Biological activity of barley secondary metabolites

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Summary. The biological activity of hordenine and gramine, secondary metabolites of barley *(Hordeum vulgare),* was tested against other plant species, a fungus *(Drechslera teres)* and armyworm *(Mythimna convecta),* and found to have significant inhibitory effect on each. The value to agriculture of exploiting these effects is discussed.

Introduction

Reese (12) defines allelochemicals as "non-nutritional chemicals produced by one organism that affect the growth, health, behaviour or population biology of other species". Recognition of a defensive-comecommunicational role for these chemicals has led to investigation of their potential in natural resistance to pests and, hence, in crop protection (see for example, I, 2. 4). The living barley plant *(Hordeum vulgare)* produces two such chemicals, hordenine and gramine (10), both alkaloids, for which such activity has been demonstrated. For example Lovett and Liu (8) demonstrated that at a concentration of 48 ppm under controlled conditions, present after 4 days growth, hordenine significantly reduced radicle length in white mustard *(Sinapis alba* L.) and gramine has been implicated in the self-defence of barley against aphids (6) and bacteria (13). Recent work in our laboratory has aimed at extending our knowledge of the biological activity of both these compounds.

Methods

Activity of hordenine against other plant species

Seed of wild oats *(Avena fatua),* prairie grass *(Bromus unioloides),* Powell's amaranth *(Amaranthus powellii)* and shepherd's purse *(Capsella bursa-pastoris)* was grown in petri dishes in the presence of a range of concentrations of hordenine from 0 to 500ppm. The lemmas and paleas were removed from the grass grains before use. This permitted synchronous germination and reduced fungal infestation. The dicot seeds were sterilised in I% bleach for 3 min and rinsed well in distilled water before use. Twenty to fifty seed were placed in each dish, according to size; each treatment was replicated 5 times. The length of the first seminal root or radicle was measured at 3-4 days after germination. In order to follow up the effects of hordenine over a longer period and to investigate hordenine as a possible source of nitrogen, a second experiment was run in which 20 seed of prairie grass were sown in sand in pots in the presence or absence of hordenine (I.5mM/g sand) and subjected to three levels of nitrogen nutrition (applied as 10, 6 and 3 mM in solution) (treatments H ION, H6N and H3N with hordenine and treatments ION, 6N and 3N without hordenine). Growth, as plant height, was monitored over 3 weeks.

Activity of hordenine and gramine against Drechslera teres

Drechslera teres was cultured on potato dextrose agar (9.5 g/200 mL) in the presence of a range of concentrations of hordenine and gramine (0, 0.25, 1.0, 5.0, lOnnM). Each treatment was replicated 6 times. The diameter of the central black area of the colonies was measured three days after innoculation.

Activity of hordenine and gramine against armyworm (Mythimna convecta) larvae

Armyworm larvae were fed diets (3) containing concentrations of 0, 0.25, 1.0, 5.0, 10.0 and 50mM hordenine and 0, 0.25, 1.0, 5.0, 10.0 and 15mM gramine. Larval weight of 20 larvae for each treatment was measured one week after the larvae were introduced to the diets.

Results and discussion

Plant species were not all affected in the same way by the presence of hordenine at the post-germination stage (Table I). Wild oats and shepherd's purse are examples of weeds that have a long history of association with cereal crops and may be expected to have some tolerance for hordenine (5). Wild oats demonstrated apparent tolerance, growth being significantly reduced only at high concentrations (500 ppm). Conversely, growth of shepherd's purse was significantly reduced at low levels of hordenine (25 and 5Oppm), at which the other species showed mild stimulation of growth. Prairie grass and Powell's amaranth have no established association with cereal crops. Whereas prairie grass was moderately

susceptible to hordenine (growth reduced at 200 ppm), Powell's amaranth was, like wild oats, significantly affected only at 500 ppm. These results are consistent with those of Overland (11) who found that the effects of barley allelochemicals varied from species to species.

Table 1. Seedling radicle length of 4 species grown under different concentrations of hordenine.

	Radicle length (mm)					
	Hordenine concentration (ppm)					
Species	0	25	50	100	200	500
Avena fatua	27.98±3.02		29.82±2.46	30.20±1.65	27.78±3.01	18.50±3.01
Bromus unioloides	25.88±2.22		29.00±1.50	28.67±1.80	22.22±4.18	5.85±1.02
Amaranthus powellii	15.92±4.19	17.84±1.95	19.18±2.38	16.52±0.77	15.12±2.34	11.60±0.32
Capsella bursa-pastoris	9.50±1.76	6.45±0.45	5.70±0.54	4.52±0.58	4.20±0.97	4.54±0.45

Results for the pot experiment are presented in Table 2 and indicate that for 5 days after emergence, irrespective of the level of nitrogen applied, growth of prairie grass was slowed by 1.5 mM hordenine/g sand (approx. 300 ppm) and that after 2 weeks it was significantly stimulated. At this stage there was no effect of nitrogen level, suggesting that seed reserves may not have been exhausted or that, at the levels of nitrogen applied, hordenine acted as a nitrogen source great enough to negate the nitrogen effect. Since, among the control treatments, the highest level of nitrogen supported greater growth. the latter may be the case. More importantly, the reduction of growth in the presence of hordenine in the first 6 days of growth may be enough to give a competing crop of barley an advantage.

Table 2. Height of prairie grass seedlings grown in the presence or absence of hordenine and at three different levels of nitrogen.

	Plant height (mm)					
Days after emergence	10N[a]	6N	3N	H10N	H6N	H3N
2	13.0 ± 1.87	12.8 ± 3.96	15.8 ± 3.27	8.6 ± 1.34	7.8 ± 2.05	9.2 ± 1.92
5	34.0 ± 4.53	33.4 ± 2.51	35.6 ± 2.70	29.0 ± 2.74	27.8 ± 1.92	27.4 ± 3.97
9	58.4 ± 3.36	53.2 ± 4.66	53.2 ± 3.56	54.0 ± 2.00	53.0 ± 5.86	50.6 ± 5.86
11	71.6 ± 2.70	64.6 ± 6.39	63.8 ± 6.30	71.6 ± 4.10	73.0 ± 5.43	68.8 ± 5.31
14	88.8 ± 4.02	82.8 ± 6.80	78.6 ± 7.83	98.2 ± 4.49	95.0 ± 6.16	93.2 ± 5.67
17	101.0 ± 6.82	93.8 ± 8.64	88.8 ± 9.26	108.0 ± 2.00	104.4 ± 7.27	106.8 ± 11.30
20	103.6 ± 7.06	97.4 ± 7.54	93.0 ± 9.92	118.2 ± 8.17	111.0 ± 7.38	113.8 ± 9.63
23	125.4 ± 9.66	104.0 ± 11.47	94.9 ± 8.20	142.4 ± 13.35	132.6 ± 10.97	122.6 ± 8.11

[a]See Methods

Table 3 shows the effect of hordenine and gramine on the growth of *Drechslera teres* and, again, indicates a possible defensive role for these compounds against other species. Hordenine is more effective against this pathogen than gramine, greatly reducing growth at 5mM whereas gramine was only effective at 10mM. These figures are high (>1000 and 1750 ppm respectively), however, the authors have found concentrations of 2000 mg/g dry weight of hordenine in barley root tissue (unpublished data) and 1500 mg/g fresh weight of gramine in leaf tissue (9). It seems possible, therefore, that these compounds may play a role in defence of barley against fungal pathogens.

Table 3. Effect of hordenine and gramine on the growth of the central black colony of *Drechslera teres.*

Concentration (mM)	Diameter of colony[a] (mm)	
	Hordenine	Gramine
0	17.33 ± 0.88	13.20 ± 0.37
0.25	14.93 ± 0.60	14.60 ± 0.40
1.0	14.00 ± 0.73	13.00 ± 0.68
5.0	12.67 ± 0.99	12.17 ± 0.60
10.0	12.33 ± 0.80	10.20 ± 0.20

[a]3 days after inoculation

The effect of hordenine and gramine on growth of armyworm larvae is less clear (Table 4). While growth was reduced by both compounds there was no consistent trend. Further work is in progress with "high" and "low" gramine cultivars in order to determine a possible role for gramine as an anti-feedant or growth inhibitor with respect to armywomi.

Table 4. Effect of hordenine and gramine on the growth of armyworm larvae.

Concentration (mM)	Larval weight[a] (mg)	
	Hordenine	Gramine
0[b]	4.72 ± 0.80	13.22 ± 1.26
0.25	5.80 ± 0.88	13.37 ± 0.91
1.0	5.31 ± 0.85	10.91 ± 0.80
5.0	7.03 ± 0.86	13.14 ± 0.82
10.0	4.27 ± 0.53	10.77 ± 0.81
15.0		9.54 ± 1.40
50.0	3.44 ± 0.62	

[a]At one week

[b]Differences in larval weight of zero treatments can be attributed to differences in larval hatching date.

Together, these data demonstrate a wide range of activity for both compounds against other species. The implications for agriculture are equally wide ranging in respect of the possibilities for reducing the input of synthetic biocides into the growing crop.

Our work has indicated the potential of hordenine and gramine to contribute to the self-defence of barley in a number of ways. Hordenine is inhibitory to radicle growth of some other plant species. Both are inhibitory to the growth of a fungal pathogen and may affect the growth of armyworm. Together, this evidence supports the contention (7) that selection to increase allelochemical content in crops such as barley, as a means of self defence, adds a further dimension to biological control. In turn, this may contribute to reduced pesticide use and to the development of ecologically and economically more sustainable agricultural systems.

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References

1. Alkohafi, A.. Rupprecht, J.K.. Anderson, J.E., McLaughlin. J.L., Mikolajczak, K.L. and Scott. B.A. 1989. In "Insecticides of Plant Origin", (Eds.) J.T. Arnason, B.J.R. Philogene and P. Morand. ACS Symposium Series No. 387, American Chemical Society, Washington D.C., pp.25-43.

2. Anaya, A.L., Ramos, L., Cruz, R., Hernandez, J.G. and Nava, V. 1987. J. Chem. Ecol. 13:20832101.

3. Del Soccoro, A. 1991. Ecology of armyworms in the New England and mid-North Coast areas of New South Wales. M.Ru.Sci. Thesis, U.N.E.

4. Green, M.B. and Hedin, P.A. 1986. "Natural resistance of plants to pests", American Chemical Society, Washington. D.C.

5. Holm, L.G., Plucknett. D.L., Pancho, J.V. and Herberger, J.P. 1977. The World's worst weeds. East-West Centre. Hawaii, 609pp.

6. Kanehisa. K., Tsumuki, H., Kawada, K. and Rustamani, M.A. 1990. Appl. Ent. Zool. 25(2):25 I - 259.

7. Lovett. J.V. 1991. Biological Agriculture and Horticulture, 8:89-100.

8. Lovett, J.V. and Liu, D.L. 1987. Proc. 4th Aust. Agron. Conf. Melbourne. August 1987, p 229.

9. Lovett, J.V. and Hoult, A.H.C. 1992. Proc. 6th Aust. Soc. Argon. Conf., Armidale. pp.426-429.

10. Mann, J.D., Steinhart, C.E. and Mudd, S.H. 1963. J. Biol. Chem. 238(2):676-681.

11. Overland. L. 1966. Amer. J. Bot. 53(5):423-432.

12. Reese, J.C. 1979. In "Herbivores: their interaction with secondary plant metabolites", (Eds.) G.A. Rosenthal and D.H. Jansen, Academic Press, New York, 309-330.

13. Sepulveda, B.A. and Corcuera, L.J. 1990. Phytochem. 29(2):465-468.