Eötvös Loránd University

Ph.D. School of Biology
Head of Ph.D. School: Prof. Dr. Anna Erdei

Ph.D. Program of Ethology
Head of Ph.D. Program: Prof. Dr. Ádám Miklósi

Gábor Markó

Herbivore-plant interactions in Hungarian juniper forests

Outline of Ph.D. Thesis

Supervisor:

Dr. Vilmos Altbäcker
Associate Professor, DSc

Department of Ethology, Faculty of Science,
Eötvös Loránd University, Budapest,
Hungary

2012

Introduction

Herbivores consume a number of toxic plants to cover their energy and nutrient requirement especially when the food is short (Bryant et al., 1985). Therefore, the chemical quality of the available plants is fundamentally determining the survival of the herbivores. So for consumers, it is particularly important to distinguish the edible plants from the less palatable or toxic plants (Provenza et al., 1998).

Virtually all plants produce plant secondary metabolisms (PSMs) to turn their chemical quality even more unpalatability and to decrease the risk of herbivore consumption (Freeland & Janzen, 1974). The PSMs contribute to chemical protection and their mechanisms are described by the plant defence theory (Stamp, 2003). The theory attempts to explain the plant chemical responses against the tissue damage caused by animal consumers as well as the complex mechanisms of the PSMs accumulation (Ortiz et al., 2002).

Herbivore pressure may transform the vegetation structure at the landscape level. The impact of consumers mainly depend on the species composition and density (Mysterud, 2006). In source limited habitats the toxic food consumption could be quite crucial for the herbivores, because in the toxic plant species are highly abundant in these environments (Coley et al., 1985). In the Hungarian juniper-poplar shrublands the main source of local differences in the vegetation pattern are local herbivores by their seasonal consumption (Kertész et al., 1993). The main herbivores of the juniper shrubland are the European rabbit (*Oryctolagus cuniculus*) and domestic sheep (*Ovis aries*), and their juniper consumption might alter the juniper populations (Katona & Altbäcker, 2002).

The common juniper (*Juniperus communis*), as the dominant pine species of the juniper shrublands, is rich in monoterpene dominated essential oils. The terpenoid components have a very important role in the plant chemical defence mechanism and they are quite effective against the browsing herbivores. The efficiency depends on the quality and quantitiy of the essential oil, which characteristics are highly varied among sites, seasons and individuals.

Objectives

In my thesis, several investigations are presented about the herbivore-plant interactions which were carried out both in the sand dunes of juniper-poplar habitat in the Kiskunság National Park, and in the laboratory. Firstly, three studies are shown about the food choice mechanisms and juniper browsing in rabbit and sheep. Secondly, a correlative study is presented about the juniper chemical defence mechanism measuring essential oil yield influenced by many biotic and abiotic factors. Finally, we documented vegetation fragmentation due to selective grazing of wild rabbits in a natural environment.

We present studies aimed at solving the following problems:

1. The role of essential oil quality and quantity of common juniper in the herbivore browsing; short and long term consequences of the juniper browsing.
2. The effect of odor and essential oil composition on sheep juniper browsing.
3. Experimental test of naïve rabbit food choice, modified by the quality of individual plants, yield and composition of essential oils, and odors.
4. Analysis of *Juniperus communis* chemical defence via essential oil yield and its relationship with several biotic and abiotic factors.
5. Comparison of the relative importance of three vegetation shaping factors, such as herbivory, shading and allelopathy, which are expected to act at landscape level.

Materials and Methods

1. The effect of essential oils of common juniper on sheep browsing

In this study we measured the seasonal pattern of essential oil yield of *Juniperus communis* shrubs browsed by sheep. Browsed and unbrowsed junipers were randomly selected for comparing the essential oil yield. The quality of the extracted essential oils was also analysed using gas chromatography.

The effects of juniper browsing were studied in two different spatial scales. First, we documented the effect of sheep juniper browsing at Orgovány along a gradient of sheep presence which decreased toward the middle of the forest from the edge. Based on the browsing pattern the effect of the herbivore presence on the vegetation structure was estimated. Second, we measured the herbivore impact associated to the juniper at the population level. The juniper age distribution

was measured for detecting long term impacts at three, geographically isolated population with different browsing traditions. One hundred shrubs around ten randomly chosen points were quatified at each site.

2. The effects of odor and essential oil composition on sheep juniper browsing

We randomly selected heavily browsed and unbrowsed juniper shrubs in the Orgovány site to determine the essential oil composition. Twigs from the same juniper individuals were also measured by electronic-nose device, as an objective analytical tool for odor sensing pattern, to determine the complex odor-pattern in each juniper individuals. The number of essential oil components and the output variables of electronic-nose measurement were separately reduced by Principal Component Analysis (PCA). Afterwards the juniper individuals were classified based on the Principal Components (PC) of GC and Electronic-nose. The browsing status was analysed by odor and essential-oil pattern using General Linear Mixed Models. The possible role of the essential oil components in the food choice was analysed by Factor Analysis.

3. Impact of odor and essential oil on the food choice of naïve domesticated rabbits

We studied the quality-dependent juniper consumption in naïve domesticated rabbit using food choice tests in laboratory settings. We compared the within and between species effects to detect sensitivity differences among the different junipers. Firstly, rabbits could choose between twigs from two different ornamental juniper species, which were visually and chemically different. Secondly, rabbits choose from twigs either browsed or avoided by sheep. The essential oil composition and odor pattern of these twigs were already known.

The influence of essential oil yield and quality on rabbit consumption were also tested. We presented food chops treated by either a monoterpene or oxygenated monoterpene-dominated essential oils, both in low and in high dose, and measured food consumption.

The odor-based food choice was examined in naïve and experienced rabbits. The experimental animals were divided in two groups; the first group was allowed to consume juniper twigs, while the other group was fed with hay. Few weeks later untreated food pellets with odorized filter paper were presented to both groups. The scented filter papers were odorized by highly aromatic juniper essential oil as the treated food, and by neutral sesame oil as the control food. Consumption differences were measured between the differentially scented foods, and the effects of previous feeding experience.

4. The role of the essential oil yields in the chemical defence of common juniper

The essential oil content of *Juniperus communis* probably acts as a means of plant chemical defence. Therefore, the relationship between essential oil yield and other abiotic and biotic factors were investigated in a correlative study. We randomly selected juvenile and adult juniper individuals from the Bugac, Bócsa and Orgovány population and measured their morphological traits. Sex ratio of each juniper population was determined, and the yield of their essential oil was also quantified.

5. The effects of rabbit grazing on the intercanopy vegetation around the juniper patches

Factors possibly decreasing the vegetation density surrounding juniper patches were compared in the Tatárszentgyörgy juniper shrubland. We tested the impact of herbivory by comparing intercanopy grassland vegetation surrounding pairs of junipers occupied or unoccupied by rabbits. Around junipers, we estimated the vascular plant composition by traditional coenological survey and CropScan. Beyong grazing, variation in grass cover might reflect the directional impact of shading, so we compared the grass cover of the sunlit southern side to the shaded northern edge of junipers. Vascular plant cover could also be lowered by allelopathy. If this is the case, a symmetrical zone of reduced plant cover on both sides of junipers is expected. The allelopathic effect of junipers was also tested experimentally using lettuce seed bioassay in the lab.

Having found an herbivory-related impact on vascular plant cover in junipers occupied by rabbits only, their diet was analysed by microhistological pellet analysis to see if plants which have reduced cover around occupied junipers are especially common in the rabbit diet.

Results and Conclusions

1. The effect of juniper essential oils on sheep browsing

We found negative relationship between juniper browsing status and essential oil yield. The essential oil yield varied seasonally; it was lowest in summer and showed the highest level in winter. We also found significant differences between heavily browsed and unbrowsed individuals in the level of certain essential oil components; δ-3-carene and β-myrcene were present in higher concentration in the unbrowsed individuals, while the α-pinene was found to be dominant in the browsed shrubs. The potential juniper browsers, sheep and rabbit, had different seasonal presence in the shrubland. Sheep were present only in spring and fall by the traditional pastoral system, while the rabbits browsed the juniper in winter, when their food supply is relatively short. Thus,

the efficiency of the chemical defence of juniper shrubs varied among seasons as well as showed differences against the local herbivores.

We explored the spatial distribution pattern of the browsed juniper shrubs and found that the ratio of the browsed junipers decreased from the shrubland edge toward to the centre of Orgovány juniper forest, indicating that sheep were the dominant juniper browsers in the Orgovány shrubland The Bugac site instead. shows signs of rabbit browsing resulting in a senescent age distribution with low regeneration potential, while the other two sites, such as Bócsa and Orgovány, show stable age class distribution, where the individual frequency steadily decreases in the older age classes. The local herbivore species composition and their relative density could be the source of the juniper age distribution differences among sites.

2. The effects of odor and essential oil composition in juniper browsing by sheep

Browsing status *Juniperus communis* individuals was successfully classified based on the ir essential oil pattern and odor matrix. Based on their molecular size and structure, the essential oil components were grouped into tree classes: dominant monoterpenes, sesquiterpenes and other monoterpenes by factor analysis. We could also describe three main chemotypes based on the dominant monoterpenes: δ-3-carene, sabinene and α-pinene. The δ-3-carene chemotype was described first in our population in this species.

The essential oil mixture of juniper is composed of numerous terpenoid components with highly diverse physiological effects. Herbivores select for palatability based on the integrated effect of PSMs instead of sensing certain components during their foraging. Thus, the odor of plants may enable herbivores to evaluate the chemical quality of the available food. The plant odor pattern seems to reflect the PSMs composition, and can be considered as a reliable signal to herbivores.

3. The impact of juniper odor and essential oil on the food choice of naïve domestic rabbits

Naïve rabbits were able to discriminate between two juniper species and to detect small chemical differences within one species. As they also choose twigs browsed by sheep, food choice mechanisms could overlap between sheep and rabbit.

Food pellets treated with artificial essential oil mixtures were presented to naïve rabbits. Essential oil yield and/or their dose-dependent odor intensity, but not the quality were found to affect consumption, as rabbits ate smaller amount from the high dose food than from the low terpene food.

The odor significantly modified the food choice and the previous juniper experience was also important in the naïve rabbit consumption. The food treated with juniper odor was less preferred than the unscented control, and the experienced animals fed significantly lower volume from the juniper-scented pellets. The odor intensity is proportional to the aromatic toxin content. Therefore, the results could be interpreted as feeding neophobia involving an aversion against the intensive scented food containing high level essential oil.

4. The role of the essential oil yield in the chemical defence of common juniper

The essential oil of the common juniper is an effective part of their chemical defence against the herbivores. We found several factors which is associated with terpenoid yield, such as sex, vertical position of the shoots, age and *Carulaspis* infection.

The sex of individuals affects the phenology of junipers. There is a strong trade-off between chemical defence and growth during the resource allocation at the individual level; males growing taller and their essential oil yield is higher than in females. This could be explained by the sex-dependent allocation pattern; females invest more sources to produce seeds during reproduction, so they have less sources to allocate into chemical defence. The lifespan of females is shorter than that of males, which could be the consequence of the weaker chemical defence level against herbivores or other pathogen organisms. Such sex-dependent resource allocation and survival would result in an age dependent sex ratio shift at the population level, and indeed a male-biased sex ratio was found in our study sites. Our results fit to the predictions of "allocation cost of phenotypic defence subhypothesis" by Stamp (2003).

The *Juniperus communis* individuals infected by scale insects showed higher level of volatile compounds compared to the non-infected individuals. The infection-associated increase in the essential oil yield could be explained by the induction of a chemical defence mechanism. Nevertheless, the accumulated essential oil would not act on the insects, because the production and allocation of essential oils are spatially separated from the pulp sucked tissues. These results suggest the presence of an inducible defence mechanism in the common juniper.

5. The effect of rabbit grazing, shading and allelopathy on the intercanopy vegetation around juniper patches

By analyzing the possible factors behind the variation in plant cover in sand grasslands surrounding junipers, we were able to separate the effects of herbivory, shading, and allelopathy in creating bare sand rings around certain shrubs. As the grass was only open around junipers

containing rabbits, the spatial variation in the sand grass vegetation surrounding junipers could be attributed to the presence of herbivores.

We found no indication of allelopathy at the edge of junipers, even though our bioassay suggested the possibility for such interaction. The dense vegetation in the dripline of unoccupied shrubs indicated that plant-plant interaction had a negligible impact compared to grazing.

The effect of herbivory was evident in a zone of a few meters from the edge of the juniper. The decrease of the cover of certain plant species was clearly attributed to rabbits, as we found that the thes plants were eaten by the local herbivores. In this study we found that rabbits make the vegetation sparse around the occupied junipers, which implies that such rabbit-grazed bare sand rings can act as natural firebreaks by increasing large scale patchiness, thus altering the spread of fire.

References

BRYANT, J. P., REICHARDT, P. & CLAUSEN, T. 1985. Plant carbon/nutrient balance - implications for chemical defense. *Abstr Pap Am Chem S* 190:91.

COLEY, P. D., BRYANT, J. P. & CHAPIN, F. S. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895-899.

FREELAND, W. J. & JANZEN, D. H. 1974. Strategies in herbivory in mammals: the role of plant secondary compounds. *Am Nat* 110:269-289.

KATONA, K. & ALTBÄCKER, V. 2002. Diet estimation by faeces analysis: sampling optimisation for the European hare. *Folia Zool* 51:11-15.

KERTÉSZ, M., SZABÓ, J. & ALTBÄCKER, V. 1993. The Bugac rabbit project. Part I: description of the study site and vegetation map. *Abstr Bot* 17:187-196.

MYSTERUD, A. 2006. The concept of overgrazing and its role in management of large herbivores. *Wildlife Biol* 12:129-141.

ORTIZ, P. L., ARISTA, M. & TALAVERA, S. 2002. Sex ratio and reproductive effort in the dioecious *Juniperus communis* subsp *alpina* (Suter) Celak. (*Cupressaceae*) along an altitudinal gradient. *Ann Bot-London* 89:205-211.

PROVENZA, F. D., VILLALBA, J. J., CHENEY, C. D. & WERNER, S. J. 1998. Self-organization of foraging behaviour: From simplicity to complexity without goals. *Nutr Res Rev* 11:199-222.

STAMP, N. 2003. Out of the quagmire of plant defense hypotheses. *Q Rev Biol* 78:23-55.

Published papers related to the Ph.D. thesis

Published papers in peer-reviewed international scientific journals

Markó G., Novák I., Bernáth J., Altbäcker V. (2011) Both gas chromatography and an electronic nose reflect chemical polymorphism of Juniper shrubs browsed or avoided by sheep. ***Journal of Chemical Ecology*** 37(7): 705-713. **IF: 2,486**

Markó G., Ónodi G., Kertész M., Altbäcker V. (2011) Rabbit grazing as the major source of intercanopy heterogeneity in a juniper shrubland. ***Arid Land Research and Management*** 25(2): 176-193. **IF: 0,449**

Markó G., Gyuricza V., Bernáth J., Altbäcker V. (2008) Essential oil yield and composition reflect browsing damage of junipers. ***Journal of Chemical Ecology*** 34(12): 1545-1552. **IF: 2,327**

Published papers in Hungarian scientific journals

Markó G., Gyuricza V., Bernáth J., Bisztray Gy. D., Altbäcker V. (2006) A borókarágást befolyásoló tényezők és hatásuk a Kiskunsági Nemzeti Park borókásaira. ***Természetvédelmi Közlemények*** 12: 165-178.

Other papers

Szenczi P., Bánszegi O., Dúcs A., **Markó G.**, Gedeon Cs. I., Németh I., Altbäcker V. (2011) Morphology and function of communal mounds of overwintering Mound-building mice (*Mus spicilegus*). ***Journal of Mammalogy*** 92(4): 852-860. **IF: 1,541**

Gedeon Cs. I., **Markó G.**, Németh I., Nyitrai V., Altbäcker V. (2010) Nest material selection affects nest insulation quality for the European ground squirrel (*Spermophilus citellus*). ***Journal of Mammalogy*** 91(3): 636-641. **IF: 1,541**

Book chapters

Markó G., Ónodi G., Csatádi K., Németh I., Váczi O., Bernáh J., Botta-Dukát Z., Kertész M., Altbäcker V. The effects of herbivory and grazing on vegetation. In: Kovács-Láng E., Molnár E., Kröel-Dulay Gy., Barabás S. (szerk.) The KISKUN LTER: Long-term ecological research in the Kiskunság, Hungary. Vácrátót: Hungarian Academy of Sciences Institute of Ecology and Botany, 2008. pp. 61-63. (ISBN:978-963-8391-37-7)

Conferences

Gedeon Cs., **Markó G.**, Németh I., Nyitrai V., Altbäcker V. (2008): The importance of nest material selection in nest insulation quality in the European ground squirrel (*Spermophilus citellus*). Proceedings of 2nd European Ground Squirrel Meeting (EGSM). Svatý Jan pod Skalou, Csehország. Lynx. 39 (2) 346-347.

Markó G., Gyuricza V., Bernáth J., Altbäcker V. (2007) Effects of herbivore browsing on the morphology and essential oils contents of juniper shrubs; a field phenomenon. 41st Congress of the International Society for Applied Ethology, Merida, Mexikó

Szenczi P., Bánszegi O., Dúcs A, Gedeon Cs., **Markó G.**, Németh I., Altbäcker V. (2007) Position of nests and mound morphology in relation to different soils in the mound-building mouse (*Mus spicilegus*). (előadás); Xth Magyar Etológiai Társaság Kongresszusa, Göd

Szenczi P., Bánszegi O., Dúcs A, Gedeon Cs., **Markó G.**, Németh I., Altbäcker V. (2007) The mound-building mouse as a "family entrepreneur": reduced reproduction and aggression as the basics for cooperative survival. (előadás); X. Magyar Etológiai Társaság Kongresszusa, Göd

Markó G., Bernáth J., Altbäcker V. (2006) A *Juniperus* illóolajának hatása a növényevők boróka rágására a Kiskunsági Nemzeti Park Ősborókásaiban. 7. Magyar Ökológus Kongresszus, Budapest

Markó G., Bernáth J., Bisztray Gy., Altbäcker V. (2004) A legelésző állatok boróka fogyasztása a Kiskunsági Nemzeti Parkban. Proc. Magyar Etológiai Társaság IX. Kongresszusa, Göd