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ASSESSMENT OF TOXIC BAIT EFFICACY IN FIELD TRIALS BY COUNTS OF BURROW OPENINGS

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ABSTRACT: The Levant vole, *Microtus guentheri*, is a pest of most of the field crops in Israel. It lives in gallery systems, the openings of which are clearly visible, and is active above ground mainly at night. Its activity was assessed by plugging the burrow openings (holes) and, after three nights, counting the number of reopened holes. The efficacy of control by whole wheat toxic baits was determined by counting the number of reopened holes before and after the treatment in random squares. Three chemicals were tested: sodium fluoroacetate, zinc phosphide and brodifacoum. The bait was applied either by hand, airplane, or centrifugal spreader. The period between treatment and post-treatment count was one week for the two acute rodenticides, and at least two weeks for the anticoagulant baits. Satisfactory results were obtained: 1) by a 2% zinc phosphide bait, with 0.7 g inserted manually into the voles' holes, or distributed at rates between 4.2 to 12.2 kg/ha; 2) by a 0.05% sodium fluoroacetate bait, with 0.2 g being inserted manually into the holes, or distributed at rates of 1.6 to 4.0 kg/ha; and 3) by 0.005% brodifacoum bait, with 3 g being inserted manually into the burrows, or broadcast at rates of 5.8 and 8.7 kg/ha.

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INTRODUCTION

The Levant vole, *Microtus guentheri* (Danford and Alston) (Microtidae), is the main field rodent species involved in mass depredation of field crops and orchards in Israel (Bodenheimer 1949; Moran and Keidar 1993; Wolf 1977). As the distribution of this species is limited to the Balkans, northeastern Asia, and Cyrenaica (Gromov and Polyakov 1992), the control methods and rodenticides used against voles in other parts of the world has to be checked carefully before being adopted for use under the conditions in Israel.

The Levant vole is distributed in heavy soils. It is sociable, living in family colonies, each consisting of a network of galleries, nest chambers and food chambers. The burrows, 5 cm in diameter, consist each of three to eight galleries, which lead into one breeding chamber and one or two storage rooms. Sometimes several holes lead to the same gallery. Between the holes, well-trodden runs may be perceived (Bodenheimer 1949). This structure of the vole burrows enables one to use the reopened holes (RH) count method recommended in the EPPO guidelines for *Microtus arvalis* (EPPO 1975). These guidelines recommend a plot of at least 1 ha with four random squares, 10 x 10 m each, plugging the holes, counting the RH after one week, plugging them again, and counting the RH on the next day. Other authors used to wait only one day after the burrow plugging before the counting and treatment (Saxena and Bhasin 1990). In Israel, the number of holes reopened 72 h after being plugged was taken as an index of the number of voles in 0.1 ha (Bodenheimer 1949) or in 0.2 ha (Naftali and Wolf 1964) single squares.

The Levant vole is mainly nocturnal. Food is collected at night and dragged into the burrows for ongoing consumption and for storage. The main stores are grains or seeds (Bodenheimer 1949). Serious economic damage is caused to cereals, fodder crops and certain vegetables. In addition, damage is inflicted on young trees by gnawing the bark and the roots of saplings. Wheat, barley, clover, alfalfa, and other field crops are depredated immediately after germination, green plants are consumed through the winter and in the autumn, ears are cut and grain stored. Lack of control measures, or even faulty timing of control, may result in losses exceeding 50% of the yield of cereal and fodder crops (Wolf 1977). Because of the sociability of the voles, their colonies often create distinct cuts in the crop, 2 to 10 m in diameter. Since 1964, voles in Israel have been controlled by a whole wheat bait treated with 0.2% fluoroacetamide (Naftali and Wolf 1964), inserted by hand into the burrow entrances with common teaspoons, or spread by mechanical devices (tractor-mounted centrifugal spreader, airplane, or hand-carried spreader) at the rate of 2 to 3 kg/ha (Wolf 1977). The bait grains are dyed brilliant green, as a safety measure, and to deter birds (Kalmbach 1943) and humans.

The use of fluoroacetamide for the control of field rodents is somewhat controversial in Israel. There are findings on poisoning of game birds by consumption of fluoroacetamide bait spread for field rodents control (Shlosberg et al. 1975). On the other hand, there are no adequate field or laboratory records, confirming that the treatment by the fluoroacetamide baits at the recommended and registered rates is dangerous to non-target species. Nevertheless, laboratory tests were performed to investigate the possibility of replacing the active ingredient by other toxicants. We chose the acute rodenticides sodium fluoroacetate (henceforth abbreviated to SFA), and zinc phosphide (ZP) (Moran 1991); slow-acting rodenticides--the second-generation anticoagulants brodifacoum (BFC) (Moran 1993a) and difethialone (Moran 1993b); and cholecalciferol (Moran, in preparation). The field trials presented here were performed in view of the results of these laboratory experiments and were planned to search for the lowest broadcasting rate sufficient to control the vole population, thus reducing the amount of toxicant in the field, and also cost.

METHODS

Assessment of Control Efficacy

Whole wheat baits were prepared by the manufacturers: 0.05% SFA bait (ROSH-80®), dyed green, 2% ZP bait (NAVRON®), which is typically black (Yewnin-Joffe Industry Ltd., Tel-Aviv), and 0.005% BFC bait, dyed red (Tivonchem Ltd., Haifa). The trials were carried out in seven fields, sized 6.4 to 31.0 ha, in which no control operations were conducted since the previous season: a) arable fields (wheat and vetch), in the first stage of growth; or b) a fodder field (alfalfa), just after reaping, when the vegetation cover was minimum, allowing both easy localization of the vole holes and an efficient control operations (Table 1). The fields were divided at random into more-or-less equal area treated and untreated plots. The treatments were conducted with the same techniques and equipment as in common control operations, and without interfering with the farming routine. In each plot, four random 20 x 20 m squares (except eight circles per plot, 6 m in diameter, in one of the trials) were sampled (Table 2). The squares were located no less than 20 m from the edges of the treated plots (thus leaving a 40 m buffer zone between squares treated differently, following to some extent, EPPO guidelines). In each trial, one plot remained untreated, serving as a control. The size of the treated and untreated plots was no less than 1 ha each (EPPO 1975), the minimum needed to include the squares, the buffer zones, and the plot margins. As the vole burrows are usually aggregated in form of colonies, the squares were sampled at random on sites with vole activity only. All the holes inside each square were plugged with soil. Three days later the RH were counted (preTRH). Then the baits were applied. The field was revisited a week later in the case of acute rodenticides, and two weeks later in the case of the anticoagulant baits; in some trials this period had to be extended because of weather interferences (Table 2). All the holes were plugged, by the same procedure, and the RH were counted three days later (postTRH). The mortality rate assessment for every square (m) was calculated according to the formula (Edge and Olson-Edge 1990):

$$(1) \qquad m = 100 - \frac{\text{post TRH}}{\text{preTRH}} \times 100$$

Hence, the mean mortality rate for n sampling units in the treated plot (M) was:

$$(2) \qquad M_T = n^{-1} \Sigma m$$

M_T is an estimate of the efficacy of the control in the plot. The mean population changes in the untreated plot (M_U) were calculated in the same way. The control efficacy attributed to the toxic bait (E) was calculated according to Abbot's formula (Busvine 1971):

$$(3) \qquad E = \frac{M_T - M_U}{100 - M_U} \times 100$$

henceforth termed "corrected control."

RESULTS AND CONCLUSIONS

The rate of vole activity in the fields, assessed by the average of the preTRH count, was variable (Table 1). It was relatively low (11.3 to 19.4 holes per sampling unit) in trials 1, 6 and 7, and higher (25.0 to 59.0 openings) in trials 3, 2 and 4. In trial 5, and in the untreated plot in trial 4, the field was very heavily populated by voles, with 153.2 and 197.2 holes per square which were uniformly distributed over the field. This kind of distribution was concurrent with a low control value (Figure 5), as discussed thereinafter.

The results of the field trials are represented in the following figures: the control estimates (means of the sampling units of every plot) in Figures 1 to 7; and the corrected control values, pooled together according to the bait toxicants, in Figures 8 to 11. In the untreated plots, the mean mortality rate (mean control) was mostly small (between -15.4% and 3.4% in five of the trials). This means that the overall natural changes (mortality, reproduction, or other activity patterns) in the vole populations are small. In these trials the calculation of the corrected control, using Abbott's formula, produced values comparable between plots. When this ratio was above 20% (in trials 1 and 3) it resulted in rather dubious values of the corrected control, as Abbott's correction is not recommended for experiments with control mortalities above 20% (Busvine 1971). In these cases the corrected mortality rates have to be considered not as reliable assessments of the control efficacy, but only as an emphasis of the differences between the control efficacies in the different treatments. The high mortality rates in the untreated plot in trial 1 (78.6%) were concurrent with a frost which occurred at the fourth night after the treatment (Table 2). The "negative mortality" in trials 2, 5 and 6 indicates growth in the vole activity or, perhaps, in reproduction as well.

Below, the control efficacies of the different baits and techniques are compared, using the corrected control values, and pooling together results from different trials. As the broadcasting by a tractor-mounted spreader resulted in bait grain coverage on the ground no different from the airborne broadcasting, both techniques were pooled together.

The control by 2% ZP bait was 94.4% (corrected control) when applied manually. The mean control rates of the treatments with the SFA formulations (92.9% and 87.2%) are not sufficient to estimate their efficacies: as mentioned before, the corrected control values presented in Figures 1 and 8 (66.7% and 40.3%) are quite dubious because of the heavy mortality in the untreated plot. On the other hand, they demonstrate somewhat better performance of the ZP formulation. The values of the corrected control by broadcast ZP bait were parallel to the increase in the distribution rates: 0.9 kg/ha resulted in 5.4% control; 2.8 kg/ha in 58.3%; 4.2 kg/ha in 71.1%, and 12.2 kg/ha in 80.2% control (Figure 9). In trial 1, the higher concentration of sodium fluoroacetate formulations applied by hand (0.1%) was more efficient (66.7% corrected control) than the 0.05% treated bait (40.3%). However, this low corrected control can be disregarded, as noted above, and as supported by the relatively high control rates achieved with 0.05% SFA (77.3%, 70.0% and 77.0%, respectively) in trials 2, 6

Table 1. Description of the test fields.

Trial No.	Location	Crop	Field Area (ha)	No. of holes[a] (mean±SE)	Month and Year	Special weather occurrence[b]
1	Tal-Shahar	Vetch	17.5	11.3± 3.1	Jan 1989	Frost (4)
2	Gat	Wheat	16.5	48.0±11.1	Feb 1989	Clear
3	Bet-Hanan	Wheat	8.5	25.0± 3.2	Dec 1989	Clear
4	Bet-Arif	Wheat	6.4	59.0± 7.2[c]	Mar 1991	Rain (7,5)[d]
5	Kefar-Rupin	Alfalfa	6.4	153.2±14.1	Jun 1991	Clear
6	Beror-Hayil	Wheat	17.7	17.0± 1.4	Jan 1993	Rain (11)
7	Gal-On	Wheat	31.0	19.4± 4.3	Dec 1993	Rain (12)[e]

[a]Average of all the units sampled in this field during the pretreatment reopened holes counting.
[b]In parentheses; days after treatment.
[c]In the treated plots only; in the untreated this parameter was 197.2±26.2.
[d]First number--after the manual treatment; second number--after the broadcasting.
[e]Part of one of the plots was irrigated five days after the treatment.

and 7, attained by relatively low broadcast rates (1.6, 4.0 and 2.7 kg/ha, respectively). To sum up, these findings show that 2% ZP bait, applied manually at a rate of 0.7 g per vole hold, and broadcast at a rate between 4.2 and 12.2 kg/ha, and SFA 0.005% baits, applied manually at a rate of 0.2 g per hole, or broadcast at rates of 1.6 to 4.0 kg/ha, were sufficiently efficient to control Levant voles.

The efficacy of manual application of 0.005% BFC treated bait was high (Figures 4 and 6), and did not change whether 3 g/hole (91.4% corrected control), or 6 g/hole (92.9%) were applied. Manual applications directly into the burrow openings are known to be more efficient in vole control than mechanical spreading because the toxic bait is more accessible to the rodents. Indeed, in trial 6 the manual application of BFC baits achieved better results (91.4%) than the broadcasting by air of 5.8 and 8.7 kg/ha (60.0% and 69.1%, respectively). On the other hand, trial 7 showed that sometimes the same rates of broadcasting could result in efficacies (84.4% to 98.2%) rather similar to the manual treatment.

BFC-treated bait (0.005%) was quite effective in three of the experiments (Figures 4, 6 and 7), but failed badly in trial 5 (a corrected control rate of 31.1% to 51.2%), although the application rates 7.5, 17.0 and ca. 25 kg/ha seemed to be sufficient. This trial differed from the three others in two characteristics: 1) the extremely heavy infestation (153.2 openings per square unit), threefold and more than in the other plots--a situation that necessitates retreatments (Naftali and Wolf 1964; Moran et al. 1988); and 2) the relatively short activity period, 11 days instead of 14. Moran (1993a) found that the average time to mortality following consumption of a sufficient amount of bait to cause 100% mortality in a laboratory vole population is 7.8±1.6 days, with a range of 6 to 10 days. Adding a few days to allow the voles to feed and gather bait results in a minimum lag of 14 days being necessary between the treatment and the posttreatment hole plugging.

In trial 6 the control rate of broadcast 0.005% BFC bait was quite similar to that achieved with the treatments with the 0.05% SFA commercial bait (ROSH-80®) (Figure 6); in trial 7 its efficacy was much better (Figure 7). One could suppose that the control efficacy is a result of the relatively long control activity periods in trials 6 and 7 (18 and 28 days, respectively), but rainy weather in these plots 12 and 11 days, respectively, after the treatments actually shortened this period, and the partial irrigation of the 8.7 kg/ha plot in trial 7, reduced somewhat the efficacy of the treatments. Only in the case of an extraordinary high broadcasting rate, as happened in trial 4 (17.3 kg/ha), could the rodents gather enough bait grains--reaching toxic amounts before the rainfall five days after the treatment.

The performance of sufficient field trials to check the variety of field crops and damage rates was perforce limited; this was due to three reasons. First, the distribution of vole colonies adequate to formate into test plots occurred in two kinds of field crops, arable and fodder fields only. Second, the maximum number of field trials a year was limited by the seasonal farming procedures relevant to Israel: 1) in wheat, the trial had to be conducted in the winter months, from the start of the rainy season to early spring, between sprouting and the time when wheat reaches a height of 60 cm (taller plants could conceal the reopened holes); 2) in alfalfa, the crop is reaped in spring and summer. It was possible to secure the necessary 21-day time span between the removal of the alfalfa bales and the next reaping in springtime only, when plant growth was relatively slow. Third, the neglected plots, infested by field rodents to an extent adequate to field trials, were few and hard to located. On the other hand, extremely high infestations, as in trial 5, were not suitable for a single-treatment test.

Table 2. Details of the rodenticide applications

Trial No.	Vehicle of Application	Formulation[a]	Rate (kg/ha)	No. of sampling units[b]	Period between treatment and postTRH plugging (days)
1	manual	ZP 2%	0.7	4	7
	manual	SFA 0.1%	0.2	4	7
	manual	SFA 0.05%	0.2	4	7
	---	untreated	---	2	
2	airplane	ZP 2%	12.2	4	7
	airplane	SFA 0.05%	1.6	3	7
	---	untreated	---	3	7
3	airplane	ZP 2%	0.9	4	8
	airplane	ZP 2%	2.8	4	8
	airplane	ZP 2%	4.2	4	8
	---	untreated	---	4	
4	tractor	BFC 0.005%	17.3	4	16
	manual	BFC 0.005%	6 g	4	14
	untreated		4		
5	tractor	BFC 0.005%	7.5	4	11
	tractor	BFC 0.005%	17.0	4	11
	tractor	BFC 0.005%	≈25	4	11
	untreated	---	4		
6	manual	BFC 0.005%	3 g	8 [c]	28
	airplane	BFC 0.005%	5.8	8 [c]	28
	airplane	BFC 0.005%	8.7	8 [c]	28
	airplane	SFA 0.05%	4.0	8 [c]	28
	---	untreated	---	8 [c]	
7	airplane	BFC 0.005%	2.9	4	18
	airplane	BFC 0.005%	5.8	4	18
	airplane	BFC 0.005%	8.7	4	18
	airplane	SFA 0.05%	2.7	4	18
	---	untreated	---	4	

[a]BFC-brodifacoum; SFA-sodium fluoroacetate; ZP-zinc phosphide.
[b]20x20 m squares, except c-circles, 6 m in diameter.
[c]postTRH-posttreatment reopened holes.

One of the major difficulties in the development of toxic rodent baits is to deduce from the laboratory experiment to the planning of a field trial. It is important to solve this problem since the number of fields for trials is limited. In previous work Moran (1993a) had estimated from laboratory results the range of 0.005% BFC bait treatment in the field tests, as follows: application of 6 g bait per vole hole, or a distribution of 6 to 12 kg/ha. The results of our work meet that estimate. Conducting field trials based on such estimates saves time and expense.

The trials were carried out according to the general pattern of the EPPO guidelines (EPPO 1975), which were modified slightly. The recommended one-week inspection period of the RH seems too long: in our study three days were sufficient. Also, there is no need for a second plugging and inspection after one day. The period of reinspection after the baiting was not defined in the EPPO guidelines. The periods described here, one week for acute toxicants and two weeks for anticoagulants, provided sufficient time for the toxic bait to be accepted by the rodents, and to be active physiologically, ending with the death of the majority of the vole population.

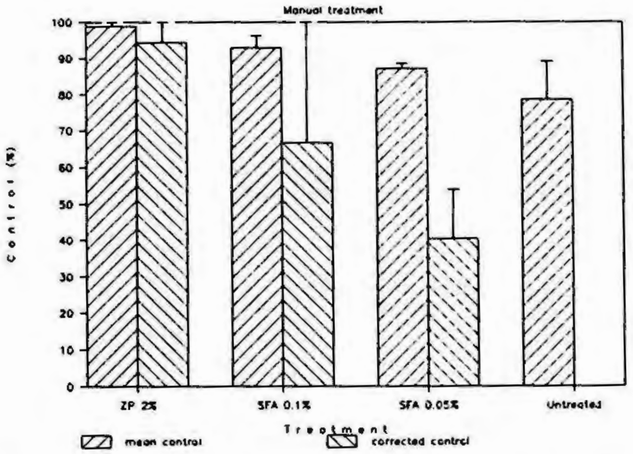

Figure 1. Mean control rates in the treated plots (±SE), and the values corrected according to the untreated plots (±SE) in trial 1 (manual treatment).

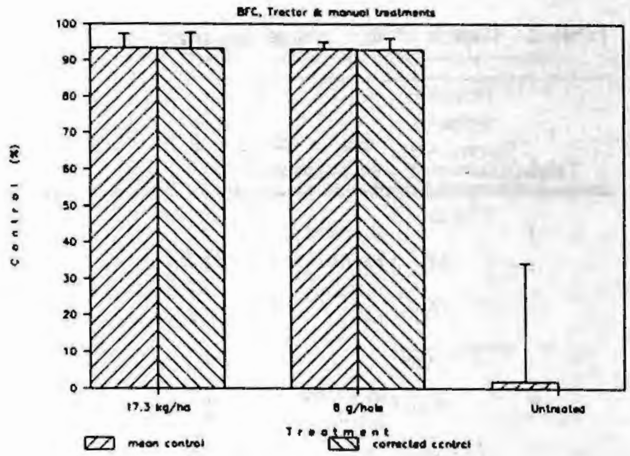

Figure 4. Mean control rates in the treated plots (±SE), and the values corrected according to the untreated plots (±SE) in trial 4 (BFC, tractor and manual treatments).

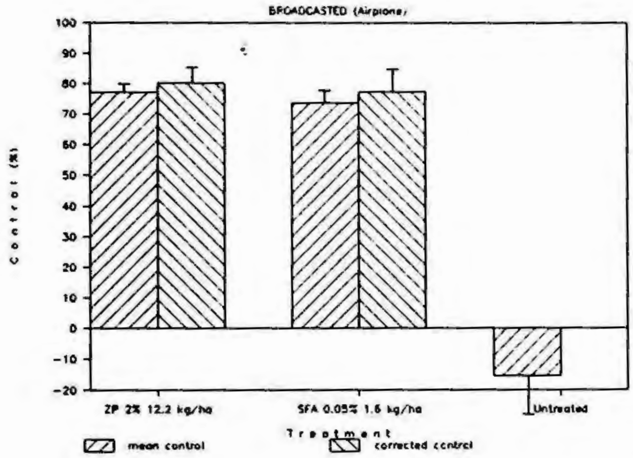

Figure 2. Mean control rates in the treated plots (±SE), and the values corrected according to the untreated plots (±SE) in trial 2 (broadcasted by airplane).

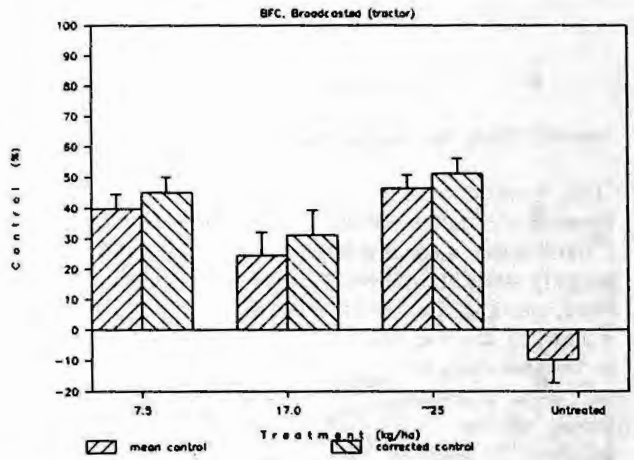

Figure 5. Mean control rates in the treated plots (±SE), and the values corrected according to the untreated plots (±SE) in trial 5 (BFC, broadcasted by tractor).

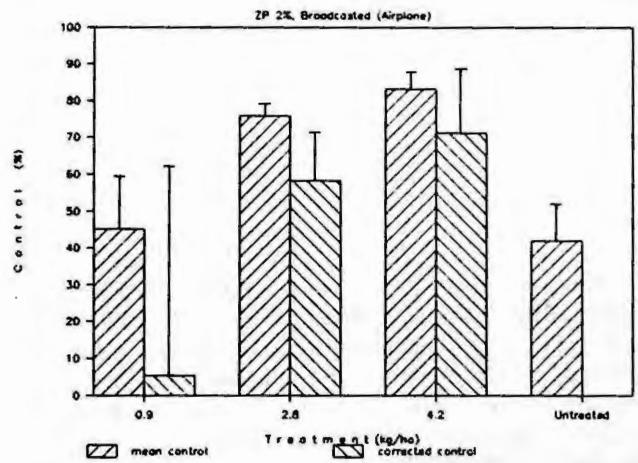

Figure 3. Mean control rates in the treated plots (±SE), and the values corrected according to the untreated plots (±SE) in trial 3 (ZP 2%, broadcasted by airplane).

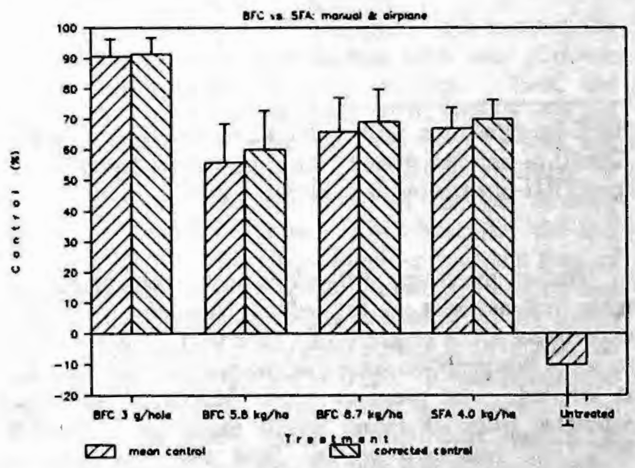

Figure 6. Mean control rates in the treated plots (±SE), and the values corrected according to the untreated plots (±SE) in trial 6 (BFC vs. SFA, manual and broadcast by airplane).

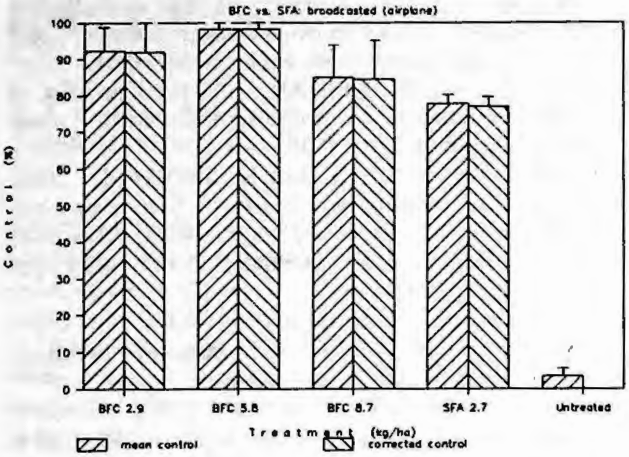

Figure 7. Mean control rates in the treated plots (±SE), and the values corrected according to the untreated plots (±SE) in trial 7 (BFC vs. SFA, broadcast by airplane).

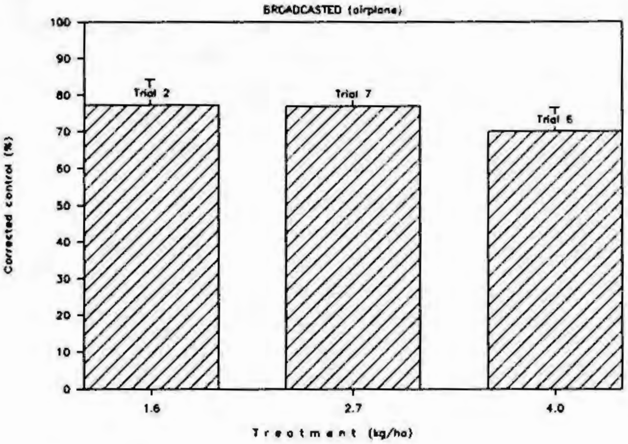

Figure 10. Control by SFA 0.05% bait broadcasted, results pooled from the broadcasted plots according to the active ingredients of the bait (corrected control rates ±SE).

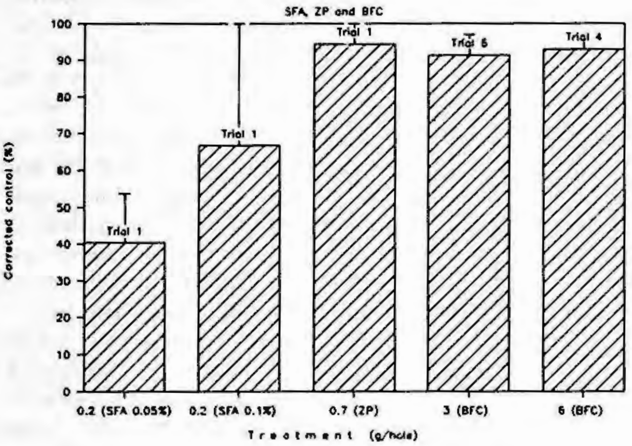

Figure 8. Control by inserting SFA, ZP and BFC bait manually into the voles' holes, pooled results of trials 1, 4 and 6 (corrected control rates ±SE).

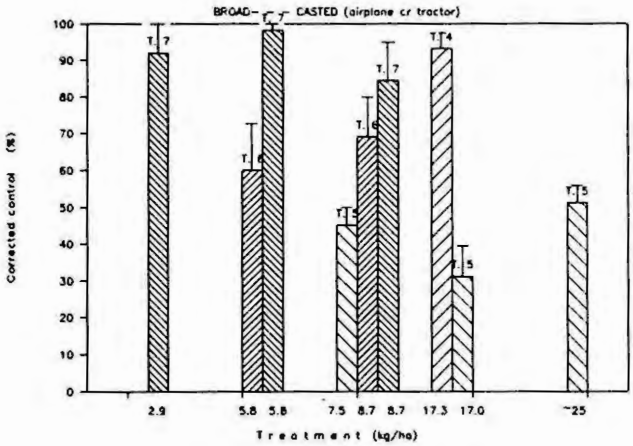

Figure 11. Control by 0.005% brodifacoum bait broadcasted, results pooled from the broadcasted plots according to the active ingredients of the bait (corrected control rates ±SE).

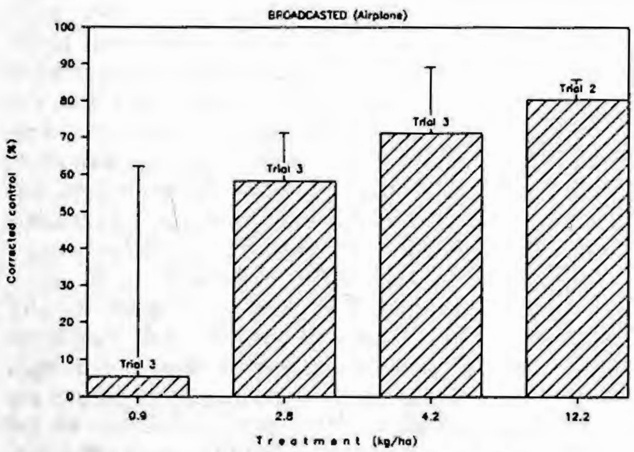

Figure 9. Control by broadcasted (airplane) ZP 2% bait, results pooled from the broadcasted plots according to the active ingredients of the bait (corrected control rates ±SE).

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