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QUINOLIZIDINE ALKALOIDS IN *Ormosia arborea* SEEDS INHIBIT PREDATION BUT NOT HOARDING BY AGOUTIS (*Dasyprocta leporina*)

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Abstract—Quinolizidine alkaloids (QAs) are secondary compounds found in seeds of many species of plants, possibly protecting them against pathogens and seed predators. QAs were isolated from *Ormosia arborea* seeds and bioassayed against red-rumped agoutis (*Dasyprocta leporina*, Rodentia: Caviomorpha) to verify if they inhibit seed predation and food hoarding (seed dispersal). Three treatments were used: (1) seeds of *O. arborea*, (2) palatable seeds of *Mimusops coriacea* (Sapotaceae) treated with MeOH, and (3) seeds of *M. coriacea* treated with QAs dissolved in MeOH in similar concentration to that present in *O. arborea*. Palatable seeds were significantly more preyed upon than seeds treated with QAs and *Ormosia* seeds, but QAs did not influence hoarding behavior. QAs in *O. arborea* may have a strong effect in avoiding seed predation by rodents, without reducing dispersal.

Key Words—Atlantic rainforest, seed defense, seed predation, seed dispersal, Rodentia, Caviomorpha, secondary compounds, chemical defense, mimetic seeds, quinolizidine alkaloid.

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INTRODUCTION

Seed predation affects the reproductive success of higher plants. Temporal patterns of seed production (masting), seed dispersal, seed morphology such as hardness and weight, and chemical defenses are traits suggested to be related to seed defense (Janzen, 1969, 1971). Many fruits and seeds are defended by secondary metabolites toxic to insects, vertebrates, and fungi (Janzen, 1971; Janzen et al., 1986; but see Ehrlen and Eriksson, 1993).

Ormosia arborea (Vell.) Harms (Leguminosae) is a large tree that occurs both in semideciduous and evergreen forests in southern Brazil (Rudd, 1965). *O. arborea* seeds are bright red and black, and are considered as "mimetic" (van der Pijl, 1982), referring to a large group of brightly colored seeds (usually red and black, or red alone) that lack a digestible tissue reward for their putative seed dispersers. These colorful seeds are presumed to resemble arils or fleshy fruits that deceive avian frugivores. Mimetic seeds occur in several plant families, but especially in the Leguminosae (mainly in Papilionoideae and Mimosoideae) (Galetti, 2002). Although the seed dispersal systems of *Ormosia* are still unclear (Peres and van Roosmalen, 1996; Foster and Delay, 1998; Galetti, 2002), recent work has shown that *Ormosia arborea* seeds can remain for long periods on the forest floor (up to 18 months) without appreciable depletion by seed predators or pathogens (Galetti, 2002). Seeds usually fall close to the parental tree, creating a conspicuous seed shadow. The conspicuousness of the seeds on the forest floor, long exposure time, and clumped distribution make them vulnerable to potential seed predators, such as agoutis and other rodents, peccaries, and tinamous.

Ormosia seeds contain quinolizidine alkaloids (Kinghorn et al., 1988; Nasution and Kinghhorn, 1993). These substances (hereafter QAs) are a group of secondary compounds present in many seeds of legumes, that may provide protection against natural enemies (Wink, 1993a). Although the strongly deterrent action of QAs has been demonstrated in invertebrate and vertebrate metabolism (Wink, 1993a), few studies focus on the influence of alkaloids in seed predation and predator behavior (Rankin, 1978). We tested whether the presence of the QAs in *Ormosia arborea* seeds reduces seed predation or influences food hoarding by red-rumped agouti (*Dasyprocta leporina*).

METHODS AND MATERIALS

Organisms. O. arborea occurs in low density, mostly near hilltops or on steep slopes (Lorenzi, 1992). Seeds of *O. arborea* were collected in the Estação Ecológica de Caetetus, Gália, São Paulo, where a long-term study on seed dispersal of *O. arborea* is underway (Galetti, 2002). *Mimusops coriacea* (DC.) Miq. (Sapotaceae), whose seeds were used as a palatable control, is an exotic tree that occurs in Southeast Brazil, spontaneously or cultivated, mainly near beaches. The seeds are similar to *Ormosia* in weight (1.22 ± 0.16 g versus 0.99 ± 0.16 g for *Ormosia* seeds) ($N = 30$ for both), and preliminary studies showed that agoutis ate most of them; there are no recorded QAs in Sapotaceae (Wink, 1992). Seeds were collected in São Sebastião and Campinas, São Paulo. The red-rumped agouti *Dasyprocta leporina* Husson (Rodentia: Dasyproctidae) is a relatively large brown rodent, with a blackish or reddish rump (Emmons and Feer, 1997); it occurs in many different habitats, including cerrados and evergreen forests. Agoutis are mainly frugivorous and granivorous (Henry, 1999), and are important seed dispersers and predators (Forget, 1992; Peres and Baider, 1997). They are especially important seed dispersers (by hoarding seeds) for some large-seeded neotropical plants (Asquith et al., 1999).

QA Purification and Characterization. QAs were extracted from 120 seeds using the same methodology as for free bases of pyrrolizidine alkaloids (Trigo et al., 1996). Tentative identification of QAs was done by gas chromatography–mass spectrometry (GC-MS) analysis, in a HP-6890 GC system equipped with a fused silica capillary column (HP-5MS, Sigma, 5% diphenyl–95% dimethylsiloxane, 30 m × 0.25 mm × 0.25 μm) coupled to an HP-5973 mass detector, based on their RIs and mass fragmentation (Kinghorn et al., 1988; Wink et al., 1991; Wink, 1993a; 1995). The RIs (retention index) were calculated by coinjection of *n*-alkanes following van den Dool and Kratz (1963). Confirmation of molecular weight was done by PCI using methane as the ionization gas. For quantification, alkaloids were analyzed in a GC system as above, equipped with a fused silica capillary column (DB5, J&W Scientific, 5% diphenyl–95% dimethylsiloxane, 30 m × 0.25 mm × 0.25μm) coupled with an FID. Commercial sparteine was used as an external standard. The conditions of injection in both analyses were, injector temperature 250°C, detector temperature 280°C, temperature program 70–300°C at 4°C/min, split = 20:1, carrier gas He, 1ml/min.

Seed Predation and Hoarding Behavior. Experiments were carried out in the Bosque dos Jequitibás, a 10-ha urban forest park in Campinas, São Paulo (Matthes, 1980), from February 1999 to March 2000. The area has more than 60 tame agoutis (*D. leporina*) whose behavior can be observed closely. One of us (PRG) observed agoutis for 2 years in the Bosque dos Jequitibás and never saw an *O. arborea* seed in the area; so it may be assumed that these agoutis did not have any experience with these seeds.

Three different treatments were used: (1) *O. arborea* seeds, (2) *M. coriacea* seeds treated with 10 μl MeOH, and (3) *M. coriacea* seeds treated with 10 μl of QAs in MeOH, isolated from *O. arborea* seeds (concentration of QAs equivalent to the proportion found in seeds of *O. arborea*). The solvent was evaporated under an air stream before the experiments.

The bioassay protocol was to put 10 seeds of a single treatment on the forest floor; whenever an agouti found the seeds, its behavior was recorded. The number of seeds eaten, hoarded, or rejected by agoutis was determined by direct observation.

The observations were made as follows: the observer sat at least 5 m from the station and observed for at least 1 hr after an agouti arrived. Fifteen minutes after the animal left, the observation sequence was concluded. Behavior was recorded in three categories: preyed, hoarded, or rejected (seeds still intact 15 min after the agouti visit). Each treatment was replicated 15 times.

Comparison among treatments for the proportion of seed preyed upon and hoarded were done by using a nonparametric Kruskal–Wallis test, since the data do not fit a normal distribution (Zar, 1999).

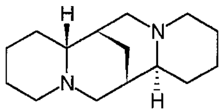
RESULTS AND DISCUSSION

Eleven QAs were found in the seeds of *O. arborea* (Table 1 and Figure 1); ormosanine (**4**) and panamine (**5**) (or its isomers) were the main alkaloids. The concentration of alkaloids found was 3.54 mg/g dry weight (about 2.95 mg per seed). QAs were not previously described in this species, but all of the known structures have been recorded in other *Ormosia* species (Kinghorn et al., 1988).

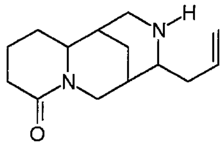
TABLE 1. QUINOLIZIDINE ALKALOIDS IN *Ormosia arborea* SEEDS

Quinolizidine alkaloids[a]	RIs	Mass fragmentation m/z (EI)[b]	Relative abundance	Concentration (mg/g dry weight)[c]
Sparteine (**1**)	1776	$[M]^+$ 234 (14), 205 (3), 193 (26), 150 (13), 137(100), 122 (15), 110 (22), 98(83)	2	0.07
Angustifoline (**2**)	2141	$[M]^+$ 234 (nd), 193 (100), 150 (13), 112 (87)	6	0.21
Lupanine (**3**)	2159	$[M]^+$ 248 (31), 193 (15), 149 (49), 136 (100), 98 (24)	1	0.04
Unknown	2462	$[M]^+$ 315 (40), 231 (42), 122 (21), 98 (55), 84 (100)	7	0.25
Ormosanine (**4**) or isomers	2487	$[M]^+$ 317 (14), 234 (32), 151 (31), 98 (51), 84 (100)	1	0.04
Ormosanine (**4**) or isomers	2498	Idem	1	0.04
Ormosanine (4) or isomers	2518	Idem	17	0.60
Ormosanine (**4**) or isomers	2543	Idem	7	0.25
Unknown	2595	$[M]^+$ 327 (7), 313 (70), 270 (34), 231 (70), 215 (42), 122 (28), 98 (100)	15	0.53
Panamine (**5**) or isomers	2618	$[M]^+$ 315 (17), 272 (7), 217 (100), 98 (43)	7	0.25
Panamine (**5**) or isomers	2679	Idem	36	1.26

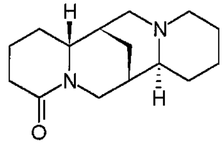
[a] Tentatively identified by comparison with RIs and mass fragmentation (Wink, 1993a; Wink et al., 1991, 1995).
[b] Molecular ion confirmed by PCI.
[c] Given by GC-FID analysis.



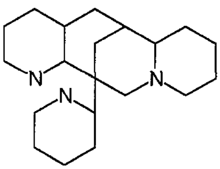
Sparteine (**1**)



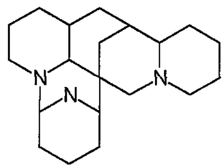
Angustifoline (**2**)



Lupanine (**3**)



Ormosanine (**4**)



Panamine (**5**)

FIG. 1. Quinolizidine alkaloids present in *Ormosia arborea* seeds. Tentatively identified by comparison with RIs and mass fragmentation (Wink, 1993a; Wink et al., 1991, 1995). Stereochemical configuration does not necessarily represent the true configuration.

Agoutis usually ate only a few *Ormosia* seeds in each station. In *O. arborea* and *M. coriacea* with QA treatments, an agouti picked up a seed, put it in its mouth, gnawed for few seconds, and then rejected it. The animal repeated this behavior a few times with the same and/or another seed. Agoutis often gnawed on only 1–4 seeds, eating pieces or breaking them without ingestion. In all gnawing events, in all treatments, agoutis bit through the seeds, killing the embryo.

Statistical analysis included 12 stations of *M. coriacea* with MeOH treatment, 6 of *Ormosia* seeds, and 11 of *M. coriacea* with QA alkaloids (other stations were excluded by absence of agouti visitation). The three treatments differ significantly in seed predation proportions (Kruskal–Wallis test, $H = 11.149$, $df = 2$, $P = 0.004$) (Figure 2A). The multiple comparison test showed *M. coriacea* seeds treated with solvent only were more preyed upon than the other treatments ($P = 0.002$ when compared to *M. coriacea* with QAs, and $P = 0.027$ to *O. arborea*); the percentage of seeds preyed upon by agoutis did not differ between *O. arborea* and *M. coriacea* treated with QAs ($P = 0.664$). The presence of QAs did not influence hoarding behavior. *M. coriacea* without QAs was hoarded in a similar frequency (0–6 seeds per station) to *O. arborea* and *M. coriacea* with QAs (Kruskal–Wallis, $H = 1.570$, $df = 2$, $P = 0.456$) (Figure 2B).

These assays demonstrate that QAs reduce the proportion of seeds preyed upon even by naive agoutis. Seed predation should be even more reduced in areas where agoutis are exposed to these unpalatable seeds for long time. In

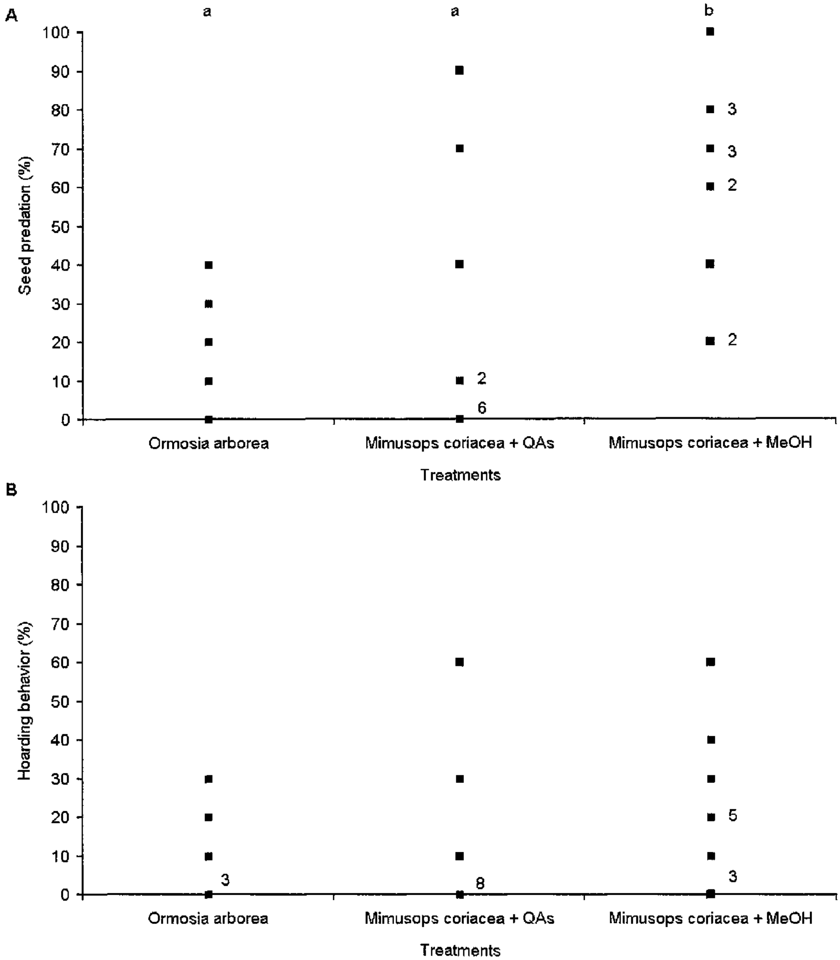


FIG. 2. Responses of *Dasyprocta leporina* in relation to *Ormosia arborea* seeds and palatable *Mimusops coriacea* seeds treated or not with QAs by (A) Seed predation; (B) Hoarding behavior. The results are shown as dispersion points, with each point being a station. The numbers on the right side of the points represent stations with similar results; if there is no number, the points represent only one station. The different letters over the dispersion data represent significant differences at the 0.05 level (Kruskal–Wallis test).

these areas, agoutis should avoid gnawing seeds with QAs by associating their features (odor, form, color) with QA presence. Wink (1993a,b) also stated that QAs have deterrence activity against vertebrates such as sheep, hares, and rabbits.

In this study, QAs did not influence the number of seeds hoarded by naive agoutis. However, after retrieval of hoarded seeds, *O. arborea* seed predation by agoutis should be deterred by QA presence. Thus, a reduction in *O. arborea* seed hoarding (and, as a consequence, seed dispersal) by agoutis should occur with their experience with QA deterrence.

Seeds such as those of *O. arborea* with long dormancy and long fruiting periods may depend on both chemical and morphological defenses in order to reduce predation and increase survival. Mechanisms that promote a reduction in the number of seeds preyed upon before or after seed dispersal, such as chemical protection, can be important for plant fitness (Janzen, 1971). The presence of secondary compounds (like QAs) in *O. arborea* seeds seems to be important in the deterrence of large rodents.

As seeds are the ultimate products of plant fitness (Janzen, 1971), it is probable that seed defense will be important, and selection, when possible, will act to improve it. We recommend comparative and experimental studies to elucidate how secondary compound evolution and seed ecology are related.

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REFERENCES

ASQUITH, N. M., TERBORGH, T., ARNOLD, A. E., and RIVEROS, C. M. 1999. The fruits the agouti ate: *Hymenaea courbaril* seed fate when its disperser is absent. *J. Trop. Ecol.* 15:229–235.

EHRLEN, J. and ERIKSSON, O. 1993. Toxicity in fleshy fruits—a non-adaptive trait. *Oikos* 66:107–113.

EMMONS, L. H. and FEER, F. 1997. Neotropical Rainforest Mammals: A Field Guide. 2nd edn. The University of Chicago Press, Chicago, Illinois.

FORGET, P.-M. 1992. Seed removal and seed fate in *Gustavia superba* (Lecythidacea). *Biotropica* 24:408–414.

FOSTER, M. S. and DELAY, L. S. 1998. Dispersal of mimetic seeds of three species of *Ormosia* (Leguminosae). *J. Trop. Ecol.* 14:389–411.

GALETTI, M. 2002. Seed dispersal of mimetic seeds: parasitism, mutualism, aposematism or exaptation? pp. 177–191, *in* D. Levey, W.R. Silva, and M. Galetti (eds.). Seed Dispersal and Frugivory: Ecology, Evolution and Conservation. CABI Publishing, New York.

HENRY, O. 1999. Frugivory and the importance of seeds in the diet of the orange-rumped agouti (*Dasyprocta leporina*) in French Guiana. *J. Trop. Ecol.* 15:291–300.

JANZEN, D. H. 1969. Seed-eaters versus seed size, number, toxicity and dispersal. *Evolution* 23:1–27.

JANZEN, D. H. 1971. Seed predation by animals. *Annu. Rev. Ecol. Syst.* 2:465–492

JANZEN, D. H., RYAN, C. A., LIENER, I. E., and PEARCE, G. 1986. Potentially defensive proteins in mature seeds of 59 species of tropical leguminosae. *J. Chem. Ecol.* 12:1469–1480.

KINGHORN, D., HUSSAIN, R. A., ROBBINS, E. F., BALANDRIN, M. F., STIRTON, C. H., and EVANS, S. V. 1988. Alkaloids of Papilionoideae. 3. Alkaloid distribution in seeds of *Ormosia, Pericopsis* and *Haplormosia*. *Phytochemistry* 27:439-444.

LORENZI, H. 1992. Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil. Editora Plantarum Ltda., Nova Odessa., São Paulo.

MATTHES, L. A. F. 1980. Composição florística, estrutura e fenologia de uma floresta residual do planalto paulista: Bosque dos Jequitibás (Campinas). MSc thesis. Universidade Estadual de Campinas, Campinas, São Paulo.

NASUTION, M. P. and KINGHORN, D. 1993. (+)-13α-4′-Hydroxytigloyloxy lupanine from *Ormosia krugii*. *Phytochemistry* 32:1603–1605.

PERES, C. A. and BAIDER, C. 1997. Seed dispersal, spatial distribution and population structure of Brazilnut trees (*Bertholletia excelsa*) in southeastern Amazonia. *J. Trop. Ecol.* 13:595–616.

PERES, C. A. and VAN ROOSMALEN, M. G. M. 1996. Avian dispersal of "mimetic seeds" of *Ormosia lignivalvis* by terrestrial granivores: Deception or mutualism? *Oikos* 75:249–258.

RANKIN, J. M. 1978. The influence of seed predation and plant competition on tree species abundances in two adjacent tropical rain forests. Ph.D. thesis. University of Michigan, Ann Arbor, Michigan.

RUDD, V. E. 1965. The American species of *Ormosia* (Leguminosae). *Contribut. US Nat. Herb.* 32:279–284.

TRIGO, J. R., BROWN K. S., JR., WITTE, L., HARTMANN, T., ERNST, L., and BARATA, L. E. S. 1996. Pyrrolizidine alkaloids: Different acquisition and use patterns in Apocynaceae and Solanaceae feeding ithomiine butterflies (Lepidoptera: Nymphalidae). *Biol. J. Linn. Soc*. 58:99–123.

VAN DEN DOOL, H. and KRATZ, P. D. 1963. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *J. Chromatogr.* 11:463–471.

VAN DER PIJL, L. 1982. Principles of Seed Dispersal in Higher Plants. 3rd Edition Springer-Verlag, Berlin.

WINK, M. 1992. The role of quinolizidine alkaloids in plant insect interactions, pp. 133–169, *in* E. A. Bernays (ed.). Insect–Plant Interactions. Vol. IV. CRC-Press, Boca Raton, Florida.

WINK, M. 1993a. Quinolizidine alkaloids, pp. 197–239, *in* P. Waterman (ed.). Methods in Plant Biochemistry, Vol. 8. Academic Press, London.

WINK, M. 1993b. Production and application of phytochemicals from an agricultural perspective, pp. 171–223, *in* T.A. van Beek and H. Breteler (eds.). Phytochemistry and Agriculture. Proceedings of the Phytochemical Society of Europe, Vol. 34. Claredon Press, Oxford.

WINK, M., MEISSNER, C., and WITTE, L. 1995. Patterns of quinolizidine alkloids in 56 species of the genus *Lupinus*. *Phytochemistry* 38:139–153.

WINK, M., MONTLLOR, C. B., BERNAYS, E. A., and WITTE, L. 1991. *Uresiphita reversalis* (Lepidoptera: Pyralidae): carrier-mediated uptake and sequestration of quinolizidine alkaloids obtained from the host plant *Teline monspessulana*. *Z. Naturforsch.* 46c:1080–1088.

ZAR, J. H. 1999. Biostatistical Analysis. 4th Edition Prentice Hall, Upper Saddle River, New Jersey.