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APPLICATION OF GRAIN BAITS TO CONTROL COMMON VOLE *MICROTUS ARVALIS* (PALLAS, 1778) IN ALFALFA CROPS, SERBIA

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Abstract - In order to compare the efficacies of conventional (cholecalciferol and bromadiolone) and new (sodium selenite) rodenticides, applied in the grain bait formulation on the whole-grain of wheat (*Triticum aestivum*) and triticale (*Triticasecale*) in alfalfa crops, experiments were conducted at two sites near Belgrade, Serbia, in the spring of 2009, using a standard EPPO method. The presence of rodent populations, their spatial distribution and density indices were evaluated by pretreatment census and rodenticide efficacy by counting active holes, 14 and 28 days after treatment. The average *Microtus arvalis* numbers of 158/ha and 184/ha were found to cause 7.4% and 9.6% alfalfa green biomass yield decreases, respectively. Twenty-eight days after treatment, the average efficacy of grain bait formulation (on wheat and triticale grains) of sodium selenite and cholecalciferol was 81%, while bromadiolone which had a higher efficiency, 85%, in the control of the common vole in alfalfa crops. The analysis of variance (ANOVA) showed that the origin of active substances, bases and associated interactions a.s x based on the efficacy-investigated grain baits did not have a statistically significant impact on the expression efficiency of the tested baits. Triticale grains can be used as carriers of active substances, sodium selenite, cholecalciferol or bromadiolone in preparation baits. Control of *M. arvalis* with the new rodenticide, sodium selenite, gave efficacy results about equal to that of cholecalciferol and bromadiolone and, therefore, provided a possible alternative rodenticide for vole control in alfalfa.

Key words: Sodium selenite, bromadiolone, cholecalciferol, triticale, *Microtus arvalis, Serbia*

INTRODUCTION

In Serbia, several rodent species inhabit and cause yield losses in alfalfa crops simultaneously, primarily the common vole *Microtus arvalis* (Pallas, 1778), striped field mouse *Apodemus agrarius* (Pallas, 1771) and hamster *Cricetus cricetus* (Linnaeus, 1758) (Ružić, 1971; Jokić et al., 2007, 2010; Vukša et al., 2009). Because of its exceptional adaptability to different environmental conditions, and the yield losses to plant production it causes, common vole has become the most important species of small rodents (Mackin-Rogalska, 1981; Jacob, 2003). Voles prefer the plant parts with high protein content (White, 1978; Bergeron and Jodoin, 1987; Hartley et al., 1995), and their need for food rich in protein becomes particularly pronounced in the winter, when the green part of plants are unavailable (Hammond and Wunder. 1991). Inadequate nutritional composition of food affects the general vitality, reproducibility and competitiveness among individuals (Hansson, 1971; Dienske, 1979). Over the years, the multiplication of the common vole, which is cyclic in character and usually occurs every three to four years (Ružić, 1983), in the cultivated plants, especially alfalfa, causes significant yield losses which may rise up to 90% (Tertil, 1977; Sterner et al., 1996).

Although various agricultural practices are able to reduce their numbers (Jacob, 2003) chemicals are still the main method of controlling rodent pests or maintaining their numbers at an acceptable level (Endepols, 2002; Sayed and Lynwood, 2002). A wide selection of rodenticide products with different chemical compositions, modes of action and toxicity levels have been used for the purpose in Serbia, as well as worldwide (Tomlin, 2006; Janjić and Elezović, 2008). In contrast to bromadiolone and cholecalciferol, in use from the 1970s as rodenticides and about which there is plenty of available information (Greaves et al., 1974; Grand, 1976; Marsh, 1977; Meehan, 1978, 1984; Bai et al., 1978; Gill and Redfern, 1980; Grolleau, 1983; Marshall, 1984; Lund, 1988; Šajti et al., 1989; Miljanović and Šajti, 1991; Hrgović et al., 1991, Kataranovski et al., 1994a, 1994b, 1999; Kataranovski and Kataranovski, 1997; Jurček et al., 1999; Husinec et al., 2002), the introduction of sodium selenite as a rodenticide was initiated by the company Ciklonizacija a.d., Novi Sad, Serbia, at the beginning of the 21st century. Selenium is widely distributed in soils, water, air, vegetation, and foods (Adriano, 1986; Cutter, 1989; Johnsson, 1991). It occurs most frequently in the form of sodium selenite (ATSDR, 2003). The biological importance of selenium is reflected in the fact that it is essential for humans and animals on the one hand, and toxic when in high concentration on the other (Rayman, 2000). The toxic effects of selenite may involve the formation of selenotrisulfides in the active sites of enzymes (Frenkel and Falvey, 1988, 1989). According to the research of V. Jaćević (unpublished), the LD_{50} value of sodium selenite (anhydrous, pure min 99%) in *per os* applications was 8.9 mg/kg and 11.2 mg/kg for male and female Swiss-Webster mice. Because the relationship and the number of harmful effects of rodents is a prerequisite for effective control, in the present study we aimed to determine the abundance and harmfulness of common vole in the vegetation period of alfalfa. As a widely available and important dietary element for the majority of rodent species, cereals are commonly used as base materials for rodenticide baits (Buckle, 1994). Using grain baits of different kind and base for the control of common vole in alfalfa crops, we wanted to determine the impact of active substances, bases and associated interactions a.s x base on the efficacy the investigated grain baits, and to assess whether further use of the sodium selenite rodenticide would be justified in comparison with conventional rodenticides such as cholecalciferol and bromadiolone. This research will provide information about the possibility of protecting alfalfa crops from the common vole, using sodium selenite, as well as acceptable solutions in terms of improving rodent control programs in alfalfa. In addition, we will obtain information about the possibility of using using triticale, which is a hybrid of wheat and rye, as a basis in baits of the active ingredients of bromadiolone, cholecalciferol and sodium selenite.

MATERIALS AND METHODS

Study areas

Trials were set up in alfalfa crops at two sites, Belegiš and Stari Tamiš, near Belgrade. In keeping with recommendations made by Ružić et al. (1983), the experiment was set up during February-March of 2009. The region has a moderate continental climate with warm, dry summers, and cold, snowy winters, the average annual temperature being 10.9°C and precipitation 540-820 mm.

Belegiš (44°50'N, 20°17'E) is located northwest of Belgrade, on the left bank of the Danube River. Alfalfa was cultivated on 18 ha and the field was surrounded by other plots of field crops (maize and sunflower) in three directions, and a local road to the west.

Stari Tamiš (44°51'N, 20°47'E) is situated on the right bank of the Danube, east of Belgrade. Its alfalfa field was cultivated on 45 ha and was surrounded by a canal in three directions and a local road to the south.

Evaluation of the structure and destructiveness of common vole

Using Longworth (Penlon Ltd., Abingdon, UK) live-catch traps, we conducted a pretreatment census and determined the composition and spatial distribution of populations of small rodent species in alfalfa crops and peripheral areas. Baits used in Longworth live traps were made with a mixture of fried bacon, onion, and bread (Vukićević-Radić et al., 2005). The traps were placed near active holes and the frequent runs to stimulate the success of catching, inspected at 12 h intervals, i.e. in the morning and evening, over a period of three successive days of catching.

The impact of common vole on total alfalfa green biomass yield was evaluated in May, shortly before harvest, based on the difference in alfalfa yields sampled from untreated protected and unprotected plots and dried at 60°C for 48 h (Johnson-Nistler et al., 2005). The protected area of this method was one in which a steel wire mesh was used to keep rodents away.

Experimental plots and rodenticides applied

The trials in alfalfa crops were conducted according to the standard EPPO method (1999), i.e. by applying a completely randomized trial design with four replicates in an area where the presence of common vole was established based on the appearance of active holes and catching results, at a distance of at least 30-50 m from the nearest site of other rodent species. The plot size depended on the spatial distribution of voles active holes, and was not less than 400 m^2. As the home range of common vole is known to be 145 m^2 in alfalfa (Mackin Rogalska, 1981), the chosen distance was sufficient to prevent movement of voles from plot to plot. Untreated plots, at least 50 m away from the treated ones and from sites of other rodent species, were selected as control plots.

Baits were prepared by applying active ingredients: sodium selenite, 0.1% (Alfa Aesar, France); bromadiolone, 0.005% (Duochem d.o.o., Serbia) and cholecalciferol, 0.075% (Lohmann Animal Health, Germany) on the grains of wheat and triticale. Also, based on the findings of other authors (Buckle, 1994; Marsh, 1988) and our previous studies (unpublished), with the aim of increasing the attractiveness and color of the baits we have added sugar (0.37%), chocolate (0.37%), molasses, sunflower oil and rhodamine B.

One day before treatment and assessment of each control, all holes were counted in the trial areas and refilled with soil. The next day, 10-15 g of rodenticides was applied to each newly opened (active) hole and the holes were again refilled. The treatment rate was in accordance with the manufacturer's recommendations of baits that contain the same active ingredient. To check the efficacy of sodium selenite and compare it to the other active ingredients tested, based on the number of active holes, effectiveness was evaluated 14 and 28 days after treatment.

During the trial, the alfalfa was three years old and in the overwintering stage, height tillers 10-15 cm. No other pesticide or fertilizer had been used in the trial plots for 12 months before or during the trial.

Computations and statistical analyses

Population density index (I) and percentage of rodents were calculated from the numbers and species of individuals caught, using the method described by Heroldova et al. (2007). Rodenticide efficacy was computed using Henderson-Tilton's formula (Henderson and Tilton, 1955), and the resulting data was transformed using log(x+1) before analysis. Analysis of variance (ANOVA) and Fisher's LSD test were used to compare the efficacy levels of the rodenticides applied for each site separately, and the impact of active substances, bases and associated interactions a.i x base on the efficacy-investigated grain baits. Untransformed means and standard errors are shown in the Tables. Student's t-test was applied to compare the significance of differences between the average dry weight yields of alfalfa and to compare the significance impact of wheat and triticale grain on the efficiency of the tested active substances. In all

Table 1. Small rodent species.

Species	Belegiš		Stari Tamiš	
	Representation[1] (%)	Index[2]	Representation (%)	Index
Microtus arvalis	64.0	9.3	69.0	9.7
Apodemus agrarius	9.0	1.3	-	-
Mus spicilegus	27.0	4.0	31.0	4.3

[1] percentage of each species in total number of animals captured at each site, including border areas
[2] index of abundance

Table 2. ANOVA parameters for main effects and associated interactions of active substances and bases on efficacy levels in controlling common vole in alfalfa 14 and 28 days after the treatment.

Main effect	df	Belegiš		Stari Tamiš	
		F	P	F	P
		14 day after the treatment			
Intercept	1	1071,26	< 0.05	725,02	< 0.05
A.i.	2	3,09	> 0.05	2,78	> 0.05
Base	1	1,88	> 0.05	0,38	> 0.05
A.i. x base	2	0,08	> 0.05	2,11	> 0.05
		28 day after the treatment			
Intercept	1	203,13	< 0.05	165,80	< 0.05
A.i.	2	0,63	> 0.05	0,98	> 0.05
Base	1	2,21	> 0.05	2,32	> 0.05
A.i. x base	2	2,78	> 0.05	0,37	> 0.05

Total df=18

analyses, the level of significance was at least $P<0.05$ (Sokal and Rohlf, 1995).

RESULTS

Presence of rodents and the destructiveness of the common vole

Three small rodent species were detected at the Belegiš site: common vole, striped field mouse *Apodemus agrarius* and steppe- or mound-building mouse *Mus spicilegus*, while the common vole and steppe mouse were found at Stari Tamiš (Table 1).

The average economic loss caused by common vole feeding and other activities amounted to 7.4% at Belegiš and 9.6% at Stari Tamiš. On both sites, a statistically significant impact on the economic losses in alfalfa was recorded. At Belegiš, the average alfalfa yield decreased from the 1537 kg ha^{-1} in the protected area to 1424 kg ha^{-1} in the unprotected area ($P<0.05$; $n=4$). Damage caused by the common vole in parts of the alfalfa crops ranged from 3% (minimum) to 12% (maximum). The average economic loss caused by the common vole at Stari Tamiš reached 9.6%. The average alfalfa yield of 1953 kg ha^{-1} in the protected area decreased to 1765 kg ha^{-1} in the unprotected

Table 3. Average number (MS) of active holes and standard error (SE) of common vole in alfalfa crop and rodenticide efficacies (Ef) 14 and 28 days from the beginning of the trial at Belegiš and Stari Tamiš.

Rodenticide	A[1]		A+14			A+28		
	MS	SE	MS	SE	Ef (%)	MS	SE	Ef (%)
				Belegiš				
Sodium selenite (wheat)	31.50	1.75	21.50	1.94	38.28b[2] (34.28-48.05)	6.50	0.65	82.08ab (78.16-86.78)
Sodium selenite (triticale)	27.25	1.79	16.25	1.31	44.60ab (33.88-56.32)	7.00	1.47	76.74ab (64.94-89.09)
Cholecalciferol (wheat)	31.25	2.86	18.25	0.75	45.02ab (33.37-59.33)	8.75	0.95	75.46b (65.93-79.44)
Cholecalciferol (triticale)	38.75	2.14	22.00	2.67	48.33ab (38.39-57.77)	6.25	1.60	86.43a (78.57-90.68)
Bromadiolone (wheat)	25.00	1.83	13.75	1.93	50.18ab (39.22-60.47)	6.00	1.47	80.09ab (73.75-88.82)
Bromadiolone (triticale)	26.25	1.93	12.50	1.66	56.65a (48.73-65.03)	4.00	0.71	86.66a (78.38-91.14)
Control	34.00	2.04	37.25	2.72	-	39.50	3.06	-
				Stari Tamiš				
Sodium selenite (wheat)	43.75	3.40	24.00	2.97	45.61c (39.58-56.75)	9.00	1.22	79.81a (71.19-87.37)
Sodium selenite (triticale)	42.25	2.02	20.25	1.37	52.21c (50.00-55.26)	6.25	0.85	86.09a (82.52-90.64)
Cholecalciferol (wheat)	33.50	2.02	14.50	2.33	57.39bc (48.64-66.66)	6.50	1.19	81.13a (70.43-89.45)
Cholecalciferol (triticale)	36.75	2.57	18.75	1.70	48.12c (33.33-60.97)	6.75	1.11	81.98a (69.33-88.58)
Bromadiolone (wheat)	40.25	1.93	18.00	2.74	55.00ab (45.45-74.42)	7.00	1.08	83.48a (74.44-87.68)
Bromadiolone (triticale)	44.75	2.21	15.25	1.44	65.19a (52.50-73.47)	5.25	0.48	88.95a (85.76-91.08)
Control	43.00	2.04	43.00	2.04	-	46.00	2.42	-

[1] A; A+14; A+28: Number of active holes at the beginning of the experiment and after 14 and 28 days
[2] Difference between values marked with the same letters per column per site have no statistical significance (Fisher´s LSD test; $P<0.05$)

plots ($P<0.0439$; n=4). The harmful effect of rodents in some parts of alfalfa crops ranged between 5% (minimum) and 10% (maximum).

Impact of active ingredients, bases and associated interactions a.i x base on the efficacy of the investigated grain baits

The analysis of variance showed that the efficiency of the grain baits in the control of common vole in alfalfa crops 14 and 28 days after the experiment began did not significantly depend on the kind of active ingredients, and the highest non-significant level was recorded at Belegiš ($F_{2,18}$=0.63; P>0.05), 28 day after treatment (Table 2).

In addition, we found that the grains of wheat and triticale, used as the bases of active ingredients, had no statistically significance for the expression efficiency of the tested baits, and the highest non-significant level was recorded at Stari Tamiš ($F_{2,18}$=0.38; P>0.05), 14 day after treatment. Associated interactions a.i x base on efficacy-investigated grain baits did not show statistically significant impact, and the highest non-significant level was recorded at Belegiš ($F_{2,18}$=0.08; P>0.05), 14 day after treatment.

Comparison of the efficacy of the tested baits

After 14 days, the lowest efficacy of 38.3% was displayed by sodium selenite on a wheat base at Belegiš, while bromadiolone on a triticale base was most effective at Stari Tamiš, 65.2%. Using analysis of variance, we found that the carriers of active substances, wheat and triticale, had no influence on the expression efficiency of baits in this period: the average efficiency of sodium selenite, cholecalciferol and bromadiolone was 41.4%, 46.7% and 53.4%, respectively at Belegiš and 48.9%, 52.7% and 60.1%, respectively, at Stari Tamiš.

At Belegiš, 28 days after the experiment began, the lowest efficacy was that of cholecalciferol on a wheat base at 75.5%, while bromadiolone on a triticale base was the most effective at 86.6%. At Stari Tamiš, the lowest efficacy of 79.8% was displayed by sodium selenite on a wheat base, while bromadiolone on a triticale base was most effective at 88.9%. In this period, the average efficacy of sodium selenite, cholecalciferol and bromadiolone at the Belegiš site was 79.4%, 80.9% and 83.3%, respectively, and 82.9%, 81.5% and 86.2%, respectively, at Stari Tamiš.

DISCUSSION

In the autumn-winter period, due to the lack of green plant mass, the common vole primarily feeds on roots (Ružić, 1971), while in the vegetation, cutting the tillers off caused significant damage to alfalfa (Ružić, 1983). The average common vole populations of 158/ha at Belegiš and 184/ha at Stari Tamiš caused 7.4% and 9.6% alfalfa green biomass yield decreases, respectively. These results are consistent with data reported by Babinska-Werka (1979) in a study of the relationship between common vole numbers and alfalfa losses, finding that an average vole population of 145-220/ha caused 8.7% damage to the alfalfa crop. In our earlier studies (Jokić et al., 2010), the average common vole abundance of 250-285/ha caused 15.6-21.2% alfalfa green biomass yield decreases.

Our data reveal no significant statistical difference between the efficacies of baits prepared on wheat and triticale bases as active ingredients. Based on the analysis, the new rodenticide sodium selenite had efficacy results about equal to those of cholecalciferol and bromadiolone and, consequently, provides a possible alternative rodenticide for vole control in alfalfa crops. Data on the efficacy of bromadiolone and cholecalciferol in controlling common vole in alfalfa are generally scarce, but they are altogether missing for sodium selenite. Results of our examination, 28 days after treatment, show that the average efficacy in controlling common vole in alfalfa crops of sodium selenite on wheat and triticale bases was about 81%. In the same period, the average efficiency of bromadiolone on both base was 85.0% , similar to the efficiency of the control of mixed populations of the common vole and the field mouse at 84% (Jokić et al., 2007), or the results obtained in the control of the common vole alone at 82.0% (Jokić et al., 2010). At the end of the experiment, the effectiveness of the two bases of cholecalciferol was about 81%. These results are consistent with our previous research, where the efficiency of cholecalciferol in the control of common vole in alfalfa crops was 78.0% (Jokić et al., 2010), and 89.0-95.0% (Vukša and Đedović, 2004) and 89.0% (Jokić et al., 2007) in the control of mixed populations of the common vole and field mouse in alfalfa crops.

The efficacy of a rodenticide bait may be dependent on the quality of the bait base rather than on the active ingredient (Lund 1988; Prakash et al., 2003). In our experiment, with the exception of one case with cholecalciferol after 28 days, we found that triticale grain can be used as the carrier of active substances, sodium selenite, cholecalciferol or bromadiolone in preparation baits to control common vole in alfalfa crops.

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