amounts than in cells of a sphacelial mycelium and are unique in that they contain ricinoleic acid ([R-(Z)]-12-hydroxy-9-octadecenoic acid). Ricinoleic acid has so far been considered an important biochemical indicator of morphological and biochemical differentiation of *C. purpurea* mycelium. Its level reaches a maximum closely before the onset of peptide alkaloid formation[7] and exceeds the mean level found during fermentation by as much as 49%[7]. High-producing strains of *C. purpurea* contain in their lipid fraction up to 40 mol% ricinoleate[5]. Total cell fatty acids show an analogous pattern during fermentation[7]. In contrast to production strains of *C. purpurea*, nonproduction strains contain no ricinoleic acid and their content of total lipids is substantially lower[5]. Biosynthesis of ricinoleic acid and ergot peptide alkaloids probably have a common regulatory mechanism[5,6]. Among the *Claviceps* sp. under study, i.e. *C. purpurea*, *C. paspali* and *C.* sp. SD-58 (probably *C. fusiformis*)[6], the relationship between mycelial differentiation and the occurrence of ricinoleic acid was proved only in *C. purpurea* producing peptide alkaloids[6]. These findings formed the basis of the hypothesis that ricinoleic acid is a specific chemotaxonomic marker of *C. purpurea* strains producing peptide alkaloids[6].

We studied the content of fatty acids in submerged cultures of six species of *Claviceps* deposited in the collection of this institute: *C. purpurea* 129/35, *C. purpurea* PLA 4, *C. fusiformis*, *C.* sp. SD-58, *C. paspali* MG-6, *C. paspali* FA (CCM F-731). Inoculation medium was T1[8]. A bioreactor (3 l) with medium CS2[8] (1.5 l) was inoculated with a 7-day-old submerged inoculum (120 ml). The paddle impeller speed in the bioreactor was 500 rpm, aeration rate 1 l/min, temperature 24 ± 1 °C. After 10-day cultivation the mycelium was collected by centrifugation and washed with water. Methyl esters of fatty acids were prepared by method No. 3 described by Marberry[9]. A mixture of the methyl esters was analyzed by gas chromatography-mass spectrometry on a HP 5995B (Hewlett Packard, USA) instrument on a capillary fused silica column under conditions described previously[10]. The content and composition of the alkaloid mixture was analyzed by high performance liquid chromatography[11].

In contrast to reports in the literature, ricinoleic acid was found in the mycelium of different *Claviceps* sp. (table) and is thus not a specific chemotaxonomic marker of *C. purpurea*, nor a specific indicator of synthesis of peptide alkaloids. In general, no significant correlation was found between the rate of production of alkaloids in different *Claviceps* sp. and the cellular level of ricinoleate. However, a valuable finding was that such a relationship was exhibited solely by *C. purpurea* strains producing peptide alkaloid and that the correlation between an increased level of ricinoleate in the mycelium and the differentiation of the mycelium to the sclerotial type was characteristic solely of the species *C. purpurea*.

Ricinoleic acid in mycelium of submerged cultures of various *Claviceps* sp. producing alkaloids

	Alkaloids Type	Production (μg/ml)	Ricinoleic acid*
C. purpurea 129/35	chano-I-, agro-, elymoclavine	1000–4000	1.90
C. purpurea PLA 4	peptides (cyclols)	7–10	3.24
C. paspali MG-6	lysergic acid α-hydroxyethylamide	20–40	0.30
C. paspali FA	lysergic acid α-hydroxyethylamide	1000	0.20
C. fusiformis	chano-I-, agro-, elymoclavine	1000–2000	4.39
C. sp. SD-58	chano-I-, agro-, elymoclavine	1000–2000	4.11

* Percentage of fatty acids mixture.

1 To whom all correspondence should be addressed.
2 Pažoutová, S., Pokorný, V., and Řeháček, Z., Can. J. Microbiol. *23* (1977) 1182.
3 Mantle, P. G., and Tonolo, A., Trans. Br. mycol. Soc. *51* (1968) 499.
4 Mantle, P. G., and Nisbet, L. J., J. gen. Microbiol. *93* (1976) 321.
5 Mantle, P. G., Moris, L. J., and Hall, S. W., Trans. Br. mycol. Soc. *53* (1969) 441.
6 Waiblinger, K., and Gröger, D., Biochem. Physiol. Pflanzen *163* (1972) 468.
7 Řeháček, Z., and Kozová, J., Folia Microbiol. *20* (1975) 112.
8 Pažoutová, S., Flieger, M., Sajdl, P., Řeháček, Z., Taisinger, J., and Bass, A., J. nat. Prod. *44* (1981) 225.
9 Mayberry, W. R., Int. J. Syst. Bacteriol. *34* (1984) 321.
10 Řezanka, T., Vokoun, J., Slavíček, J., and Podojil, M., J. Chromat. *268* (1983) 71.
11 Wurst, M., Flieger, M., and Řeháček, Z., J. Chromat. *150* (1978) 477.

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Biological properties of alkaloids. Influence of quinolizidine alkaloids and gramine on the germination and development of powdery mildew, *Erysiphe graminis* f.sp. *hordei*

C. Wippich and M. Wink

Institut für Mikrobiologie and Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, D-3300 Braunschweig (Federal Republic of Germany), 4 September 1984

Summary. The quinolizidine alkaloids sparteine, lupanine and 13-tigloyl-oxylupanine and the indole alkaloid gramine inhibited the germination of conidia of *Erysiphe graminis* f.sp. *hordei* Marchal and also the further development to appressoria. Half-maximal inhibitory concentrations of these alkaloids were 1–5 mmol/l. This result provides further evidence for a role of alkaloids as chemical defense compounds in plants.

Key words. Quinolizidine alkaloids; gramine; powdery mildew; *Erysiphe graminis;* inhibition of germination.

There is sufficient evidence to assume that most of the so-called secondary products or allelochemicals of plants are not waste products, but dynamic metabolites which are important for the biological fitness of the plants. These compounds either serve to attract pollinating or seed dispersing animals or repel and inhibit herbivores and microorganisms[1–11]. We have studied the biological properties of quinolizidine alkaloids (QA), common natural products of many Leguminosae, in this context[12–17]. We concluded that a minor function of QA in lupins is nitrogen transport and nitrogen storage[18,19], but that their major function is chemical defense. It could be shown experimentally that QA deter herbivores (mammals, insects, snails) from feeding, and

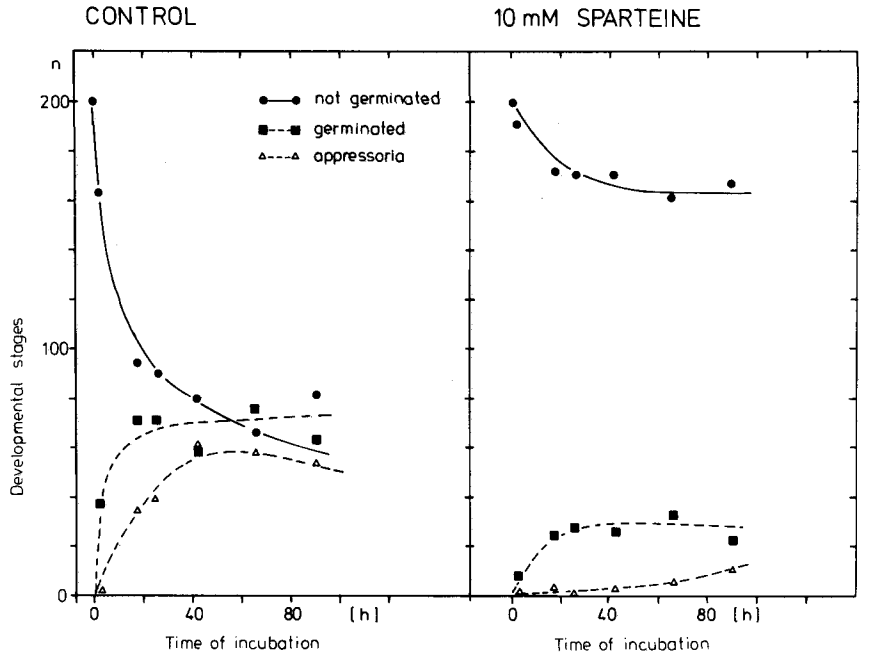

Figure 1. Time course of germination and development of *E. graminis* on microscope slides coated with agar. Infective conidia of *E. graminis* were homogeneously distributed on agar-coated slides and incubated at 17 °C in the dark. Development of the mildew was followed by light microscopy. A) control; B) Agar containing sparteine (10 mmol/l equivalent to 0.2%), adjusted to pH 6.5.

Influence of Alkaloids on germination and development of *Erysiphe graminis*

Parameter	Developmental stages (%) I $\bar{x} \pm s$	II $\bar{x} \pm s$	III $\bar{x} \pm s$	IV $\bar{x} \pm s$
A)				
Control	34.1 ± 8	17.1 ± 3	38 ± 4	10.6 ± 2
+ Lupanine				
250 µg	82.0 ± 7***	11.4 ± 4**	6.1 ± 4***	0.3 ± 0.5***
2,500 µg	92.6 ± 3***	5.0 ± 2***	2.3 ± 2***	0.1 ± 0.3***
25,000 µg	98.1 ± 3***	1.8 ± 2***	0.2 ± 0.6***	0
+ 13-Tigloyl-oxylupanine				
30 µg	86.3 ± 8***	8.2 ± 4***	3.8 ± 3***	1.7 ± 2***
315 µg	97.2 ± 3***	2.8 ± 3***	0	0
1575 µg	98.1 ± 2***	1.8 ± 2***	0.1 ± 0.3***	0
B)				
Control	36.2 ± 5	13.7 ± 2	35.8 ± 4	14.3 ± 3
+ Lupanine	71.2 ± 5***	11.8 ± 6	13.3 ± 5***	3.2 ± 2***
+ Sparteine	73.2 ± 5***	14.7 ± 3	11.0 ± 3***	1.3 ± 1***
+ Gramine	73.0 ± 8***	12.7 ± 2	12.5 ± 8***	1.5 ± 1***

Leaf segments of barley (length about 20 mm, weight 30 mg) were infected with *E. graminis* (strain GI-I) conidia and incubated in agar-filled petri dishes at 20 °C and 14 h light period for 70 h. Then the numbers of conidia, appressoria and secondary hyphae were determined by light microscopy. Prior to infection defined alkaloid solutions were applied onto the leaf segments. A) Application of the alkaloids as free bases with ethyl ether as solvent. B) Leaf segments were dipped into aqueous alkaloid solutions (alkaloid concentration 10 mmol/l) adjusted to pH 6 which contained about 0.1% Tween 20. In control experiments the leaf segments were treated with the appropriate solvents. Data represent mean values of 6 (B) and 9 (A) experiments. I, conidia not germinated; II, conidia germinated; III, appressoria; IV, secondary hyphae. Differences between treatments and controls were determined by t-test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

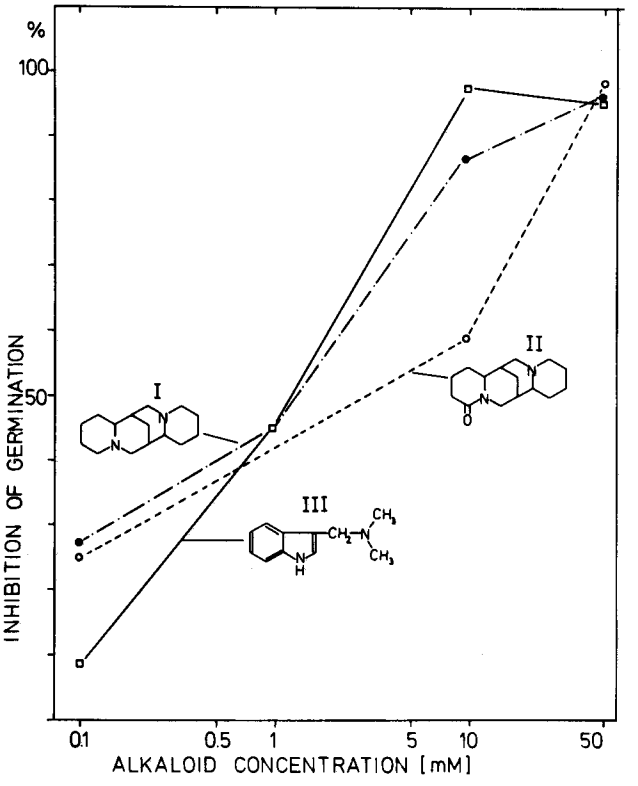

Figure 2. Influence of alkaloid concentrations on the germination of *E. graminis* conidia. Experimental conditions as in figure 1. The developmental stages were analyzed after 40 h. I, sparteine; II, lupanine; III, gramine.

inhibit the growth of bacteria and phytopathogenic fungi[15-17] and the germination of other plants[12]. Since we had investigated only a few fungal species in this context, it was necessary to enlarge our studies on fungal pathogens. An interesting candidate is the powdery mildew of cereals, *Erysiphe graminis*, a well-studied species of agricultural interest. In this communication evidence is presented that QA, such as sparteine, lupanine and 13-tigloyl-oxylupanine and the indole alkaloid gramine (a natural alkaloid of cereals), inhibit the germination of *E. graminis* f.sp. *hordei* Marchal conidia and their further development up to appressoria formation.

Primary leaves of barley (*Hordeum vulgare* cv. Golden promise), which are susceptible to infection by *Erysiphe graminis*, were either suspended in alkaloid solutions for 10 sec or alkaloids

were directly applied onto the leaves. Infective conidia of *E. graminis* were evenly dusted over the alkaloid-treated leaf segments and incubated at 20 °C and a 14-h light period for 70 h. As can be seen from the table, QA and gramine significantly suppress the germination of *E. graminis* conidia, as compared to controls. Furthermore the number of appressoria and secondary hyphae were also reduced. Both the free alkaloid bases and the salts were effective.

These results could be confirmed in another set of experiments. Microscope slides were coated with agar on which conidia readily germinate and develop into appressoria (fig. 1). However if the agar contained suitable concentrations of alkaloids, a marked inhibition of germination and further development could be observed (fig. 1b). Half-maximal inhibition was recorded at alkaloid concentrations between 1 and 5 mmol/l (equivalent to 0.02–0.1 %) (fig. 2). The inhibitory concentrations of QA against mildew are lower than those recorded against other phytopathogenic fungi[14] but are in the same range as against gram-positive bacteria[14]. The actual concentrations of QA in lupins fall in the range of 5–20 mmol/kg in leaves, of 5–40 mmol/kg in stems and of 100–200 mmol/kg in seeds and seedlings[19, 20]. Furthermore QA are concentrated in a strategically suitable position in peripheral tissues[20], especially the epidermis and subepidermal layers, which have to ward off a microbial or herbivore attack. It is thus likely that QA can account for the resistance of alkaloid-rich ('bitter') lupins against powdery mildews. Since no defense is absolute it is not surprising to find a specialized mildew, *E. pisi*, infecting lupins as well as other Leguminosae; a mildew, however, which according to preliminary observations (Wink unpublished) can degrade QA.

Our results support the assumption that QA have a broad spectrum of biotoxic properties, the mechanisms of which are still unknown. There is growing evidence that most alkaloids and other toxins, often thought to be evolved in response to specific and specialized herbivores, may be broad spectrum defenses, just like tannins and other digestion-inhibitors. A challenging question is whether we can exploit the properties of alkaloids to protect cereals against powdery mildew, either by selecting plants with a high concentration of the natural indole alkaloid of cereals, i.e. gramine, or by spraying plants with exogenous alkaloids.

1 Swain, T., A. Rev. Plant Physiol. *28* (1977) 479.
2 Levin, D. A., A. Rev. Ecol. Syst. *7* (1976) 121.
3 Levinson, H. Z., Experientia *32* (1976) 408.
4 Nahrstedt, A., Pharmazie i.u. Zeit *8* (1979) 129.
5 Zenk, M., Ber. dt. Bot. Ges. *80* (1967) 573.
6 Waller, G. R., and Nowacki, E., Alkaloid biology and metabolism in plants. Plenum Press, New York 1978.
7 Harborne, J. B., Pure appl. Chem. *49* (1977) 1403.
8 Harborne, J. B., Introduction to ecological biochemistry. Academic Press, London 1982.
9 Fraenkel, G., Science *129* (1959) 1466.
10 Stahl, E., Jena. Z. Naturw. *22* (1888) 557.
11 Whitacker, R. H., and Feeny, P. P., Science *171* (1971) 757.
12 Wink, M., Planta *158* (1983) 365.
13 Wink, M., Z. Naturforsch. *38c* (1983) 905.
14 Wink, M., Z. Naturforsch. *39c* (1984) 548.
15 Wink, M., Z. Naturforsch. *39c* (1984) 553.
16 Wink, M., Proc. 3rd Int. Lupin Conf. *325* (1984).
17 Wink, M., Hartmann, T., Witte, L., and Rheinheimer, J., Z. Naturforsch. *37c* (1982) 1081.
18 Wink, M., and Witte, L., Planta *161* (1984) 519.
19 Wink, M., Plant Evol. Syst., in press.
20 Wink, M., Heinen, H. J., Vogt, H., and Schiebel, H. M., Plant cell reports *3* (1984) 230.

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Object retrieval preferences of Norway rats: an evolutionary generalization of behavior

R. J. Wallace

Department of Zoology, Australian National University, Canberra (Australia), 6 July 1984

Summary. Prior studies have shown that object retrieval (including food hoarding) by domestic rats can occur in the context of different motivations. The present experiments show that retrieval preferences related to two motivational systems, feeding and gnawing, are ordered by object features related to either or both systems. Object retrieval in this species is apparently guided by a generalized value system. The capacity to order alternatives across specific motivational systems has evident selective advantage and may reflect a general adaptive principle.

Key words. Hoarding; economic value; motivational integration; evolutionary adaptation.

Norway rats (*Rattus norvegicus*, fam. Muridae)[1-4] and other rodent species (e.g. *Rattus fuscipes* (fam. Muridae)[5], *Mesocricetus auratus* (fam. Cricetidae)[6], *Cricetomys gambianus* (fam. Cricetidae)[7], *Neotoma cinerea* (fam. Cricetidae)[8], *Sciurus vulgaris* (fam. Sciuridae)[9], *Tamiasciurus hudsonicus* (fam. Sciuridae)[10], *Geomys bursarius* (fam. Geomyidae)[11], *Heterocephalus glaber* (fam. Bathyergidae)[12] transport a variety of objects on occasion to their homes or other sheltered locations. That transport of this kind is related to hoarding and food storage has been argued previously[5, 13, 14]. For *Rattus* species there is evidence that such transport, or 'object retrieval', can occur in association with more than one motivational system. In these animals the following properties of objects can support retrieval preferences: sweetness, wateriness, partibility, novelty[4, 5, 14-16]. The associated motivational systems are feeding, drinking, gnawing and exploring. This variety of relationships to object retrieval must have arisen from multiple selection pressures acting at command levels in the retrieval system. However, the above results do not show the degree to which retrieval preferences based on different properties of objects can be coordinated. The present experiments suggest that for domestic *R. norvegicus*, these preferences are always ordered consistently, i.e., that objects of different kinds are retrieved in conformity with a scaling process that can always be represented in one dimension. This implies that retrieval in this species is governed by a unified system that is related to more than one motivational context.

This report describes the choices of domestic rats within the behavior sequence that produces retrieval. To impose choice behavior on this sequence, a Y-maze was used for the 'hoarding alley'; rats could then choose between objects presented in the two arms by entering one arm or the other. The pair of objects presented on a single trial was drawn from a set of three or four alternatives, which allowed properties of choice based on sets of pairs to be examined. (The size of the set of all alternatives was limited because of the time required to present all possible pairs by positions and to allow subjects to approach asymptotic be-