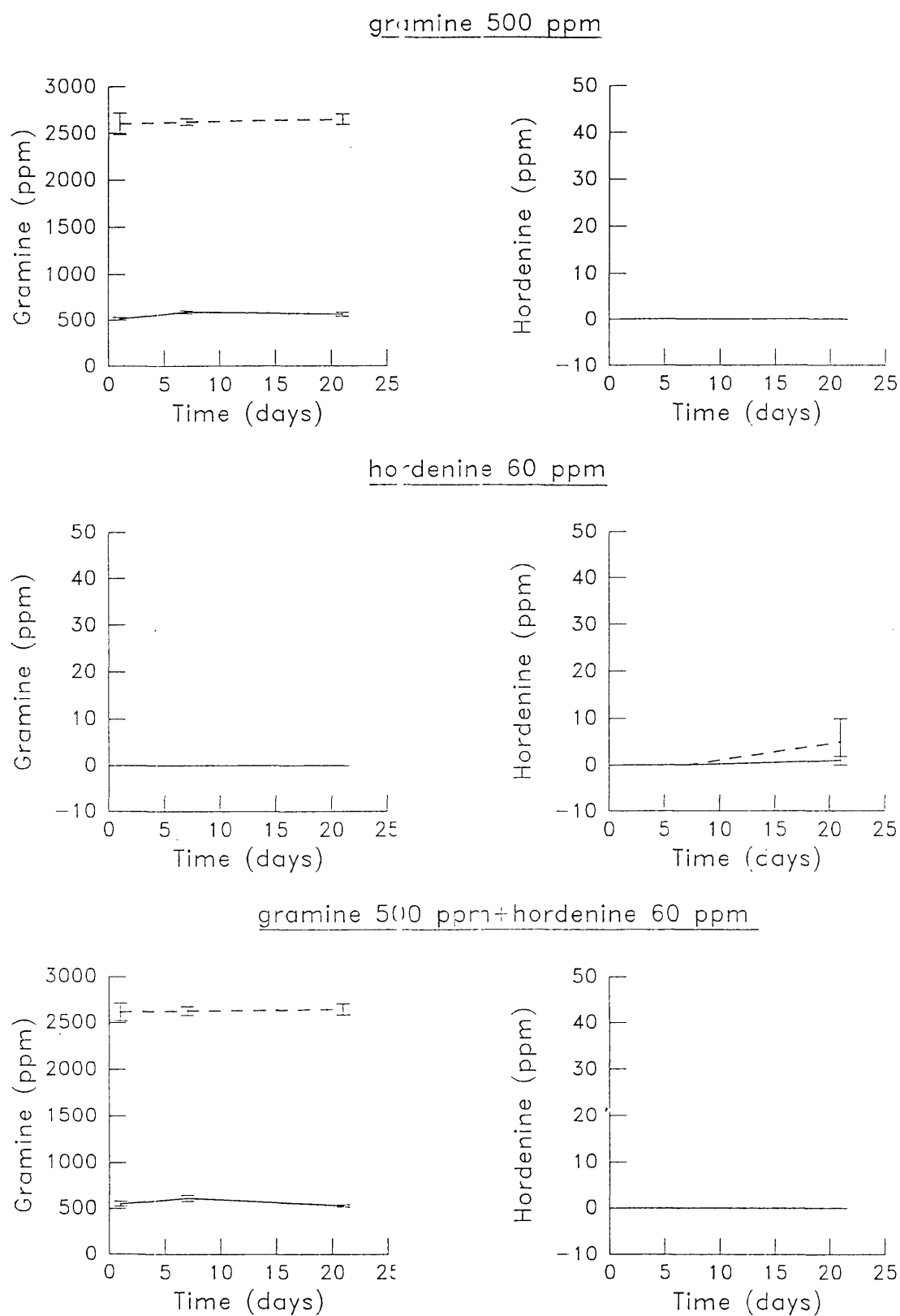
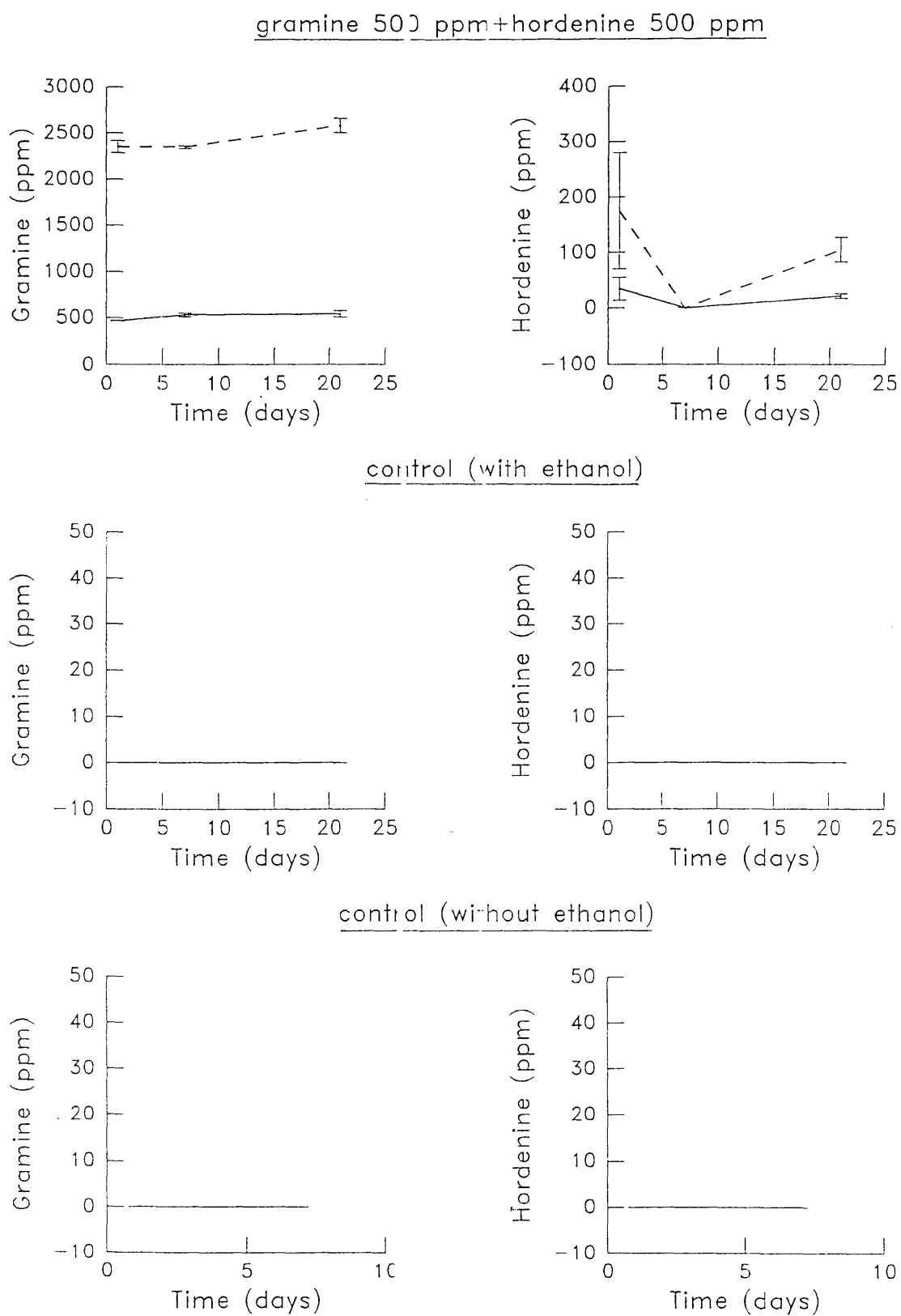


Figure 4.4. (continued)

4.3.4 Experiment 7 - Effect of gramine and hordenine on *A. ipsilon* reared on artificial diets

4.3.4.1 Survival

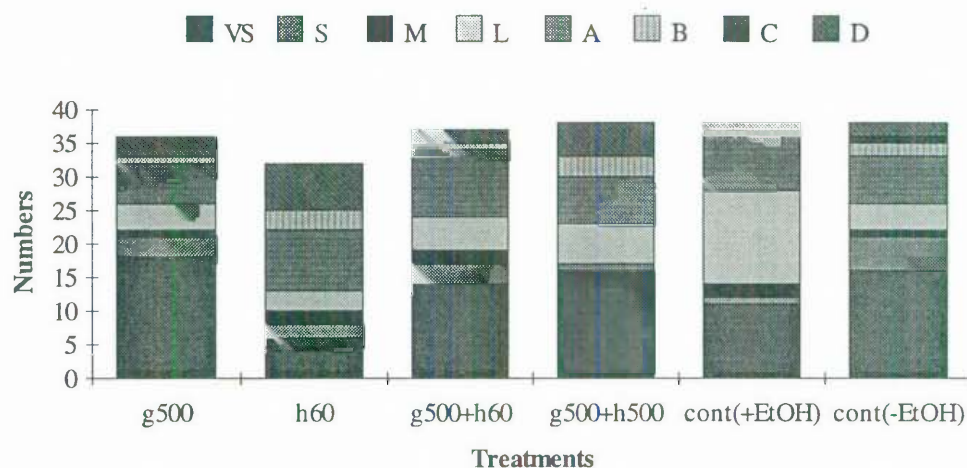
Numbers of *A. ipsilon* surviving to each category are shown in Table 4.41.

Table 4.41. Survival numbers of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine (Numbers surviving to stages: VS = very small larva, S = small larva, M = medium larva, L = large larva, A = larva died during pupation, B = malformed pupa, C = died before adult emergence and D = severely malformed adult). Initial N was 40.

Treatments	VS	S	M	L	A	B	C	D
1. 500 ppm gramine	22	19	18	14	8	7	7	4
2. 60 ppm hordenine	34	32	30	27	18	15	15	8
3. 500 ppm gramine + 60 ppm hordenine	26	23	21	16	6	5	5	3
4. 500 ppm gramine + 500 ppm hordenine	24	23	23	17	10	7	7	2
5. control (with ethanol)	29	23	26	12	4	3	3	2
6. control (without ethanol)	24	19	18	14	7	5	4	2

Figure 4.5 shows that most insects died when they were very small or during pupation and the lowest mortality was found in diet containing hordenine 60 ppm.

Figure 4.5. Numbers of dead larvae, dead pupae, malformed pupae, dead adults and malformed adults of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine (VS = very small larva; S = small larva; M = medium larva; L = large larva; A = larva died during pupation; B = malformed pupa; C = died before adult emergence; and D = severely malformed adult, g500 = gramine 500 ppm; h60 = hordenine 60 ppm; g500+h60 = gramine 500 ppm+hordenine 60 ppm; g500+h500 = gramine 500 ppm+hordenine 500 ppm; cont(+EtOH) = control with ethanol; and cont(-EtOH) = control without ethanol).



A χ^2 analysis of the numbers surviving to the large larvae stage is shown in Table 4.42.

Table 4.42. Survival of *Agrotis ipsilon* (large larva) reared on artificial diets containing gramine and hordenine.

Treatments	Survived	Died	Total
1. 500 ppm gramine	14 (16.7)	26 (23.3)	40
2. 60 ppm hordenine	27 (16.7)	13 (23.3)	40
3. 500 ppm gramine + 60 ppm hordenine	16 (16.7)	24 (23.3)	40
4. 500 ppm gramine + 500 ppm hordenine	17 (16.7)	23 (23.3)	40
5. control (with ethanol)	12 (16.7)	28 (23.3)	40
6. control (without ethanol)	14 (16.7)	26 (23.3)	40
Total	100	140	240

The numbers in parenthesis are expected counts based on the null hypothesis, the numbers that died are cumulative to the large larval stage. The treatments were significantly different ($\chi^2 = 14.74$, $df = 5$, $p < 0.025$). Initial N was 40.

Further χ^2 analyses using 2x2 contingency tables gave a significant difference between the 60 ppm hordenine treatment and the control (without ethanol) treatment ($\chi^2 = 8.46$, $df = 1$, $p < 0.005$).

χ^2 analyses were also carried out on cumulative mortality to earlier developmental stages. There were no significant differences between treatments for very small larvae ($\chi^2 = 10.68$, $df = 5$, $p > 0.05$) but significant differences for small larvae ($\chi^2 = 13.75$, $df = 5$, $p < 0.025$) and medium larvae ($\chi^2 = 11.34$, $df = 5$, $p < 0.05$). A χ^2 analysis of the number of larvae which survived the pupal period is shown in Table 4.43.

Table 4.43. Survival of *Agrotis ipsilon* (larvae died during pupation) reared on artificial diets containing gramine and hordenine.

Treatments	Survived	Died	Total
1. 500 ppm gramine	8 (7.4)	6 (6.6)	14
2. 60 ppm hordenine	18 (14.3)	9 (12.7)	27
3. 500 ppm gramine + 60 ppm hordenine	6 (8.5)	10 (7.5)	16
4. 500 ppm gramine + 500 ppm hordenine	10 (9.0)	7 (8.0)	17
5. control (with ethanol)	4 (6.4)	8 (5.6)	12
6. control (without ethanol)	7 (7.4)	7 (6.6)	14
Total	53	47	100

The numbers in parenthesis are expected counts based on the null hypothesis. The treatments were not significantly different ($\chi^2 = 5.81$, $df = 5$, $p > 0.25$).

A χ^2 analysis of the number surviving as complete pupae is shown in Table 4.44.

Table 4.44. Survival of *Agrotis ipsilon* (malformed pupa) reared on artificial diets containing gramine and hordenine.

Treatments	Survived	Died	Total
1. 500 ppm gramine	7 (6.3)	1 (1.7)	8
2. 60 ppm hordenine	15 (14.3)	3 (3.7)	18
3. 500 ppm gramine + 60 ppm hordenine	5 (4.8)	1 (1.3)	6
4. 500 ppm gramine + 500 ppm hordenine	7 (7.9)	3 (2.1)	10
5. control (with ethanol)	3 (3.2)	1 (0.8)	4
6. control (without ethanol)	5 (5.6)	2 (1.5)	7
Total	42	11	53

The numbers in parenthesis are expected counts based on the null hypothesis. The treatments were not significantly different ($\chi^2 = 1.40$, $df = 5$, $p > 0.90$).

A χ^2 analysis of the number of larvae which survived the adult period was invalid because only one pupa died before adult emergence in control diet without ethanol and no pupae died before emergence in other treatments. A χ^2 analysis of the number surviving as complete adults is shown in Table 4.45.

Table 4.45. Survival of *Agrotis ipsilon* (severely malformed adult) reared on artificial diets containing gramine and hordenine.

Treatments	Survived	Died	Total
1. 500 ppm gramine	4 (3.6)	3 (3.4)	7
2. 60 ppm hordenine	8 (7.7)	7 (7.3)	15
3. 500 ppm gramine + 60 ppm hordenine	3 (2.6)	2 (2.4)	5
4. 500 ppm gramine + 500 ppm hordenine	2 (3.6)	5 (3.4)	7
5. control (with ethanol)	2 (1.5)	1 (1.5)	3
6. control (without ethanol)	2 (2.1)	2 (2.0)	4
Total	21	20	41

The numbers in parenthesis are expected counts based on the null hypothesis. The treatments were not significantly different ($\chi^2 = 2.01$, $df = 5$, $p > 0.75$).

There were no significant differences between treatments in overall survival of insects to the adult stage (Table 4.46).

Table 4.46. Overall survival of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

Treatments	Viable adults	Died or non-viable	Total
1. 500 ppm gramine	4 (3.5)	36 (36.5)	40
2. 60 ppm hordenine	8 (3.5)	32 (36.5)	40
3. 500 ppm gramine + 60 ppm hordenine	3 (3.5)	37 (36.5)	40
4. 500 ppm gramine + 500 ppm hordenine	2 (3.5)	38 (36.5)	40
5. control (with ethanol)	2 (3.5)	38 (36.5)	40
6. control (without ethanol)	2 (3.5)	38 (36.5)	40
Total	21	219	240

The numbers in parenthesis are expected counts based on the null hypothesis. The treatments were not significantly different ($\chi^2 = 8.61$, $df = 5$, $p > 0.10$). Initial N was 40.

4.3.4.2 Time to pupation

The one-way analysis of variance showed that there were significant differences between treatments ($p = 0.021$) in time to pupation of *A. ipsilon*. Nevertheless, there were no significant differences between treatments when using the Bonferroni Multiple Comparisons Procedure (Table 4.47).

Table 4.47. Time to pupation (days) of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

Treatments	n	Time to pupation (Mean±se)
1. 500 ppm gramine	8	34.88±1.66
2. 60 ppm hordenine	18	30.89±0.87
3. 500 ppm gramine + 60 ppm hordenine	6	33.50±1.84
4. 500 ppm gramine + 500 ppm hordenine	10	35.60±1.75
5. control (with ethanol)	4	31.00±0.41
6. control (without ethanol)	7	29.86±0.80

The treatments had significant differences ($p = 0.021$) by one-way analysis of variance but there were no significant differences by the Bonferroni Multiple Comparisons Procedure (Devore and Peck 1986).

There was a highly significant difference ($p = 0.002$) between the sexes in time to pupation of *A. ipsilon* reared on artificial diets when data from all treatments were combined. Females had a longer time to pupation than males (Tables 4.48 and 4.49).

Table 4.48. Time to pupation (days) of male and female *Agrotis ipsilon* reared on artificial diets (all treatments combined).

Sexes	n	Days to pupation (Mean±se)
Males	20	30.15±0.51
Females	33	34.00±0.85

Table 4.49. Analysis of variance on time to pupation of male and female *Agrotis ipsilon* reared on artificial diets (all treatments combined).

SOURCE	DF	SS	MS	F	P
SEXES	1	184.6	184.6	10.91	0.002
ERROR	51	862.5	16.9		
TOTAL	52	1047.1			

The general linear model showed that there were no significant differences ($p = 0.092$) between treatments in time to pupation of *A. ipsilon*. However, there was a significant difference between the sexes ($p = 0.017$). The interaction effect between treatment and sex was not significant ($p = 0.847$) (Table 4.50).

Table 4.50. GLM analysis of time to pupation of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

SOURCE	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	5	251.72	159.14	31.83	2.05	0.092
SEX	1	127.35	95.83	95.83	6.17	0.017
TREAT*SEX	5	30.99	30.99	6.20	0.40	0.847
ERROR	41	637.07	637.07	15.54		
TOTAL	52	1047.13				

4.3.4.3 Weight of pupae

There were no significant differences ($p = 0.611$) between treatments in weight of pupae of *A. ipsilon* (Table 4.51).

Table 4.51. Weight of pupae of *Agrotis ipsilon* (mg) reared on artificial diets containing gramine and hordenine.

Treatments	n	Weight of pupae (Mean±se)
1. 500 ppm gramine	8	551.0±35.6
2. 60 ppm hordenine	18	520.1±26.8
3. 500 ppm gramine + 60 ppm hordenine	6	501.7±44.9
4. 500 ppm gramine + 500 ppm hordenine	10	483.3±35.8
5. control (with ethanol)	4	528.5±53.0
6. control (without ethanol)	7	569.3±22.8

The treatments had no significant differences ($p = 0.611$) by one-way analysis of variance.

Data shown in Table 4.52 indicated that there was no significant difference ($p = 0.149$) in weight of pupae of male and female *A. ipsilon* reared on artificial diets when data from all treatments were combined.

Table 4.52. Weight of pupae (mg) of male and female *Agrotis ipsilon* reared on artificial diets (all treatments combined).

Sexes	n	Weight of pupae (Mean \pm se)
Males	20	496.3 \pm 18.4
Females	33	539.0 \pm 19.7

non significant ($p = 0.149$) by one-way analysis of variance

The general linear model showed that there were no significant differences between treatments ($p = 0.500$), and between the sexes ($p = 0.609$) in weight of pupae of *A. ipsilon*. The interaction effect between treatment and the sex was also not significant ($p = 0.243$) (Table 4.53).

Table 4.53. GLM analysis of weight of pupae of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

SOURCE	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	5	40030	45865	9173	0.88	0.500
SEX	1	23991	2751	2751	0.27	0.609
TREAT*SEX	5	72834	72834	14567	1.40	0.243
ERROR	41	425364	425364	10375		
TOTAL	52	562220				

4.3.4.4 Sex ratio

There were no significant differences between treatments in sex ratio of pupae ($p > 0.25$) and sex ratio of adults ($p > 0.50$) (Table 4.54).

Table 4.54. Sex ratio of *Agrotis ipsilon* pupae and adults from larvae reared on artificial diets containing gramine and hordenine.

Treatments	Sex ratio (pupae)		Sex ratio (adults)	
	Males	Females	Males	Females
1. 500 ppm gramine	1	7	1	6
2. 60 ppm hordenine	8	10	6	9
3. 500 ppm gramine + 60 ppm hordenine	1	5	1	4
4. 500 ppm gramine + 500 ppm hordenine	4	6	3	4
5.control (with ethanol)	3	1	2	1
6.control (without ethanol)	3	4	2	2

The treatments were not significantly different ($\chi^2 = 6.11$, $df = 5$, $p > 0.25$ for sex ratio of pupae, $\chi^2 = 3.77$, $df = 5$, $p > 0.50$ for sex ratio of adults).

4.3.4.5 Time to adult emergence

Times to adult emergence of *A. ipsilon* reared on artificial diets with and without gramine and hordenine were not significantly different ($p = 0.073$) (Table 4.55).

Table 4.55. Time to adult emergence (days) of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

Treatments	n	Time of adult emergence (Mean±se)
1. 500 ppm gramine	7	47.43±1.73
2. 60 ppm hordenine	15	43.87±0.75
3. 500 ppm gramine + 60 ppm hordenine	5	46.40±1.63
4. 500 ppm gramine + 500 ppm hordenine	7	48.43±2.18
5.control (with ethanol)	3	44.67±0.33
6.control (without ethanol)	4	42.75±1.03

The treatments had no significant differences ($p = 0.073$) by one-way analysis of variance.

There was a significant difference ($p = 0.041$) in time to adult emergence of male and female *A. ipsilon* reared on artificial diets when data from all treatments were combined (Tables 4.56 and 4.57). Females took longer to adult emergence than males.

Table 4.56. Time to adult emergence (days) of male and female *Agrotis ipsilon* reared on artificial diets (all treatments combined).

Sexes	n	Time of adult emergence (Mean±se)
Males	15	43.80±0.74
Females	26	46.50±0.87

Table 4.57. Analysis of variance on time to adult emergence of male and female *Agrotis ipsilon* reared on artificial diets (all treatments combined).

SOURCE	DF	SS	MS	F	P
SEXES	1	69.3	69.3	4.49	0.041
ERROR	39	502.9	15.5		
TOTAL	40	572.2			

The general linear model showed that there were no significant differences between treatments ($p = 0.188$), and between the sexes ($p = 0.136$) in time to adult emergence of *A. ipsilon*. The interaction effect between treatment and the sex was also not significant ($p = 0.865$) (Table 4.58).

Table 4.58. GLM analysis of time to adult emergence of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

SOURCE	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	5	162.47	120.86	24.17	1.61	0.188
SEX	1	46.99	35.28	35.28	2.35	0.136
TREAT*SEX	5	27.73	27.73	5.55	0.37	0.865
ERROR	29	435.06	435.06	15.00		
TOTAL	40	672.24				

4.3.4.6 Duration of pupal stage

There were no significant differences between treatments in duration of pupal stage of *A. ipsilon* (Table 4.59).

Table 4.59. Duration of pupal stage (days) of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

Treatments	n	Duration of pupal stage (Mean±se)
1. 500 ppm gramine	7	13.43±0.20
2. 60 ppm hordenine	15	13.67±0.27
3. 500 ppm gramine + 60 ppm hordenine	5	14.20±0.20
4. 500 ppm gramine + 500 ppm hordenine	7	13.43±0.37
5. control (with ethanol)	3	13.33±0.33
6. control (without ethanol)	4	14.00±0.41

The treatments had no significant differences ($p = 0.558$) by one-way analysis of variance.

Duration of pupal stage of male and female *A. ipsilon* reared on artificial diets was not significantly different when data from all treatments were combined (Table 4.60).

Table 4.60. Duration of pupal stage (days) of male and female *Agrotis ipsilon* reared on artificial diets (all treatments combined).

Sexes	n	Duration of pupal stage (Mean±se)
Males	15	13.73±0.18
Females	26	13.62±0.18

non significant ($p = 0.676$) by one-way analysis of variance

The general linear model showed that there were no significant differences between treatments ($p = 0.727$), and between the sexes ($p = 0.735$) in duration of pupal stage of *A. ipsilon*. The interaction effect between treatment and the sex was also not significant ($p = 0.802$) (Table 4.61).

Table 4.61. GLM analysis of duration of pupal stage of *Agrotis ipsilon* reared on artificial diets containing gramine and hordenine.

SOURCE	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	5	2.95	2.34	0.47	0.56	0.727
SEX	1	0.23	0.10	0.10	0.12	0.735
TREAT*SEX	5	1.91	1.91	0.38	0.46	0.802
ERROR	29	24.03	24.08	0.83		
TOTAL	40	29.22				

4.3.4.7 Gramine and hordenine concentration in artificial diets

For treatment 1 (gramine 500 ppm), gramine concentration in new diets (the diets before feeding by larvae) varied from 458.0 to 466.0 ppm fresh weight (2039.1 to 2180.5 ppm dry weight) and gramine concentration in old diets (the diets left from larvae feeding) varied from 513.0 to 521.2 ppm fresh weight (1825.1 to 2073.5 ppm dry weight). No hordenine was found in both new and old diets.

For treatment 2 (hordenine 50 ppm), gramine concentration in new diets varied from 0.7 to 2.3 ppm fresh weight (3.1 to 10.4 ppm dry weight) and hordenine concentration was 41.3 ppm fresh weight (187.0 to 188.3 ppm dry weight). The old diets of treatment 2 had gramine concentrations of 2.4 ppm fresh weight (8.8 to 9.8 ppm dry weight) and hordenine concentrations from 15.2 to 20.7 ppm fresh weight (61.6 to 77.9 ppm dry weight).

For treatment 3 (gramine 500 ppm+hordenine 60 ppm), gramine concentration in new diets varied from 462.3 to 484.1 ppm fresh weight (2063.2 to 2116.8 ppm dry weight) and hordenine concentration in new diets varied from 39.2 to 39.9 ppm fresh weight (171.6 to 177.8 ppm dry weight). For the old diets, gramine concentration varied from 529.8 to 532.1 ppm fresh weight (1948.6 to 2124.8 ppm dry weight) and hordenine concentration varied from 16.5 to 13.5 ppm fresh weight (66.2 to 67.3 ppm dry weight).

For treatment 4 (gramine 500 ppm+hordenine 500 ppm), gramine concentration in new diets varied from 493.9 to 533.5 ppm fresh weight (2203.6 to 2417.9 ppm dry weight) and hordenine concentration in new diets varied from 260.6 to 273.9 ppm fresh weight (1157.2 to 1242.1 ppm dry weight). Gramine concentration in the old diets varied from 519.4 to 556.3 ppm fresh weight (2006.6 to 2155.6 ppm dry weight) and hordenine concentration varied from 112.0 to 129.4 ppm fresh weight (463.7 to 467.1 ppm dry weight).

For treatment 5 (control with ethanol), gramine and hordenine concentration in the new diets varied from 1.9 to 3.4 ppm fresh weight (7.2 to 15.5 ppm dry weight) and 3.1 to 6.4 ppm fresh weight (14.2 to 27.3 ppm dry weight), respectively. For the old diets, gramine and hordenine concentration varied from 3.3 to 3.7 ppm fresh weight (11.8 to 13.7 ppm dry weight) and 2.1 to 3.0 ppm fresh weight (8.4 to 9.5 ppm dry weight), respectively.

For treatment 6 (control without ethanol), gramine concentration in new diets varied from 0.0 to 0.6 ppm fresh weight (0.0 to 2.6 ppm dry weight) and hordenine concentration in new diets varied from 2.4 to 5.1 ppm fresh weight (10.9 to 23.5 ppm dry weight). Gramine was not found in the old diets and hordenine concentration varied from 0.0 to 4.3 ppm fresh weight (0.0 to 18.2 ppm dry weight) (Figure 4.6 and Appendix M).

Figure 4.6. Gramine and hordenine concentration (ppm) in artificial diet used for feeding *Agrotis ipsilon* (The concentrations of gramine and hordenine at the 1st, 10th, 11th and 24th days were the concentrations of the first batch/new diet, the first batch/old diet, the second batch/new diet, the second batch/old diet, respectively). — fresh weight ----- dry weight

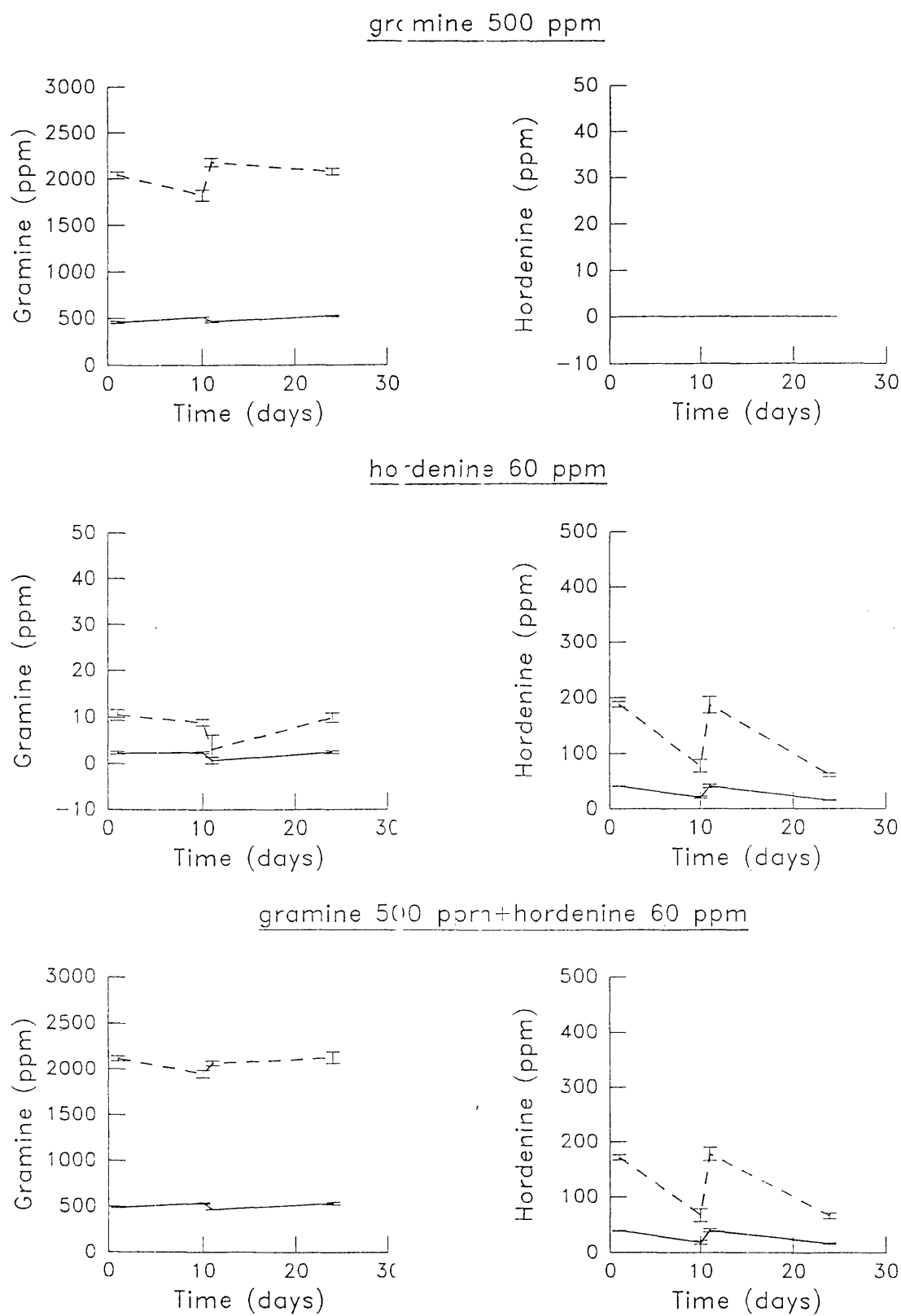
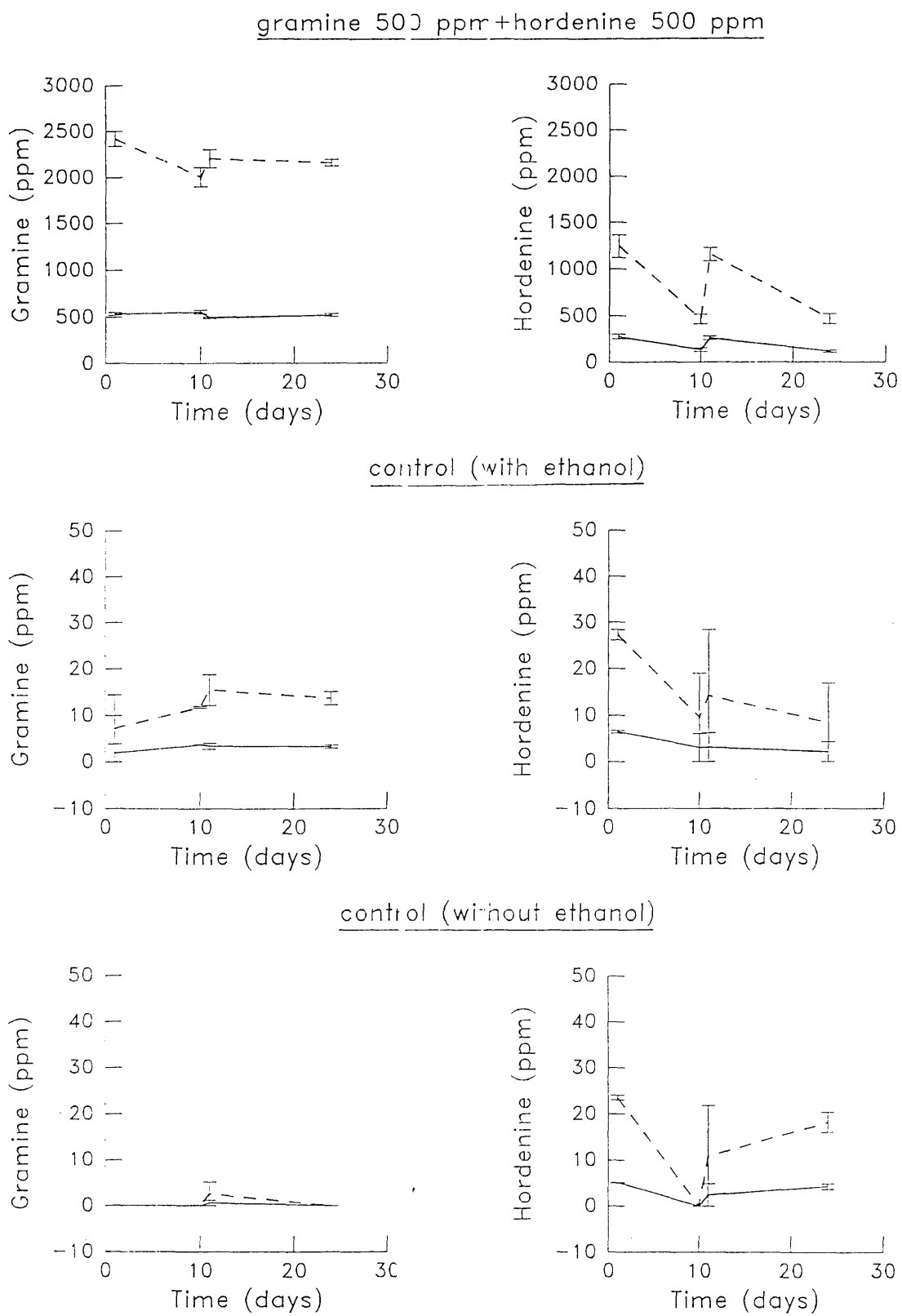


Figure 4.6. (continued)

4.4 DISCUSSION

The effects of gramine and/or hordenine when incorporated in artificial diets on survival, growth and development of insects are summarized in Table 4.62.

Table 4.62. Summary of effects of gramine and hordenine incorporated in artificial diets on *M. convecta*, *H. punctigera* and *A. ipsilon*.

	Gramine	Hordenine	Gramine+hordenine ²	Ethanol
<i>M. convecta</i>				
Survival to pupation	NS	negative (p<0.05)	negative (p<0.001)	not tested
Survival to emergence	NS	NS	negative (p<0.001)	not tested
Sex ratio at pupation	NS	NS	NS	not tested
Sex ratio at emergence	NS	NS	NS	not tested
Duration of larval stage	negative (p = 0.001)	NS	NS ¹	not tested
Duration of pupal stage	NS	NS	NS	not tested
Weight of pupae	NS	NS	NS	not tested
<i>H. punctigera</i>				
Survival to pupation	NS	NS	NS	negative (p<0.025)
Survival to emergence	NS	NS	NS	NS
Sex ratio at pupation	NS	NS	NS	NS
Sex ratio at emergence	NS	NS	NS	NS
Duration of larval stage	NS	NS	NS	NS
Duration of pupal stage	NS	NS	NS	NS
Weight of pupae	NS	NS	NS	negative (p<0.001)
<i>A. ipsilon</i>				
Survival to pupation	NS	positive (p<0.005)	NS	NS
Survival to emergence	NS	NS	NS	NS
Sex ratio at pupation	NS	NS	NS	NS
Sex ratio at emergence	NS	NS	NS	NS
Duration of larval stage	NS ¹	NS	NS ¹	NS
Duration of pupal stage	NS	NS	NS	NS
Weight of pupae	NS	NS	NS	NS

1 = possible negative effect, but sample sizes were too small for reliable statistical analysis, due to high mortality.

2 includes gramine 500 ppm+hordenine 60 ppm and gramine 500 ppm+hordenine 500 ppm, because both treatments tended to show similar effects.

NS = no statistically significant effect

4.4.1 Effects of gramine and hordenine on survival of insects

4.4.1.1 Effects of gramine and hordenine on survival of *M. convecta*

The results of Experiment 5 (Tables 4.2, 4.3 and Figure 4.1) showed that gramine 500 ppm, when incorporated in artificial diet, showed no detrimental effect on survival of *M. convecta*. Hordenine 60 ppm when incorporated in artificial diet showed only a weak detrimental effect on survival of the insect to the pupal stage, and no significant effect on survival to the adult stage. On the other hand, no insect survived on the diet containing gramine 500 ppm+hordenine 60 ppm (the concentrations of gramine and hordenine found in barley leaves cv.Lara). This suggests that gramine and hordenine have synergistic effects in reducing survival of *M. convecta*. However, the diet containing gramine 500 ppm+hordenine 500 ppm did not show more detrimental effect on survival of *M. convecta* when compared to the diet containing gramine 500 ppm+hordenine 60 ppm. This result did not support the hypothesis that gramine and hordenine had higher synergism when they were combined in equal concentrations (Liu 1991). In Chapter 3-Experiment 2, the results showed that larvae could survive (80%) on barley (cv.Lara) which contains gramine 500 ppm+hordenine 60 ppm. On the other hand, larvae could not survive on the diet containing such amounts of gramine and hordenine in this experiment. The reason why the survival patterns of the insect were different between these experiments is not known. It is possible that there may be some other differences between barley and the artificial diet that may affect survival of the insect, in combination with the allelochemicals being studied.

4.4.1.2 Effects of gramine and hordenine on survival of *H. punctigera*

From Experiment 6, the results showed that there were no significant differences between treatments in survival of *H. punctigera* to the pupal and adult stage (Table 4.62). However, ethanol showed significant negative effect on survival to the pupal stage. Since there were some difficulties in recovering hordenine from the *Helicoverpa* artificial diet (see Section 4.4.3), it is possible that *H. punctigera* were not exposed to hordenine even in the diets which were supposed to have contained it. Thus, the absence of detrimental effects of hordenine and of detrimental interactions between hordenine and gramine (as occurred with *M. convecta*) has not been conclusively demonstrated.

4.4.1.3 Effects of gramine and hordenine on survival of *A. ipsilon*

The results of Experiment 7 showed that there were no significant differences in survival of *A. ipsilon* reared on artificial diets containing different gramine and hordenine concentrations when the larvae were very small. However, total survival of *A. ipsilon* was significantly different between treatments at the small, medium and large larval stages, and at the pupal stage (Tables 4.42-4.44). This significance was largely because the hordenine 60 ppm treatment had significantly higher survival than the others. This result is apparently anomalous, and it disappeared when survival through to the adult stage was examined. There were no significant differences at this stage. The results need to be considered in the light of the fact that survival of *A. ipsilon* reared on artificial diets containing different gramine and hordenine concentrations was very low even with the control diet. This may be because the diet used in this experiment was armyworm diet which may be unsuitable for *A. ipsilon*. However, preliminary work showed that it was more suitable than the *Helicoverpa* diet (A. Nesic, pers. comm. 1993). An artificial diet that may be more suitable for *Agrotis* larvae is the pinto bean diet developed by Reese *et al.* (1972). Unfortunately, this diet contains many synthetic ingredients which could not be obtained in time for the experiment. The poor survival of *A. ipsilon* in the control diet means that detrimental effects of allelochemicals might have been masked. As with *H. punctigera*, it is not possible to confidently state that gramine and hordenine have no detrimental effects on survival of *A. ipsilon*.

4.4.2 Effects of gramine and hordenine on the growth and development of insects

4.4.2.1 Effects of gramine and hordenine on the growth and development of *M. convecta*

The results of Experiment 5 showed that time to pupation of *M. convecta* reared on diets containing different gramine and hordenine concentrations showed highly significant differences when the data were analysed by both one-way analysis of variance (Table 4.4) and the general linear model (Table 4.6). Time to pupation of *M. convecta* reared on diet containing 500 ppm gramine was significantly longer than that of larvae reared on control diet. Time to pupation on diets containing hordenine 60 ppm and control were not significantly different. Because no larvae survived in the diet containing gramine 500 ppm+hordenine 60 ppm, data on the growth and development of *M. convecta* reared on this diet could not be collected. Time to pupation of *M. convecta* reared on diet containing gramine 500 ppm+hordenine 500 ppm tended to be

longer than that of control but there was no significant difference between this treatment and the control when the data were analysed by the Bonferroni Multiple Comparisons Procedure. This may be because the number of surviving larvae in this treatment was very low (4). Time to pupation of the insect reared on diets containing gramine 500 ppm, hordenine 60 ppm and gramine 500 ppm+hordenine 500 ppm were also not significantly different. In addition, the one-way analysis of variance and the general linear model also showed that there were no significant differences in time to pupation of male and female *M. convecta* (Tables 4.5 and 4.6).

Weights of pupae of *M. convecta* reared on diets containing different gramine and hordenine concentrations were not significantly different when the data were analysed by both one-way analysis of variance (Table 4.7) and the general linear model (Table 4.9). Moreover, there were no significant differences in weight of pupae of male and female *M. convecta* (Tables 4.8 and 4.9).

As with time to pupation, the one-way analysis of variance and the general linear model showed that there were highly significant differences in time to adult emergence (Tables 4.11 and 4.13). Times to adult emergence of the larvae reared on diets containing gramine 500 ppm and gramine 500 ppm+hordenine 500 ppm were significantly longer than that of larvae reared on control diet. However, times to adult emergence of the insect reared on diets containing gramine 500 ppm and gramine 500 ppm+hordenine 500 ppm were not significantly different. There were also no significant differences in times to adult emergence of *M. convecta* reared on diets containing hordenine 60 ppm and control diet. These trends were similar to those obtained with times to pupation, which is not surprising since those times are a major components of times to emergence. In addition, times to adult emergence of male and female *M. convecta* were not significantly different (Tables 4.12 and 4.13) though duration of the pupal stage of males and females was significantly different (Table 4.15 and 4.16). Males had longer duration of pupa stage than females (a result similar to the result of Experiment 4 in Chapter 3).

These results demonstrated that the development of *M. convecta* was delayed when the larvae were reared on the diets containing gramine 500 ppm and gramine 500 ppm+hordenine 500 ppm. This result was contrast to the result of Experiment 4 in Chapter 3 which showed that there were no significant differences in the development of the insect reared on the diets containing gramine 500 ppm and control. However, it could support the result of Experiment 2 in Chapter 3 which showed that the barley cv.Lara (the cultivar with approximately 500 ppm of gramine) showed detrimental effects on the development of the insect compared with a cultivar with low gramine

levels. Therefore, it is likely that gramine can have negative effects on the growth and development of *M. convecta*. Moreover, gramine and hordenine at the concentrations found in leaves of cv.Lara (gramine 500 ppm+hordenine 60 ppm) showed synergistic effects in reducing survival of *M. convecta*. Survival to pupation with gramine 500 ppm alone was 84% of the control (16/30 vs 19/30), and with hordenine 60 ppm alone it was 58% of the control (11/30 vs 19/30). However, with both allelochemicals present survival was zero. Thus, the combination reduced survival more than would be expected on the basis of a simple additive effect. Synergistic effects of these allelochemicals at the concentrations found in barley cv.Lara on the development of the insect could not be established in this experiment, because of the low survival rate of insects in combined gramine/hordenine treatments. However, the available data suggest that such effects might occur (Tables 4.4 and 4.11).

4.4.2.2 Effects of gramine and hordenine on the growth and development of *H. punctigera*

The results of Experiment 6 showed that there were highly significant differences in times to pupation of *H. punctigera* reared on diets containing different gramine and hordenine concentrations when the data were analysed by both the one-way analysis of variance (Table 4.25) and the general linear model (Table 4.27). However, most of this significance was due to the fact that insects on the control (without ethanol) diet developed more quickly than those on any other diet. There were no significant differences in times to pupation of the insect reared on diets containing gramine and/or hordenine and the control with ethanol. The detrimental effect of ethanol in the diet was unexpected. *H. punctigera* feeds on flowers and fruiting structures, and might be expected to be exposed, under natural conditions, to ethanol in fermenting nectar and therefore tolerate it. The one-way analysis of variance and the general linear model showed that there was no significant difference in time to pupation of male and female *H. punctigera* (Tables 4.26 and 4.27).

The one-way analysis of variance (Table 4.28) and the general linear model (Table 4.31) showed that weights of pupae reared on diets containing different gramine and hordenine concentrations had highly significant differences. Again, most of this significance was associated with detrimental effects of ethanol. Weights of pupae were significantly different between the sexes of the insect (Tables 4.29-4.31). Male pupae were heavier than females. The sex ratio of pupae reared on different diets varied, though not significantly (Table 4.32). The GLM analysis (Table 4.31) showed that both factors were significant, indicating that the effect of treatment was not an artifact of variations in the sex ratio.

The one-way analysis of variance (Table 4.33) and the general linear model (Table 4.36) showed that time to adult emergence of *H. punctigera* reared on diets containing different gramine and hordenine concentrations had highly significant differences. This effect was again due mostly to ethanol, and was to be expected since time to pupation is an important component of time to emergence. Moreover, time to adult emergence of *H. punctigera* reared on the diets was also significantly different between the sexes (Tables 4.34-4.35). Male pupae took longer to emerge than female pupae. Duration of the pupal stage of male *H. punctigera* was also longer than that of females (Tables 4.38 and 4.39). As with weight of pupae, time to adult emergence of the insect may be affected by the sexes as well as treatments, but the effects of treatment were not an artifact of variable sex ratios, because they were also detected by the GLM analysis.

4.4.2.3 Effects of gramine and hordenine on the growth and development of *A. ipsilon*

From Experiment 7, the one-way analysis of variance showed that time to pupation of *A. ipsilon* reared on diets containing different gramine and hordenine concentrations were significantly different (Table 4.47), but the Bonferroni Multiple Comparisons test was unable to detect any significant differences. The general linear model showed no significant differences between treatments (Table 4.50). There was significant difference in time to pupation of male and female *A. ipsilon* (Tables 4.48-4.50). Females had longer time to pupation than males, but the sex ratio in different treatments were different (though not significantly so) (Table 4.54). Although time to pupation of *A. ipsilon* reared on diets containing different gramine and hordenine concentrations were shown to be significantly different by the one-way analysis of variance, the result of the general linear model may be more reliable because of the low numbers surviving and the variable sex ratio between treatments.

The one-way analysis of variance (Table 4.51) and the general linear model (Table 4.53) showed that weights of pupae of *A. ipsilon* reared on diet containing different gramine and hordenine concentrations were not significantly different. There were also no significant differences in weight of pupae between the sexes (Tables 4.52 and 4.53).

The one-way analysis of variance (Table 4.55) and the general linear model (Table 4.58) showed that times to adult emergence of *A. ipsilon* reared on diets containing different gramine and hordenine concentrations were not significantly

different. However, time to adult emergence of the insect reared on diets containing gramine 500 ppm, gramine 500 ppm+hordenine 60 ppm and gramine 500 ppm+hordenine 500 ppm tended to be delayed when compared to control diets, both with and without ethanol. The one-way analysis of variance showed that there were significant differences in time to adult emergence of male and female *A. ipsilon* (Tables 4.56 and 4.57) while the general linear model showed that there were no significant differences between the sexes (Table 4.58). As with time to pupation, the result of the general linear model may be more reliable because the numbers of survival and the sex ratios of the insects reared on different treatments were very variable. In addition, duration of the pupal stage was not significantly different between male and female *A. ipsilon* (Tables 4.60 and 4.61).

The effects of gramine and hordenine on the growth and development of *A. ipsilon* in this experiment were not clear. Although gramine 500 ppm, gramine 500 ppm+hordenine 60 ppm and gramine 500 ppm+hordenine 500 ppm tended to delay the development time of the insect, the statistical analysis did not show any significant effects. This may be because the low numbers of survivors and the variable sex ratios masked any such effects. It would be desirable to repeat these experiments using a diet which gave better survival.

4.4.3 Gramine and hordenine concentrations in artificial diets

Most of the gramine incorporated in both armyworm and *Helicoverpa* diets was recovered (Figures 4.2, 4.4 and 4.6). On the other hand, much of the hordenine incorporated in both diets was not recovered. The hordenine incorporated in armyworm diets was recovered better than in *Helicoverpa* diets and almost all the hordenine incorporated in *Helicoverpa* diets was not found. It is possible that hordenine incorporated in the diets may have reacted with some of the other chemicals in the diets or been degraded. Hordenine could react with ascorbic acid (a chemical incorporated in both armyworm and *Helicoverpa* diets) but the reaction would need high temperature (more than 50°C while the hordenine was incorporated into artificial diets at the temperature below 50°C). The reason why the apparent loss of hordenine was more severe in *Helicoverpa* diet than armyworm diet is not clear. The two diets vary in their natural ingredients (maize meal and dried barley for armyworm diet, and soyabeans for *Helicoverpa* diet). There are also variations in the proportions (but not the presence or absence) of some synthetic ingredients (see Appendix A and F). The most probable explanation for the deficiency of hordenine is that it reacted with some ingredient or ingredients, present in large amounts in the *Helicoverpa* diet, in such a way that it could not be extracted by acetic acid (D. Tucker, pers. comm. 1994). What is not known is

whether this reaction meant that the hordenine was not available to the insects. If it was not, then the absence of effects of hordenine (alone and in combination with gramine) on *Helicoverpa* compared with *Mythimna* might be an artifact of the experimental methods. It might also mean that the effects on *M. convecta* have been underestimated in this work. For these reasons, further work on improving assays for hordenine in artificial diets is required.

4.5 GENERAL DISCUSSION

These results demonstrated that gramine and hordenine alone (gramine 500 ppm and hordenine 60 ppm) did not reduce survival of *M. convecta*, *H. punctigera* and *A. ipsilon*. In fact, the survival of *A. ipsilon* tended to be highest in artificial diet containing hordenine 60 ppm. It is interesting that there was no survival of *M. convecta* when the insect was reared on diet containing gramine and hordenine at the concentrations found in barley leaves cv.Lara (gramine 500 ppm+hordenine 60 ppm). This result suggests that gramine and hordenine at the concentrations occurring naturally in barley leaves were effectively synergistic. Other studies also show the synergistic effects of plant allelochemicals on insects. For example, myristicin and xanthotoxin, the chemicals found in leaves of the plants in family Umbelliferae, had synergistic effects on survival and development time of *H. zea* (Berenbaum and Neal 1985) and different phenolic compounds from *S. bicolor* had synergistic effects on feeding behaviour of *L. migratoria* (Adams and Bernays 1978). However, the concentrations of gramine and hordenine that showed adverse effect on survival of *M. convecta* when incorporated in artificial diets did not show significant adverse effects on survival of *H. punctigera* and *A. ipsilon* (though this may be an artifact of the experimental method). Furthermore, gramine and hordenine when incorporated in artificial diet at the equal concentrations (gramine 500 ppm+hordenine 500 ppm) did not reduce significant numbers of survival of these insects when compared to that of the diet containing gramine and hordenine at the concentrations found in leaves of cv.Lara. This result did not support the result of Lui (1991) which demonstrated that the effects of gramine and hordenine on white mustard were higher when they were combined in equal concentrations.

Gramine and possibly gramine+hordenine when incorporated in artificial diets delayed the development of *M. convecta* while hordenine 60 ppm alone did not show significant adverse effect. However, the development time of the insect reared on diets containing gramine alone and gramine+hordenine were not significantly different demonstrating that the combination of these allelochemicals in artificial diet tended to have no synergistic effects on the development of the insect. Effects of gramine and hordenine on the growth and development of *H. punctigera* and *A. ipsilon* were not clear

because of the effects of the survival numbers and sex ratios of the insects, and (for *H. punctigera* especially) difficulties in recovering hordenine from the diet. Therefore, the effects of these allelochemicals on the growth and development of these insects can not be concluded yet.

Other organisms that are affected by gramine and hordenine include weeds. Gramine and hordenine affected radicle elongation (Liu and Lovett 1987, cited by Lovett 1989, Liu and Lovett 1990) and cytology (Lovett *et al.* 1989) of white mustard. However, these allelochemicals tended to delay the growth rather than stop germination.

The bioassay using artificial diets to study the effects of allelochemicals on insects is subject to some problems. Artificial diets normally have one of two problems: they are suboptimal or they are superoptimal. Not all insects are easily reared on artificial diets and for this reason, suboptimal diets have sometimes been used to verify the effects of specific chemicals. Such suboptimal diets can cause stress of the insects even without any addition of test chemicals. Therefore, the impact of test chemicals can not be confidently evaluated. On the other hand, superoptimal diets frequently enhance growth of the insects, subsequently, resistance of the insects to test chemicals may be increased (Wolfson 1988). Of course, the same criticisms can be levelled at natural diets, which might be suboptimal or superoptimal depending on the plant species, cultivar, stage of growth, nutrient level, etc. However, there is a bioassay system that is not based on artificial diets but it is based on the natural food turned into an artificial substrate. The system has been used for leaf-feeding insects such as beetles and Lepidoptera by incorporating the test chemicals into a gelatin solution and then painting the solution onto the leaf surfaces (either upper, or lower, or both). Since gelatin is a protein and may affect growth rate of some insects, the appropriate control treatment when testing chemical in gelatin solutions is gelatin-treated leaves (Wolfson and Murdock 1987).

Since plant defensive substances do not uniformly affect all potential enemies (Rhoades 1979), differences in the effects of gramine and hordenine on different insect species would be expected. Herbivores are usually exposed to toxic allelochemicals in plants, nevertheless, they can defend themselves from toxicity via biochemical defense mechanisms (Brattsten 1979). The detoxication system in insects involves many groups of enzymes (Brattsten 1979, Berentzen and Zangerl 1988). Among the enzymes which involve in the detoxication system, mixed-function oxidases (MFOs) are the most important enzymes and high activity of MFOs is perceived in the fat body and in the tissues associated with the alimentary tract in insects (Brattsten 1979). In addition, MFOs activity are affected by physiological factors including age, sex, genetic and

nutritional condition (Brattsten 1979). Mixed-function oxidases such as hydroxylase are found in midgut of corn earworm (*E. zea*) and tobacco budworm (*H. virescens*) (Yu and Ing 1984). The important pyrethroid resistant mechanism in *H. armigera* is oxidative metabolic detoxification (Forrester *et al.* 1993). Similar mechanisms may occur in *H. punctigera* because the two species are co-incident on many crops which are subjected to heavy insecticide applications even though *H. punctigera* does not show strong insecticide resistance. Since specialized herbivores with narrow host ranges are generally much more resistant to the effects of defensive substances in their host plants than generalised herbivores that do not normally eat the plant (Rhoades 1979), *H. punctigera* was expected to be more susceptible to gramine and hordenine than *M. convecta* because *H. punctigera* feeds on dicotyledons (Zalucki *et al.* 1986) whereas *M. convecta* feeds on cereals including barley (McDonald and Smith 1986). However, the results demonstrated that the combination of gramine and hordenine had an adverse effect on survival of *M. convecta* not on *H. punctigera*.

The adverse effects of some plant allelochemicals on physiological development of insect may be associated with ecdysone insufficiency (Slama 1979). The allelochemicals may inhibit ecdysis (a process of molting) of insects, by antagonizing the function of ecdysterone, antagonizing the function of juvenile hormones or inhibiting chitin synthesis (Kubo and Klocke 1986). This role of allelochemicals may make them useful as insect control agents because they are specific for organisms which molt (Kubo and Klocke 1986) and environmentally safer natural products (Slama 1979). Hence, further study on adverse effects of gramine and hordenine on ecdysis of insects may be valuable for effective pest management.

Plants that contain alkaloids are mainly dicotyledons; a few are found among the monocotyledons and gymnosperms. These plants may have toxic or repellent properties due to alkaloids which are physiologically active toward many organisms (Robinson 1979). Gramine and hordenine are two alkaloids found in barley which might be expected to be toxic or repel insect pests. As described in Chapter 3, there is some evidence showed that barley cultivars that contain high gramine content have adverse effects on feeding behaviour of aphids such as *S. graminum* and *R. padi* (Zuniga *et al.* 1985, Argandona *et al.* 1987, Zuniga *et al.* 1988). Gramine was also found to have adverse effects on biology of grasshoppers such as *L. migratoria* and *M. sanguinipes* (Bernays 1990, Westcott *et al.* 1992). Hordenine was found to have adverse effects on feeding behaviour and survival of a polyphagous aphid, *M. persicae* (Schoonhoven and Derksen-Koppers 1976) and showed significantly negative effects on survival and mean weight of *M. sanguinipes* (Westcott *et al.* 1992). These studies demonstrated that

gramine and hordenine may have both toxic and repellent properties but these allelochemicals may show different effects on different species.

Apart from aphids and grasshoppers, the biological effects of gramine and hordenine on Lepidoptera are also interesting because many serious pests are belong to this order. Therefore, the studies on the effects of these allelochemicals on Lepidoptera such as *M. convecta* and *A. ipsilon* (or other *Agrotis* species) that are serious pests of barley may be valuable for breeding cultivar resistant to these insects. If gramine and hordenine had synergistic effects on these insects, the barley cultivars that contain both gramine and hordenine may be desired. However, the concentrations of gramine and hordenine that can have negative effects on survival, the growth and development of these insects need further study. It is noteworthy that plants containing a small number of different alkaloids in relatively high concentration and high individual variability may reduce the possibility of insects developing an effective defensive mechanism. Conversely, plants that have been bred artificially for high alkaloid content are likely to be susceptible to insect attack because the variability may be lost (Robinson 1979).

Since gramine and hordenine are alkaloids, they may affect the palatability of plants used for feeding animals (Marten *et al.* 1976, Marten *et al.* 1981) or they may have adverse effects on animals (Gallagher *et al.* 1964, Barwell *et al.* 1989, Bourke *et al.* 1992). Therefore, the concentrations of gramine and hordenine in barley that can negatively affect survival, growth and development of insect pests while they do not reduce the palatability of the plant and have no adverse effects on animals should be investigated.

Furthermore, plant allelochemicals that were fed on by herbivorous insects may affect the quality of the herbivores as food for beneficial insects. Allelochemicals ingested by herbivores can have adverse effects on survival, the growth and development and behaviour of beneficial insects (Barbosa 1988, Jones *et al.* 1988, Williams *et al.* 1988, Barbosa *et al.* 1991). On the other hand, allelochemicals produced by plants can stimulate host/prey selection behaviour of beneficial insects (Nordlund *et al.* 1988). Beneficial insects can also use pheromones of their host in host location (Wood 1982, Noldus and van Lenteran 1985). Accordingly, the study on effects of gramine and hordenine on beneficial insects is of interest.

The results of this chapter suggested that gramine and hordenine incorporated in artificial diet at the concentrations found in leaves of barley cv.Lara showed adverse effects on survival of *M. convecta*. In addition, gramine 500 ppm and possibly gramine

500 ppm+hordenine 500 ppm incorporated in artificial diet also delayed the development of the insect. These results are similar to the result of Experiment 2 that showed adverse effects of barley cv.Lara on the development of *M. convecta*. These studies suggest that such concentrations of gramine and hordenine may be those that are required for breeding barley cultivars resistant to *M. convecta*. However, it may be more reliable if the effects of barley cultivars containing such concentrations (such as cv.Lara) on the insect were investigated in the field. Moreover, the effect of these barley cultivars on palatability of plants and adverse effects on animals and beneficial insects should be investigated. Although gramine and hordenine incorporated in artificial diet at the concentrations found in leaves of barley cv.Lara did not show significant adverse effects on survival, growth and development of *A. ipsilon*, these concentrations tended to delay the development of the insect. Therefore, it is possible that gramine and hordenine at the concentrations found in leaves of barley cv.Lara may also have adverse effects on *A. ipsilon* but the study on such effects needs to be repeated.