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BEER AS OLFATORY ATTRACTANT IN THE FIGHT AGAINST HARMFUL SLUGS *Arion lusitanicus* MABILLE 1868

PIWO JAKO ATRAKTANT W ZWALCZANIU SZKODLIWEGO ŚLIMAKA Z GATUNKU *Arion lusitanicus* MABILLE 1868

Abstract: In 2012-2013 a series of laboratory and field experiments were carried out to check out, if beers can be used as olfactory attractants in the fight against harmful slugs *Arion lusitanicus*. Six brands of lager beer were used for olfactory analysis (Goolman Premium, Harnas Jasne Pelve, Tatra Mocne, Kasztelan Niepasteryzowane, Lezajsk Niepasteryzowane, Wojak Jasny Pely). During laboratory and field tests it was evidenced that beers of all types were more attractive for slugs than water.

Keywords: *Arion lusitanicus*, beer, attractant, olfactometry

Introduction

Slugs *Arion lusitanicus* are considered to be common pests of many crop plants [1-5]. Currently, synthetic molluscicides are being used to the fight against them. However, it seems that these preparations can be extremely toxic for the environment [6]. Moreover, they are characterized by low effectiveness under high ambient humidity conditions, which are preferred by slugs [7]. *A. lusitanicus* is characterized by a high resistance to those compounds [8]. For this reason alternative ways to combat and deter these pests are being sought. For this purpose, various kinds of physical barriers [9, 10], molluscicides nematodes [6, 11, 12], and easily accessible natural and chemical repellents and attractants [5, 7, 13-17] are trying to be used with various degree of success. Attractants, used most frequently in the fight against slugs *A. lusitanicus*, are mainly the products of natural fermentation. Amongst them beers could be successfully used [18].

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The aim of the present study was to check out, whether the beer can be an attractant for slugs *A. lusitanicus*. Using indirect experimental methods, we wanted to see whether the beer brand, and, therefore, its different chemical composition, could be factors having an impact on its attractiveness for those animals.

Material and methods

Animals

Experiments were carried out on adult slugs *Arion lusitanicus* Mab. Results of field measurements refer only to one slug species *A. lusitanicus*. Other snail species were not taken into account.

Attractants

Six brands of lager beers were used as attractants:

- Goolman Premium - alcohol content of 5.0% by volume. Pasteurized beer, containing barley malt. Producer: Perla, Browary Lubelskie SA,
- Harnas Jasne Pelne - alcohol content of 6.0% by volume. Pasteurized beer, containing barley malt. Producer: Carlsberg Polska sp. z o.o.,
- Tatra Mocne - alcohol content of 7.0% by volume. Pasteurized beer, containing barley malt. Producer: Grupa Zywiec SA,
- Kasztelan Niepasteryzowane - alcohol content of 5.7% by volume. Unpasteurized beer, containing barley malt. Producer: Carlsberg Polska sp. z o.o.,
- Lezajsk Niepasteryzowane - alcohol content of 5.5% by volume. Unpasteurized beer, containing barley malt. Producer: Grupa Zywiec SA,
- Wojak Jasny Pelny - alcohol content of 6.0% by volume. Pasteurized beer, containing barley malt. Producer: Kompania Piwowarska.

Laboratory experiment

Laboratory experiments were carried out from August till September in two consecutive years 2012 and 2013, in the Institute of Applied Biotechnology and Basic Science. Six-stellar olfactometer OLF0001 (producer: Zienkiewicz Andrzej Zaklad Remontowo-Montazowy Aparatury Laboratoryjnej) was used. Synthetic air, with a purity 4.0, at a rate of 100 cm³/min, was flown through each arm of the olfactometer. Air was a carrier of a fragrance.

Two experimental procedures were used. In the first procedure, only one animal was located in the olfactometer. In the second one, six animals were simultaneously located there. Each experimental model consisted of 50 repetitions. Olfactory preference of the slug *A. lusitanicus* was tested for each analyzed beer brand. During all experiments, gas was passed over the surface of three samples of the beer. They were located in three randomly selected arms of the olfactometer. In other three arms of the olfactometer, gas was passed over the pure water. Time, which was left to animals to choose a suitable environment was 120 min. After that time, olfactory preference of studied slug was determined by observing, whether the animal chose the channel with the scent of beer, or water.

Field experiments

Field experiments were carried out at the same period of time, as laboratory tests. They included setting of 12 homemade traps, located randomly in the park area beside the Institute of Applied Biotechnology and Basic Science. They were built according to the method described by Hagnell et al [19]. A half of traps contained 125 cm³ of water and remaining six traps contained 125 cm³ of beer of analyzed brands. After three days of the experiment, slugs caught in traps were counted and their membership in the species were characterized. Experiments were repeated in each year of the study and for each brand of the beer.

Statistical analysis

Statistic analysis was performed using ANOVA (one way Tukey's test) with the use of Statistica 10.0 software. Due to the fact that during field experiments, analyzes of the olfactory preferences were made separately for each brand of beer, hence the tests were held in different weather conditions, statistical analysis of differences between the preferences of individual brands of beer by slugs was not performed.

Results

Figure 1 summarizes test results on olfactory preference in *A. lusitanicus*. Regardless of the brand of beer the animals chose environments with a scent of beer more often than with pure water. Above mentioned differences were significant.

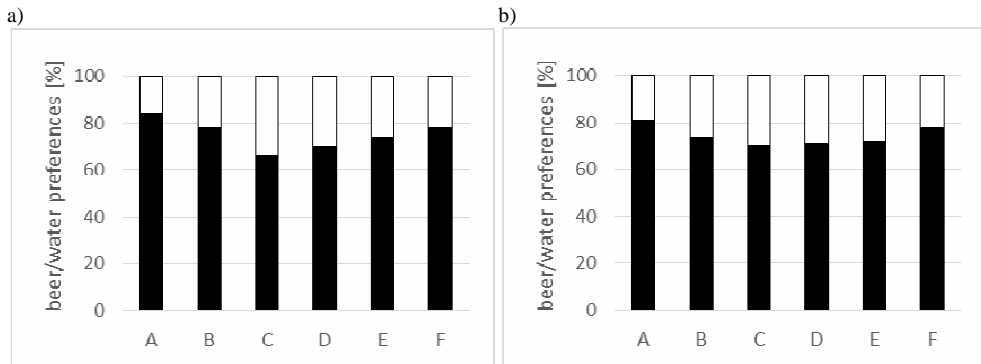


Fig. 1. A stacked column chart illustrating olfactory preferences between beer and water in *A. lusitanicus*. Laboratory tests using the olfactometer, performed on: a) isolated individuals, b) groups consisting of six individuals. A - Goolman Premium, B - Harnas Jasne Pelne, C - Tatra Mocne, D - Kasztelan Niepasteryzowane, E - Lezajsk Niepasteryzowane, F - Wojak Jasny Pelny

Regardless of whether there was a single or six animals in the olfactometer chamber, Goolman Premium was chosen most frequently among all brands of the beer studied. Conversely, Tatra Mocne was chosen least frequently (Fig. 1). A list of obtained results is given in Table 1.

Table 1

A list of statistically significant differences in the olfactory preference between subsequent beer brands by *A. lusitanicus* - laboratory tests using olfactometer

1 individual	6 individuals
A-C ($p < 0.05$)	A-B ($p < 0.05$)
C-F ($p < 0.05$)	A-C ($p < 0.05$)
	A-D ($p < 0.05$)
	A-E ($p < 0.05$)

A - Goolman Premium, B - Harnas Jasne Pelne, C - Tatra Mocne, D - Kasztelan Niepasteryzowane, E - Lezajsk Niepasteryzowane, F - Wojak Jasny Pelny

Goolman Premium beer was selected most frequently also during field experiments (83.2%) (Fig. 2), and the least frequently selected beers were Wojak Jasny Pelny (69.4%) and Kasztelan Niepasteryzowane (71.4%).

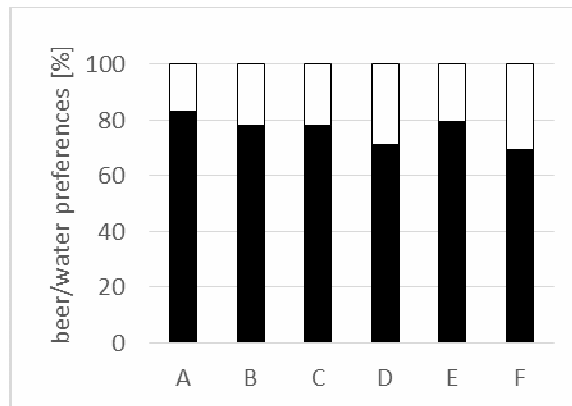


Fig. 2. A stacked column chart illustrating olfactory preferences between beer and water in *A. lusitanicus*. Field tests. A - Goolman Premium, B - Harnas Jasne Pelne, C - Tatra Mocne, D - Kasztelan Niepasteryzowane, E - Lezajsk Niepasteryzowane, F - Wojak Jasny Pelny

Table 2

Statistically significant differences in preferences between beer and water in test performed on laboratory and field tests

Laboratory tests		Field test
1 individual	6 individuals	
A ($p < 0.001$)	A ($p < 0.001$)	A ($p < 0.001$)
B ($p < 0.001$)	B ($p < 0.001$)	B ($p < 0.01$)
C ($p < 0.01$)	C ($p < 0.001$)	C ($p < 0.01$)
D ($p < 0.001$)	D ($p < 0.001$)	D ($p < 0.05$)
E ($p < 0.001$)	E ($p < 0.001$)	E ($p < 0.01$)
F ($p < 0.001$)	F ($p < 0.001$)	

A - Goolman Premium, B - Harnas Jasne Pelne, C - Tatra Mocne, D - Kasztelan Niepasteryzowane, E - Lezajsk Niepasteryzowane, F - Wojak Jasny Pelny

Discussion

Results of laboratory experiments (Fig. 1) suggest, that lager beers are effective attractants for *A. lusitanicus*. This observation was confirmed by previous studies on land snails of various species [18-21]. It has been found, that brand of beer, hence its various chemical composition [22], may influence on the attractiveness of this drink for slugs *A. lusitanicus*. Analogous reactions of snails on beers of different brands were also noted by Dankowska [18].

In laboratory tests, an additional analyzes on the influence of the group size on olfactory preferences of slugs were performed. It has been shown, that irrespective, whether there were single or six slugs in given place, their olfactory preferences were similar - the most attractive beer was Goolman Premium and the least attractive was Tatra Mocne (Fig. 1).

It was shown that both in field experiments and in laboratory experiments the beer appeared to be more attractive for *A. lusitanicus*, than water (Figs. 1 and 2). In all cases those differences were statistically significant (Table 2). Simultaneously, it was noted that the percentage of the attractiveness of various beer brands changed, comparing to results of laboratory analyzes. The most attractive for *A. lusitanicus* was Goolman Premium, while Wojak Jasny Pelný, which were on the second position among beers chosen by slugs under laboratory conditions, in field experiments appeared to be the least attractive. Those differences could be a result of changing conditions during the experiment, especially in the case of field experiments. Both ambient temperature [22, 23], chemical composition [22, 24-26] and a colour of the drink [23, 27], may have in fact a significant influence on the rate of decomposition of beer.

Since few years the slugs *A. lusitanicus* have been considered to be one of the most dangerous pests of agricultural and horticultural crops in Poland. In our country, only eight preparations against slugs, containing only three active substances: metaldehyde, calcium phosphate and iron III phosphate, are approved for use [28]. This implies the need for seek alternative methods to combat this mollusc. Our study showed, that the beer, as the cheapest and the most commonly used alcohol drink in Poland, acts as the attractant for studied slugs and can find its place in the programs of crop protection against harmful slugs of the species *A. lusitanicus*.

Conclusions

- Beer is olfactory attractant for slugs *A. lusitanicus*, dangerous pests of agricultural and horticultural crops.
- Differences in chemical composition of various beer brands can significantly influence on its attractiveness for *A. lusitanicus*.
- A number of slugs at a given place does not influence on an attractiveness of individual beer brands for *A. lusitanicus*.
- Laboratory tests conducted in accordance with established methodology are not identical with the results of tests carried out under field conditions.

References

- [1] Frank T. Slug damage and numbers of the slug pests, *Arion lusitanicus* and *Deroceras reticulatum*, in oilseed rape grown beside sown wildflower strips. *Agric Ecosyst Environ.* 1998;67:67-78. DOI: 10.1016/S0167-8809(97)00108-4.
- [2] Kozłowski J. Density of the slug *Arion lusitanicus* Mabille (Gastropoda: Pulmonata) in different microhabitats. *J Plant Prot Res.* 2000;40:158-161.
- [3] Grimm B, Paill W. Spatial distribution and home-range of the pest slug *Arion lusitanicus* (Mollusca: Pulmonata). *Acta Oecol.* 2001;22:219-227. DOI: 10.1016/S1146-609X(01)01115-8.
- [4] Kozłowski J, Kozłowski RJ. Expansion of the invasive slug species *Arion lusitanicus* Mabille, 1868 (Gastropoda: Pulmonata: Stylommatophora) and dangers to garden crops - a literature review with some new data. *Folia Malacol.* 2011;19:1-10. DOI: 10.2478/v10125-011-0005-8.
- [5] Kozłowski J, Kałuski T, Jaskulska M, Kozłowska M. Badania laboratoryjne nad ograniczeniem uszkodzeń roślin rzepaku przez ślimaka luzytańskiego (*Arion lusitanicus* Mabille). *J Plant Prot Res.* 2010;50:520-526.
- [6] Rae RG, Robertson JF, Wilson MJ. Optimization of biological (*Phasmarhabditis hermaphrodita*) and chemical (iron phosphate and metaldehyde) slug control. *Crop Prot.* 2009;28:765-773. DOI: 10.1016/j.cropro.2009.04.005.
- [7] Barone M, Frank T. Effects of plant extracts on the feeding behaviour of the slug *Arion lusitanicus*. *Ann Appl Biol.* 1999;134:341-345. DOI: 10.1111/j.1744-7348.1999.tb05274.x.
- [8] Sulzberger R. Wenn Schnecken zur Plage werden - 111 Tips zur naturgemäßen Abwehr. München: Verlag BLV.
- [9] Schüder I, Port G, Bennison J. Barriers, repellents and antifeedants for slug and snail control. *Crop Prot.* 2003;22:1033-1038. DOI: 10.1016/S0261-2194(03)00120-0.
- [10] Laznik Z, Krizaj D, Trdan S. The effectiveness of electrified fencing using copper electrodes for slug (*Airon* spp.) control with direct electric current and voltage. *Span J Agric Res.* 2011;9:894-900. DOI: 10.5424/sjar/20110903-412-10.
- [11] Speiser B, Zaller JG, Neudecker A. Size-specific susceptibility of the pest slugs *Deroceras reticulatum* and *Arion lusitanicus* to the nematode biocontrol agent *Phasmarhabditis hermaphrodita*. *BioControl.* 2001;46:311-320. DOI: 10.1023/A:1011469730322.
- [12] Iglesias J, Castillejo J, Castro R. Field test using the nematode *Phasmarhabditis hermaphrodita* for biocontrol of slugs in Spain. *Biocontrol Sci Technol.* 2001;11:93-98. DOI: 10.1080/09583150020029772.
- [13] Frank T, Friedli J. Laboratory food choice trials to explore the potential of common weeds to reduce slug feeding on oilseed rape. *Biol Agric Hortic.* 1999;7:19-29. DOI: 10.1080/01448765.1999.9754821.
- [14] Frank T, Biert K, Speiser B. Feeding deterrent effect of carvone, a compound from caraway seeds, on the slug *Arion lusitanicus*. *Ann Appl Biol.* 2002;141:1-8. DOI: 10.1111/j.1744-7348.2002.tb00200.x.
- [15] Kozłowski J, Kozłowska M. Food preferences of *deroceras reticulatum*, *arion lusitanicus* and *arion rufus* for various medicinal herbs and oilseed rape. *J Plant Prot Res.* 2004;44:239-249.
- [16] Kozłowski J, Kozłowska M. Differences in acceptability of herb plants and oilseed rape for slugs (*A. lusitanicus*, *A. rufus* and *D. reticulatum*) in food choice tests. *J Plant Prot Res.* 2008;48:461-474.
- [17] Pisarek M. Oddziaływanie wodnych wyciągów i naparów z korzeni wybranych roślin zielarskich na zerwanie ślimaków nagich z rodzaju *Arion*. *Prog Plant Prot.* 2006;46:334-337.
- [18] Dankowska E. Effectiveness of beer traps and molluscicides as means as Gastropods control. *Folia Malacol.* 2011;19:273-275. DOI: 10.2478/v10125-011-0012-9.
- [19] Hagnell J, Schander C, Nilsson M, Ragnarsson J, Valstar H, Wollkopf AM, et al. How to trap a slug: Commercial versus homemade slug traps. *Crop Prot.* 2006;25:212-215. DOI: 10.1016/j.cropro.2005.04.008.
- [20] Cranshaw WS. Attractiveness of beer and fermentation products to the gray garden slug, *Agriolimax reticulatum* (Muller) (Mollusca: Limacidae). *Colo St Tech Bull.* 1997;97:1-7.
- [21] Jankowska B, Wilk E. Effectiveness of different baits for slug catching in gardens and organic farms. *Prog Plant Prot.* 2009;49:894-899.
- [22] Vanderhaegen B, Delvaux F, Daenen L, Verachtert H, Delvaux FR. Aging characteristics of different beer types. *Food Chem.* 2007;103:404-412. DOI: 10.1016/j.foodchem.2006.07.062.
- [23] Rodríguez-Bencomo JJ, Muñoz-González C, Martín-Álvarez PJ, Lázaro E, Mancebo R, Castañé X, et al. Optimization of a HS-SPME-GC-MS Procedure for beer volatile profiling using response surface methodology: Application to follow aroma stability of beers under different storage conditions. *Food Anal Methods.* 2012;5:1386-1397. DOI: 10.1007/s12161-012-9390-x.
- [24] Perpète P, Collin S. Influence of beer ethanol content on the wort flavour perception. *Food Chem.* 2000;71:379-385. DOI: 10.1016/S0308-8146(00)00179-5.

- [25] Guido LF, Curto A, Boivin P, Benismail N, Gonçalves C, Barros AA. Predicting the organoleptic stability of beer from chemical data using multivariate analysis. *Eur Food Res Technol.* 2007;226:57-62. DOI: 10.1007/s00217-006-0508-5.
- [26] Tian JY. Determination of several flavours in beer with headspace sampling-gas chromatography. *Food Chem.* 2010;123:1318-1321. DOI: 10.1016/j.foodchem.2010.06.013.
- [27] Suárez AF, Kunz T, Rodríguez NC, MacKinlay J, Hughes P, Methner F. Impact of colour adjustment on flavour stability of pale lager beers with a range of distinct colouring agents. *Food Chem.* 2011;125:850-859. DOI: 10.1016/j.foodchem.2010.08.070.
- [28] Wyszukiwarka środków ochrony roślin <http://www.minrol.gov.pl/pol/Informacje-branzowe/Wyszukiwarka-srodkow-ochrony-roslin>. Access: 09.12.2014.

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Abstrakt: W latach 2012-2013 w warunkach laboratoryjnych i polowych przeprowadzono cykl eksperymentów mających na celu sprawdzenie, czy piwo stanowi atraktant, mogący znaleźć zastosowanie w walce ze szkodliwym ślimakiem z gatunku *Arion lusitanicus* Mab. W badaniach preferencji węchowych wykorzystano sześć piw typu lager (Goolman Premium, Harnaś Jasne Pełne, Tatra Mocne, Kasztelan Niepasteryzowane, Leżajsk Niepasteryzowane, Wojak Jasny Pełny). Zarówno podczas testów laboratoryjnych, jak i terenowych wykazano, że dla osobników *A. lusitanicus* piwa wszystkich badanych marek okazały się bardziej atrakcyjne od wody.

Słowa kluczowe: *Arion lusitanicus*, piwo, atraktant, olfaktometria