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The efficacy of three metaldehyde pellets marketed in Argentina, on the control of *Deroceras reticulatum* (Müller) (Pulmonata: Stylommatophora)

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Abstract

Management of the grey slug, *Deroceras reticulatum* (Müller) (Pulmonata: Stylommatophora), in Argentina, has been limited to the use of pelleted toxic baits containing metaldehyde. The most important molluscicides in the Argentinean market are Clartex (Rizobacter Argentina S.A.), Acay (Acay Agro S.R.L.) and Babotox (Huagro S.A.). The aim of this work was to investigate the efficacy and dose of those commercial baits against *D. reticulatum*. The molluscicides (Acay, Clartex and Babotox), at doses of 3, 4 and 5 kg ha^{-1} and dry and moist soil conditions were investigated in terraria trials. The efficacy of the molluscicides and dose level was also evaluated in field experiments at two sites at Balcarce in the southeast of Buenos Aires Province, Argentina. The proportions of dead slugs did not show significant interactions between soil conditions, molluscicides and their dose ($p > 0.05$). The proportion of dead slugs, in moist conditions, was significantly lower than under dry conditions ($p < 0.05$). Highest slug deaths were obtained with Acay 56.1%. The trend in relation to the efficacy of the molluscicides was similar in the two field trials. Acay gave the best performance, followed by Clartex and Babotox.

Additional key words: commercial molluscicides, control efficacy, slug, soil conditions.

Resumen

Eficacia de tres cebos con metaldehído registrados en Argentina, en el control de *Deroceras reticulatum* (Müller) (Pulmonata: Stylommatophora)

En Argentina, el manejo de la babosa gris *Deroceras reticulatum* (Müller) (Pulmonata: Stylommatophora) está limitado al uso de cebos tóxicos formulados como pellets con metaldehído. Los molusquicidas más importantes usados en el mercado argentino son Clartex (Rizobacter Argentina S.A.), Acay (Acay Agro S.R.L.) y Babotox (Huagro S.A.). El objetivo del presente trabajo fue investigar la eficacia y dosis de los cebos comerciales sobre *D. reticulatum*. En el laboratorio se evaluaron molusquicidas (Acay, Clartex y Babotox), dosis (3, 4 y 5 kg ha^{-1}) y condición del suelo (seco y húmedo). La eficacia de control de los molusquicidas y sus dosis se evaluaron en dos sitios ubicados en Balcarce (sudeste de la provincia de Buenos Aires, Argentina). La proporción de babosas muertas no mostró interacción significativa entre condición del suelo, molusquicida y dosis ($p > 0{,}05$). En condiciones húmedas la proporción de babosas muertas fue significativamente menor que en condiciones secas ($p < 0{,}05$) y la mortalidad mayor se obtuvo con Acay al 56,1%. La eficacia de los molusquicidas mostró una tendencia similar en los ensayos a campo y el producto más promisorio fue Acay seguido por Clartex y Babotox.

Palabras clave adicionales: babosa, condición del suelo, eficacia de control, molusquicidas comerciales.

Introduction[1]

Deroceras reticulatum (Müller), best known as the grey slug, is one of the most important pests in no-tillage sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* L.) crops in Argentina. In order of prevalence the slugs are *D. laeve* (Müller), the marsh slug, and *Milax gagates* (Draparnaud), the black-keeled slug (Costamagna *et al*., 1999). In no-tillage systems there is no mechanical disturbance of the soil or retention

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1 Abbreviations used: A.I. (active ingredient), DAA (days after application), LSD (least significance difference), SNTP (slug number in treated plots), SNUP (slug number in untreated plots).

of residues, this increases soil moisture, which provides an optimal habitat for slug survival and reproduction (Barratt *et al*., 1989).

Glen and Moens (2002) and Moens and Glen (2002) observed that slug damage to plants is caused at sowing and immediately after germination. According to Clemente (2006), environmental conditions in the southeast of Buenos Aires province, in Argentina, favour a generation of new individuals of semelparous *D. reticulatum* from the beginning of winter to the end of spring. This coincides with the sowing and the emergence of winter and summer crops, when the greatest damage occurs.

Methods for protecting crops from slug damage include cultural control (Glen *et al*., 1990; Hammond, 1996; Speiser and Hochstrasser, 1998), and biological control, using the parasitic nematodes *Pharmarhabditis hermaphrodita* (Schneider) (Ester and Geelen, 1996; Glen *et al*., 1996; France *et al.*, 2002). However, chemical control is the main strategy utilized (Chabert, 1996; Coupland, 1996; Meredith, 1996; Costamagna *et al*., 1999; Bailey, 2002; Iglesias *et al*., 2002).

Two groups of chemicals are used worldwide: metaldehyde and carbamates. These are often formulated as bait pellets and act as stomach poisons (Wedgwood and Bailey, 1988; Bourne *et al*., 1990; Henderson *et al*., 1992). Metaldehyde baits, in particular, are toxic to slugs on ingestion and cause increased slime secretion, immobilization and eventual death by loss of water (Bailey, 2002). Metaldehyde is highly specific and is not toxic to beneficial organisms such as earthworms, spiders, ants, millipedes, staphylinid and carabid beetles (Bieri, 2003).

The most important molluscicides commercially available in Argentina are Clartex (Rizobacter Argentina S.A.), Acay (Acay Agro S.R.L.) and Babotox (Huagro S.A.). They are formulated as granulated baits with metaldehyde and an attractant, usually a cereal bran or flour. In addition, they contain animal repellent, colouring and antifungal agents and are applied by broadcasting onto the soil surface.

As Argentinean bait pellets have different concentrations of the active ingredient (A.I.) metaldehyde and have unknown attractants, their performance may vary. Further, Acay, Clartex and Babotox have different physical properties (Losada, unpublished data), which could affect their effectiveness as molluscicides.

Given the lack of information on the efficacy of Acay, Clartex and Babotox against *D. reticulatum*, the aim of this work was to investigate their efficacy and the effect of dose of these commercial slug baits.

Material and Methods

Laboratory trials

The trials were conducted from August 2004 to July 2006. *Deroceras reticulatum* used in the assays were from laboratory cultures. These cultures were started from approximately 100 adult slugs originally collected between April and June 2004, from a grass park at the National Institute of Agricultural Technology (INTA), Experimental Station at Balcarce, in the southeast of Buenos Aires Province (37° 45’ S, 58° 18’ W).

Slugs were placed in individual plastic boxes (385 cm^3) for egg production. Each box contained moist soil (which allowed moisture conservation) and holes were made in the lid. This allowed for air circulation while ensuring the atmosphere was moist. The slugs were fed on dry rabbit food. The boxes were placed in a rearing chamber at 20 ± 1°C (LD:12/12 h) and were examined weekly. Soil and food were changed at the time of these observations.

Approximately 2,000 eggs were transferred using a fine camelhair brush to plastic boxes (385 cm^3) with damp filter paper and were incubated in the same chamber as the parent slugs. As eggs hatched, newly hatched slugs were placed into individual plastic boxes (385 cm^3) with moist soil and food. Slugs were examined weekly and the food and soil were changed at the same time. Slugs were weighed monthly on an electronic analytical balance (accuracy of 0.001 g). The permanent slug culture allowed the production of reproductive slugs of 400-600 mg (Clemente, 2006).

The efficacy of the three molluscicides, formulated as pelleted baits, was evaluated in terraria. The molluscicides used were Clartex (Rizobacter Argentina S.A.), Acay (Acay Agro S.R.L.) and Babotox (Huagro S.A.). Terraria measured 0.12 m^2 by 0.25 m and were filled at the bottom with 10 cm of moist soil and with a 5 cm layer of wheat dry residuals. Three soybean seeds were placed in a row with five slugs of 400-600 mg (a heavy slug infestation). Slugs were pre-starved for 48 h before being introduced into a terrarium. Litio fat was put on the upper edge of each terrarium to prevent the slugs escaping. Terraria were kept in a rearing chamber at 20 ± 1°C (LD: 12/12 h).

At the start of the assay, terraria soils contained approximately 25% moisture. During the following period, two soil moisture conditions were imposed: drought, where the terrarium was not watered, and moist, where the terrarium was sprayed at 3 mm d^{-1} during the 10 d of treatment.

Table 1. Commercial products, concentration of A.I. (metaldehyde) (%), dose A.I. and dose of molluscicide in laboratory and frield trials

			Laboratory trial	Field trials	
				Experiment A	Experiment B
Commercial product	Metaldehyde (%)	Dose A.I. (g ha^{-1})	Molluscicide dose (kg ha^{-1})	Molluscicide dose (kg ha^{-1})	Molluscicide dose (kg ha^{-1})
Acay	4	120	3	3	3
		160	4	—	4
		200	5	5	5
Clartex	5	150	3	3	—
		200	4	—	—
		250	5	5	—
Babotox	5	150	3	3	—
		200	4	—	4
		250	5	5	5
Control	—	—	—	—	—

Each molluscicide was tested at a dose of 3, 4 and 5 kg ha^{-1} (Table 1). Terraria without molluscicides were used as controls. Molluscicide, dose and soil moisture conditions were randomly distributed.

Slug deaths, undamaged and damaged plants were monitored daily during 10 days after application (DAA). Slug damage was evaluated as dead plants (no emerged seed, cut hypocotyl), damaged plants (cotyledon damage) or undamaged.

The experimental design was completely randomised with six replicates. Data on the proportion of dead slugs were analysed with a general linear model (McCullagh and Nelder, 1989) using SAS v.8 software (SAS, 2001). Differences in the proportions of undamaged, damaged and dead plants under different soil moisture conditions and for Acay, Clartex, Babotox and the control were examined using a χ^2.

Field trials

During 2004, field experiments were carried out at two sites: on a farm in Balcarce (Experiment A), and at the INTA Experimental Station, Balcarce (Experiment B). Both sites were monitored before sowing to determine an area, which was homogeneously infested with *D. reticulatum*. In Experiments A and B there were an average of 7.3 and 33.3 slugs m^{-2}, respectively.

The trials were conducted in a no-tillage soybean crop in early November 2004. The previous crop was wheat (*Triticum aestivum* L.). The plot size was 200 m^2 and they were arranged in randomised blocks with 3 replicates.

The treatments, concentration of A.I. metaldehyde (%), dose of A.I. (g ha^1) and molluscicide dose (kg ha^{-1}) for both experiments are shown in Table 1. The control was three 200 m^2 plots without molluscicide.

The treatments were applied using a hand operated mechanical spreader applicator to the seedling crop. The number of dead and live slugs was determined at 7 and 14 DAA. Two samples of 50 × 50 cm (0.25 m^2) were taken from each plot. The sampling procedure adopted was a visual inspection of residuals, at the surface and at 3 cm level in the soil.

Efficacy of control was determined using Abbott's formula (Abbott, 1925):

$$\text{Abbott's control percentage} = \left(1 - \frac{\text{SNTP}}{\text{SNUP}}\right) * 100 \quad [1]$$

where SNTP = slug number in treated plots after treatment and SNUP = slug number in untreated plots after treatment.

Protection of the crop by the treatments was assessed at 14 DAA by counting the number of plants in a 2 m-length of row plot^{-1}.

ANOVA (analysis of variance) was used to evaluate slug control and plant number among the different treatments. Levene's test was used to test homogeneity of treatment variance. Fisher's least significance difference (LSD) test was used to determine significant differences. Overall data were analysed using SAS v.8 software (SAS, 2001).

Results

Laboratory trials

At 10 DAA, there were no significant interactions for the number of dead slugs in relation to soil moisture level, molluscicide or dose ($p > 0.05$). However, significant differences were observed in relation to soil moisture level and molluscicide ($p < 0.0001$). In untreated terraria, all of the slugs remained alive.

The proportion of dead slugs in dry soil was 56.1%, which was significantly higher than 21.3% in moist soil ($p < 0.05$). The proportion of dead slugs was similar in response to Acay (56.7%) and Clartex (47.2%) ($p = 0.056$), these were significantly higher than in response to Babotox (19.4%) ($p < 0.0001$).

Symptoms of poisoning were observed in slugs before their death. Symptoms included: flaccid body, immobilization, uncoordinated muscular activity and excessive mucus secretion. After death, the bodies became harder, darker and drier.

Slug injury on soybean plants always began on cotyledons and hypocotyls and sometimes progressed to leaves. There was additional damage to germinating seed, resulting in plant death.

From the χ^2 test the proportions of undamaged, damaged and dead plants differed under dry and moist soil conditions in both treated ($p < 0.0001$) and untreated terraria ($p = 0.007$). The number of undamaged plants was higher under dry conditions than in moist soil. The proportion of dead plants was the highest in the latter (Fig. 1A).

The χ^2 tests for proportion of dead slugs differed for Acay, Clartex, Babotox and the untreated terraria ($p < 0.0001$). Assessments plant damage showed that the number of undamaged plants was higher with Acay than for Clartex and for Babotox (Fig. 1B). These observations were related to the high proportion of dead slugs achieved with Acay.

Field trials

In Experiment A, average of control of *D. reticulatum* did not differ significantly among metaldehyde pelleted bait treatments, at 7 ($p = 0.655$) and 14 DAA ($p = 0.814$). Nevertheless, Acay and Babotox at a low dose achieved seemingly lower average of control than at a high dose, but the average of control for Clartex at a high dose was seemingly lower than at a low dose (Table 2). The highest level of control was with Acay at 5 kg ha^{-1} and Clartex at 3 kg ha^{-1}, 100 and 82%, respectively. The lowest level of control was with Acay and Babotox at 3 kg ha^{-1}, 57.6% and 54.6% respectively (Table 2).

At 14 DAA, there were similar dose-response relationships among treatments: at 5 kg ha^{-1} control seemed to be higher than at 3 kg ha^{-1} for all of the molluscicides. When the products were compared at both 7 and 14 DAA, slug control was increased by Acay at 3 kg ha^{-1} and Clartex at 5 kg ha^{-1} (Table 2).

In Experiment B, the efficacy of control of *D. reticulatum* differed significantly among molluscicide treatments at both 7 ($p = 0.0195$) and 14 DAA ($p = 0.0005$). Babotox at 3 and 4 kg ha^{-1} and at both 7 and 14 DAA gave the lowest level of control, and Acay the highest (Table 3). When these results are compared with those from Experiment A, the molluscicide effectiveness was

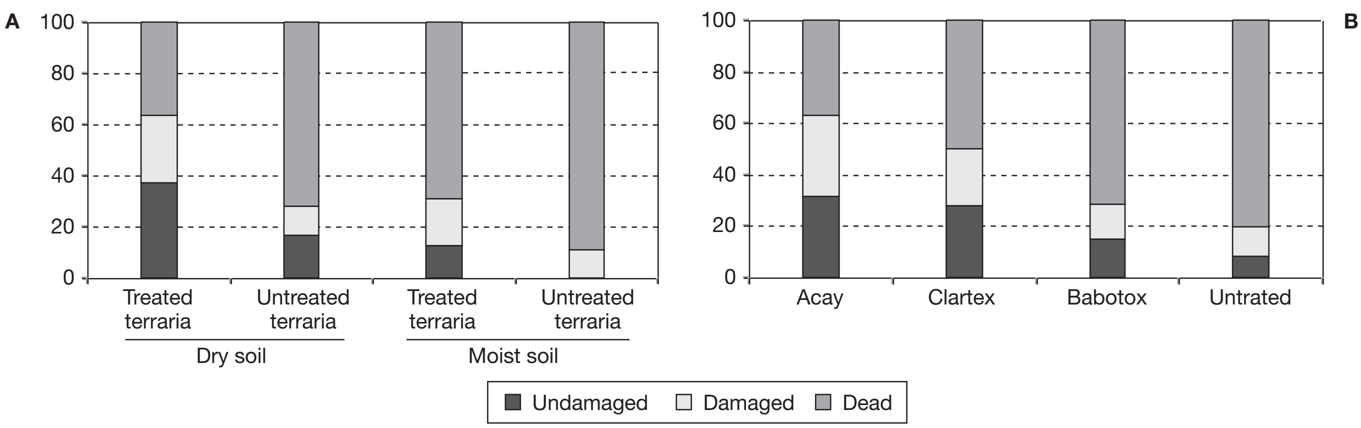

Figure 1. Percentage of undamaged, damaged and dead plants 10 days after application A) under dry and moist soil conditions and B) for Acay, Clartex, Babotox and untreated terraria.

Table 2. Average control of *Deroceras reticulatum,* standard deviations (SD), minimum (Min) and maximum (Max) values with three molluscicides at 7 at 14 days after application (DAA) in field Experiment A

Molluscicide	Dose (kg ha^{-1})	7 DAA				14 DAA			
		Control (%)	SD	Min	Max	Control (%)	SD	Min	Max
Acay	3	57.6	51.7	0	100	80	34.6	40	100
	5	100	0	100	100	93.3	11.5	80	100
Clartex	3	81.8	31.5	45.5	100	60	52.9	0	100
	5	72.7	47.2	18.2	100	80	34.6	40	100
Babotox	3	54.5	15.8	45.5	72.7	53.3	50.3	0	100
	5	63.6	41.7	18.2	100	80	34.6	40	100

Table 3. Average control of *Deroceras reticulatum,* standard deviations (SD), minimum (Min) and maximum (Max) values with two molluscicides at 7 at 14 days after application (DAA) in field Experiment B

Molluscicide	Dose (kg ha^{-1})	7 DAA				14 DAA			
		Control (%)	SD	Min	Max	Control (%)	SD	Min	Max
Acay	3	72[a]	3.5	70	76	69.2[ab]	7.7	61.5	76.9
	5	58[ab]	21.6	40	82	74.36[a]	11.8	61.5	84.6
Babotox	3	27.3[c]	47.3	0	82	18.0[cd]	16.0	0	30.8
	4	34.7[bc]	32.3	0	64	2.6[d]	4.4	0	7.7
	5	58[ab]	10.4	46	64	43.6[bc]	24.7	15.4	61.5

Means in each column followed by the same letter are not significantly different ($p > 0.05$).

similar. In all cases best performance was obtained with Acay, followed by Clartex and Babotox (Tables 2 and 3).

In Experiment A the number of plants among molluscicide treatments was significantly different ($p = 0.041$) (Table 4). More plants were observed with Acay at 3 kg ha^{-1} and 5 kg ha^{-1}, and with Clartex at 3 kg ha^{-1}, 33.3, 31.7 and 32.9 plants m^{-2} respectively. Babotox at both doses gave the lowest number of plants (26.7 and 27.5 plants m^{-2}) (Table 4).

Table 4. number of soybean plants m^{-2}, standard deviation (SD), minimum (Min) and maximum (Max) values at 14 days after application in field experiments A and B

Molluscicide	Dose (kg ha^{-1})	Experiment A				Experiment B			
		Number of plants	SD	Min	Max	Number or plants	SD	Min	Max
Acay	3	33.3[a]	1.9	31.3	35	—	—	—	—
	4	—	—	—	—	25	2.2	23.5	27.5
	5	31.7[abc]	2.6	28.8	33.8	28.8	2.3	26.5	31
Clartex	3	32.9[ab]	2.9	31.3	36.3	—	—	—	—
	5	28.3[bcd]	5.1	23.8	33.8	—	—	—	—
Babotox	3	26.7[d]	1.9	25	28.8	25.7	2.8	22.5	28
	4	—	—	—	—	24.5	3.3	21.5	31
	5	27.5[cd]	1.3	26.3	28.8	27.7	5.4	21.5	31
Control		27.5[cd]	2.5	25	30	24.3	4.3	20.5	29

Means in each column followed by the same letter are not significantly different ($p > 0.05$).

The number of plants was similar in all treatments in Experiment B (approximately 26 plants m^{-2}) and did not show any significant differences ($p = 0.568$) among molluscicide treatments (Table 4). In Experiment A the highest plant number corresponded with the best treatment for the control of *D. reticulatum* (Tables 3 and 4).

Discussion

The trials reported here describe an initial assessment of the molluscicidal activity of metaldehyde-baited pellets sold in Argentina. The results indicate that there was large variation in the effectiveness of the products, which is possibly due to several factors. It is recognized that prevailing environmental conditions can strongly influence both slug activity and molluscicide efficacy. Slugs are most active in crops at night or following rain on overcast days (Bailey, 2002).

Chabert (1996), reported that under moist conditions the effectiveness duration of metaldehyde pellets was about one week, but the duration was longer under dry conditions, approx. 12 d. In this work it was seen that in treated terraria highest slug control was found under dry conditions. This could explain why all of the slugs in untreated terraria remained alive. In this study the drought conditions imposed were not a factor in slug deaths.

It is known that higher temperatures increase metaldehyde toxicity and loss of slug body water, with consequent dehydration (Cragg and Vincent, 1952; Wright and Williams, 1980; Young, 1996). Mallet and Bougaran (1971, in Bailey, 2002) found a higher death rate in *D. reticulatum* when it is exposed to metaldehyde at above 20°C than at 16°C. This may be a reason why the efficacy of control is more variable in the field than in the laboratory trials, where the temperature was a constant 20°C.

Data from laboratory trials may not be directly applicable to field conditions in all cases, but they do provide useful information on the potential of different baits. Further, the laboratory results correlated with the data from the field trials. Highest slug control was in Experiment A, which was characterized by a lack of rain after bait application of > 50% with all products at all doses. In Experiment B, at 4 DAA, there was 17.5 mm of rain so product effectiveness dose response was more variable than in Experiment A. Rain seems to be the most important environmental factor affecting bait persistence and effectiveness, as it caused disintegration of granulated baits. Coupland (1996), considers it is important molluscicide application at sowing, when there are relatively dry conditions, because the products will remain active. Under moist conditions it is necessary, after treatment, to check for slug activity, bait pellets and in some cases, to even repeat the treatment.

On the other hand, Bailey (2002) considers that age structure of the slug population can present differences in their susceptibility to metaldehyde baited pellets. Frain and Newell (1982) suggested that juvenile *D. reticulatum* were less likely to eat molluscicidal baits and Kemp and Newell (1985) found that *D. reticulatum* of less than 80 mg weight were less susceptible to poisoning by metaldehyde baits. In this study, slugs with body weight corresponding to that of reproductive adults (i.e. the most active and representative individuals in natural conditions) were used (Clemente, 2006). This was the reason slug age was not considered.

An important observation for evaluating the toxicity of metaldehyde molluscicides is slug symptoms. As with other works (Briggs and Henderson, 1987; Wedgwood and Bailey, 1988; Bourne *et al*., 1990) the symptoms of poisoning of *D. reticulatum* were slime secretion and immobilization. It was also observed that, after death, slug bodies became harder, darker and drier.

In the laboratory plant damage was related to the environmental conditions and the control efficacy of Acay, Clartex and Babotox. Damage was more severe in moist soil possibly due to the moisture causing bait dissolution, rendering them ineffective. This agrees with the work of Friedli and Frank (1998), who reported that in field-plots, with a high slug density in humid months, a further application of molluscicide, to prevent severe slug damage, was necessary. In addition, in Experiment B, where it rained after treatment application, generally there were a lower number of plants than in Experiment A.

Although Acay, Clartex and Babotox had approximately the same concentration of metaldehyde, in the laboratory trial Acay and Clartex gave the best control. This difference can possibly be attributed to the attractiveness of the bait formulation rather than to the active ingredient (Howling, 1991). Frain and Newell (1982) suggested that companies might produce commercial slug baits by incorporating different «flavour» components into the pellets.

In this work the molluscicides tested differed in pellet size (granules). It is known that variation in molluscicide activity may be due to different physical properties. The size of Acay, Clartex and Babotox pellets was determined in Argentina by Losada (unpublished data), who found that pellets of Acay and Clartex were smaller than Babotox pellets. Thus the differences in product efficacy could be explained by different pellet size. In the terraria and field trials at the same molluscicide dose, the small size and greater number of Acay and Clartex pellets increased the probability that slugs would fed on them. This explains the good efficacy of Acay and Clartex. Coupland (1996) found that their small pellet size and the large number of pellets per gramm of formulation make them appropriate for slug control. Losada (unpublished data) confirmed this by showing the weight of 200 Acay and Clartex pellets is lower than that of 200 Babotox pellets. Therefore, in the field overall molluscicide effectiveness would be due to a combination of their attractiveness to slugs and the number of pellets m^{-2} based on application rate.

In conclusion, the data presented in this study show that:

i) Dry soil conditions improved molluscicide effectiveness.

ii) Acay and Clartex gave good control of *D. reticulatum* and the highest number of undamaged plants was found with both products.

iii) In the two field experiments (A and B) the molluscicides performed in a similar way, although Acay was the most promising, followed by Clartex and Babotox.

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