BARLEY ALLELOCHEMICALS AS SELF DEFENCE PROPERTIES AGAINST VERTEBRATE ANIMALS

A thesis submitted for the degree of Master of Rural Science of the University of New England

Irawati Sarjana (Andalas University), 1987

Department of Agronomy and Soil Science University of New England, Armidale Australia

March 1996

Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree.

I certify that to the best of my knowledge any help received in preparing this thesis, and all source used, have been acknowledged in this thesis.

.....

Irawati March 25, 1996

Acknowledgments

First of all, I gratefully appreciate my academic supervisors Prof. John V. Lovett and Dr. Juliet R. Robe ts for their patient, friendly guidance, never-ending encouragement and, particularly, their assistance in English language usage.

I also express my deep gratitude to Mrs. Anne H. C. Hoult, Mr. Walter Redlich and Mr. Dan Alter for their technical assistance in the laboratory analyses of gramine and hordenine; Mr. Grahame Chaffey, Ms. Jo Andrews, Mr. Suratman, Mr. Asril and Mr. Chalid Thalib for their help in tissue collection; Mr. John Smallshaw and all staff of the UNE Animal House for their kind assistance during all my animal house work; Ms. Annette McLeod and Ms. Isabelle Coulon for their assistance in histological techniques; Ms. Shirley Dawson (Media Resources Unit, UNE) for her guidance with the micrographs of liver tissues.

My sincere thanks also ξ o to Dr. Bob Hamilton of Agriculture Canada for his assistance with reading materials. I also wish to thank Mr. Dahlanuddin of the Department of Animal Science for his assistance with statistical analysis and word processing; Department of Agro 10my and Soil Science; Department of Physiology and my colleague postgraduate students in both departments for being helpful and friendly; all Indonesian friends in Armidal 2 for providing a family-like environment.

Finally, I must acknow edge that my study would not have been possible without a study grant from the Australian Agency for International Development (AusAID), formerly known as AIDAB, and permission from the Indonesian government. All personal support and assistance from AusAID's staff, particularly Mrs. Sheila Soebroto (Jakarta office), Mr. Bruce O'Brien and Mr. Maris Veidelis (Sydney office), both during my preparation in Indonesia and during my study in Australia, was very much appreciated.

Summary

Plant secondary metabo ites have been demonstrated to act as self defence agents against other species. Allelochemicals found in barley, gramine and hordenine, have similar effects on other plants, fungi, bacteria and insects (invertebrates). However, there has been a lack of evidence for barley allelopathy as a self defence mechanism against vertebrate an mals.

Four experiments were carried out to study the effects of the secondary metabolites of barley, gramine and hordenine, on the growth of two vertebrate species (laboratory mice and broiler chickens). The objectives of the study were to provide information on the potential value of these metabolites in self defence of barley against vertebrate pests. All experiments were conducted at the Animal House of the University of New England, Armidale, NSW. Water and feed were provided *ad libitum*. There were two different levels, 50 and 500 ppm, of both gramine and hordenine which were incorporated into the feed of the treatment groups.

The first experiment was designed to assess any possible adverse effects of both alkaloids on mice. Laboratory mice were given feeding choice between standard laboratory feed and alkaloid-containing feed. In the second and third experiments, animals (both mice and broiler chickens) were not given feeding choice but were provided with different concentrations of either gramine or hordenine in feed. The last experiment studied possible synergistic effects of the two alkaloids, gramine and hordenine, on broiler chickens.

Overall, the treatments did not significantly affect the growth of mice and chickens. However, gramine 500 ppm in feed significantly reduced feed intake in mice. Small quantities of both gramine and hordenine were recovered from the livers of both animal species through High Performance Liquid Chromatography (HPLC) analysis. However, when mice were give 1 feeding choice, the recovered gramine and hordenine

were detected from high level reatments (500 ppm) only. The amount of alkaloid recovered was associated with the concentration given in feed.

The main finding of the present study is that gramine had more effects than hordenine on the animals tested. A high concentration (500 ppm) of either gramine or hordenine was sufficient to induce changes in liver structure of the chickens. These changes were indicated by cell vacuolation even though they were not necessarily associated with reduced feed in take or growth rate of the animals. The changes are congruent with damage observed in cells of other species exposed to secondary metabolites.

Broiler chickens provided a better model for this study than did laboratory mice. Broiler chickens have a very fast growth rate and are able to seek food immediately after hatch. However, laboratory mice cannot be weaned until three weeks after birth and have attained a significant proportion (up to 80%) of their adult body weight by the time they are introduced to the experimental feeds. Therefore, the effects of allelochemicals on feed intake and growth rate in vertebrates are more likely to be demonstrated in broiler chickens than in mice.

Contents

	Page
Declaration	ii
Acknowledgment	iii
Summary	iv
List of figures	x
List of tables	xi
List of plates	xii
Chapter 1. Introduction	1
1.1. History of Agricult are	2
1.2. Pressures on Agriculture	
Chapter 2. Literature Review	6
2.1. Allelopathy	
2.2. The history of allelopathy	
2.3. Allelochemicals: secondary metabolites	
2.4. Release of allelochemicals from plants	
2.4.1. Volatilisation	
2.4.2. Root Exudation	
2.4.3. Leaching	
2.4.4. Decomposition of plant residues	
2.5. Transport of alleloc hemicals in the environment	
2.6. Factors affecting the production and activity of allelochemicals	
2.7. Activity of alleloch-micals	
2.7.1. Effects of allelochemicals on weed management	
2.7.2. Effects of allelochemicals on other organisms	
2.8. Interpretation of the effects of allelochemicals	
2.9. Occurrence of allelopathy in crop plants	
2.10. Common features of allelopathy in crops	
2.11. Barley as a case s udy for allelopathy in crops	
2.11.1. Activity of barley allelochemicals against weeds	
2.11.2. Activity of barley allelochemicals against other organisms	
2.11.3. Deficiencies in knowledge and need for further research	
Chapter 3 Materials and Mathods	12
Chapter 3. Materials and Methods	
3.1. Introduction	
3.3. Preparation of Feed	
J.J. FICHALAUNI OF FCC1	43

3.3.2. Chicken Feed	
3.4. Housing, Care and Maintenance	46
3.4.1. Mice	46
3.4.2. Chickens	46
3.5. Sacrifice of Anima s and Collection of Tissue	47
3.6. Determination of C ramine and Hordenine	47
3.7. Histological Examination	50
3.8. Experimental Design	51
Chapter 4. Preliminary Assessment of the Effects of Gramine and Hordenine on	
Vertebrates5	52
4.1. Introduction	53
4.2. Materials and Methods	53
4.3. Results	55
4.3.1. Body Weight	55
4.3.2. Feed Intake	
4.3.3. Liver Weight	57
4.3.4. HPLC Analysis of Liver	59
4.4. Discussion	
Chapter 5. Effects of Gramine and Hordenine on Mice	62
5.1. Introduction	63
5.2. Materials and Methods	63
5.3. Results	64
5.3.1. Body Weight	64
5.3.2. Feed Intake	64
5.3.3. Liver Weight	66
5.3.4. HPLC Analysis of Liver	
5.4. Discussion	
Chapter 6. Effects of Gramine and Hordenine on Broiler Chickens	72
6.1. Introduction	73
6.2. Materials and Methods	73
6.2.1. Experimental Design	73
6.2.2. Data Collection	
6.2.3. Histological Examination	74
6.3. Results	
6.3.1. Body Weigl t	74
6.3.2. Feed Intake	
6.3.3. Liver Weight	
6.3.4. HPLC Analysis of Liver	
6.3.5. Feed Conversion Ratio	
6.3.6. Histological Examination	
6.3.7. Additional Observation	
6.4. Discussion	

Chapter 7. Synergistic Effects of Gramine and Hordenine on Broiler Chickens	86
7.1. Introduction	. 87
7.2. Materials and Methods	. 87
7.3. Results	. 88
7.3.1. Body Weigh	. 88
7.3.2. Feed Intake	. 88
7.3.3. Liver Weigh	. 89
7.3.4. HPLC Analysis of Liver	. 89
7.3.5. Feed Conversion Ratio	.91
7.3.6. Histological Examination	. 92
7.4. Discussion	. 92
Chapter 8. General Discussion	. 96
8.1. General Discussion	
8.2. Conclusion	104
References	106
Appendices	age
Appendix A: The composition of Custom Mix mouse feed	
Appendix B: The composit on of Chick Starter Feed and Broiler Finisher Feed.	
	129
Appendix C: Buffered 10% Formalin (pH 7)	
Appendix D: Mobile Phase for HPLC	
Appendix E: The Histokine tte Process	
Appendix F: The Staining Process (Ehrlich's Haemotoxylin and Eosin)	
Appendix G: Body weights of mice receiving different levels of alkaloids graming	
and hordenin; in feed during the feeding choice experiment	133
Appendix H: Total feed intakes of mice receiving different levels of alkaloids	
gramine and nordenine in feed during the feeding choice experime	ent
Appendix I: Liver weights of mice receiving different levels of alkaloids gramin	ıe
and hordenine in feed during the feeding choice experiment	135
Appendix J: Liver weights of mice receiving different levels of alkaloids gramin	ıe
and hordenin :	135
Appendix K: Liver weights of chickens receiving different levels of alkaloids	
gramine and nordenine	
Appendix L: Body weights of mice receiving different levels of alkaloids gramin	ne
and hordenin; in feed	136
Appendix M: Feed intakes of chickens receiving different levels of alkaloids	
gramine and nordenine in feed	137
Appendix N: Feed conversion ratios of chickens receiving different levels of	
alkaloids grainine and hordenine in feed	137

Appendix O: Body weights of chickens receiving different combination	of
alkaloids gramine and hordenine in feed	138
Appendix P: Feed intakes of chickens receiving different combination of	alkaloids
gramine and hordenine in feed	139
Appendix Q: Feed conversion ratios of chickens receiving different com	bination of
alkaloids gramine and hordenine in feed	139

List of Figures

Page
Figure 1.1. Evolution and current perception of the relationship among
agricultural technologies
Figure 2.1. The production and release of allelochemicals from plant to the
environment12
Figure 2.2. A relationship of the factors and processes affecting the transport of
allelochemicals from the site of production to the target plant 19
Figure 2.3. The biosynthesis pathway of gramine from tryptophan
Figure 2.4. The formation of hordenine from tyrosine
Figure 3.1. The flow diagram of purification for hordenine
Figure 3.2. The flow diagram of purification for gramine
Figure 4.1. Body weight of mice for eight weeks of treatment56
Figure 4.2. Body weight of mice as percentage of initial body weight
Figure 4.3. Mouse feed ingested for eight weeks of treatment
Figure 4.4. Total amount of feed taken by mice during the experiment 58
Figure 4.5. Liver weight of mice after eight weeks of treatment
Figure 5.1. Body weight of mice over twelve weeks of experimental period 64
Figure 5.2. Body weight of mice as a percentage of initial body weight
Figure 5.3. Mouse feed ingested for alkaloid treatments
Figure 5.4. Liver weight of mice after twelve weeks of alkaloid treatments 66
Figure 6.1. Body weight of broiler chickens over seven weeks of experimental
period75
Figure 6.2. Chicken feed ingested over seven weeks of age
Figure 6.3. Liver weight of chickens at seven weeks of age
Figure 6.4. Feed conversion ratio of chickens receiving different levels of alkaloids
79
Figure 7.1. Body weight of broiler chickens over seven weeks of age
Figure 7.2. Chicken feed intake over the experimental period90
Figure 7.3. Liver weight of chickens at seven weeks of age
Figure 7.4. Feed conversion ratio of chickens exposed to combinations of different
levels of alkaloi is gramine and hordenine92

List of tables

	Page
Table 4.1. Gramine and hordenine content in liver	59
Table 5.1. Feed intakes of mice receiving different levels of alkaloids gramin	e and
hordenine in the feed	67
Table 5.2. Gramine and hordenine content in liver	68
Table 6.1. Body weight of chickens receiving different levels of alkaloids	76
Table 6.2. Gramine and hordenine content of chicken liver	79
Table 7.1. Liver weight of broiler chickens at seven weeks of age	91
Table 7.2. Gramine and hordenine content of chicken liver	91

List of plates

	Page
Plate 6.1. Micrographs of livers from chickens receiving different levels of	
gramine and hordenine incorporated into feed	82
Plate 6.2. Lesions on the feet of chickens receiving 500 ppm gramine in feed	
during weeks 4 ard 5 of the experiment	83
Plate 7.1. Micrographs of livers from chickens receiving combinations of leve	ls of
gramine and hordenine incorporated into feed	93