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EFFICACY OF FOUR MOLLUSCICIDES AGAINST THE GIANT AFRICAN SNAIL, *LISSACHATINA FULICA* (GASTROPODA: PULMONATA: ACHITINIDAE)

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ADSTRACT

The giant African snail (GAS), Lissachatina fulica Bowdich is one of the world's most pestiferous snail species. This invasive pest was discovered in Miami, Florida in Sep 2011. Shortly thereafter, an eradication program was implemented by the Florida Department of Agriculture and Consumer Services in conjunction with the United States Department of Agriculture. In the past, most mollusc eradication efforts have relied on metaldehyde and carbamate-based products that may also have deleterious effects on humans and non-target vertebrates. This study compared the efficacy of 4 commercially available molluscicides: a metaldehyde and carbamate-based bait (Ortho Bug-Geta Plus) and 3 more environmentally "friendly" formulations, including 2 iron-based baits (Ferroxx and Sluggo) and 1 boric acidbased bait (Niban) to elicit mortality in laboratory populations of GAS. Bait formulations were evaluated using a combination of choice and no-choice tests. OrthoBug-Geta Plus was the most effective molluscicide and produced mortality between 69.2% in choice tests and 71.7% in no-choice tests. Sluggo produced a mortality of 49.2% in choice tests and 59.2% in no-choice tests. Niban produced the highest mortality of all the baits evaluated in the no-choice test at 74.2% but was much less effective in choice tests with a 48.3% mortality rate. Ferroxx caused some mortality, 50.8%, but was statistically no different than Sluggo in the no-choice test. Mean percent mortality was significantly higher in adults and neonates compared to juveniles in all treatments. Sluggo, Niban and Ferroxx all proved to be significantly less toxic than Ortho Bug-Geta Plus in choice tests. Although Niban had a very high mortality rate in the no-choice tests, when given a choice mortality declined sharply indicating that this product is not very attractive to GAS. Of the 2 iron based products, Sluggo and Ferroxx, Ferroxx was less effective at 35.8% mortality in the choice tests. While only having a moderate mortality rate, Sluggo was deemed to be effective enough to incorporate into Florida's eradication program.

Key Words: Niban, Sluggo, Ferroxx, Ortho Bug-Geta Plus, molluscicide

RESUMEN

El caracol gigante africano (CGA), Lissachatina fulica, es una de las especies de caracoles más pestilentes del mundo. Esta plaga invasora fue descubierta en Miami, Florida en septiembre del 2011. Poco después, el Departamento de Agricultura y Servicios al Consumidor en conjunto con el Departamento de Agricultura de los Estados Unidos implementaron un programa de erradicación. En el pasado, la mayoria de los esfuerzos para erradicar moluscos han dependido del uso de productos de metaldehído y de base carbamato que también pueden tener efectos nocivos sobre los seres humanos y los vertebrados que no son el enfoque del tratamiento. Este estudio comparó la eficacia de cuatro moluscocidas disponibles en el mercado: un cebo con metaldehído y de base carbamato (Ortho-Geta Bug Plus) y 3 formulaciones que son más ambientalmente "amigable", incluyendo 2 cebos de base de hierro (Ferroxx y Sluggo) y 1 cebo con base de ácido bórico (Niban) para provocar la mortalidad en poblaciones de CGA en el laboratorio. Se evaluaron las formulaciones de cebo usando una combinación de pruebas de opción y no opción. OrthoBug Geta-Plus fue el moluscocida más eficaz y produciendo una mortalidad entre 69.2% en las pruebas de opción y 71.7% en las pruebas de no opción. Sluggo produjo una mortalidad del 49.2% en las pruebas de opción y 59.2% en las pruebas de no opción. Niban produjó la mortalidad mas alta de todos los cebos evaluados en la prueba de no opción a 74.2%, pero fue mucho menos eficaz en pruebas de opción con una tasa de mortalidad de 48.3%. Ferroxx causó una mortalidad del 50.8%, pero no fue estadísticamente diferente de Sluggo en la prueba de la no opción. El promedio del porcentaje de mortalidad fue significativamente mayor en los adultos y recién nacidos en comparación con los juveniles en todos los tratamientos. Sluggo, Niban y Ferroxx resultó ser significativamente menos tóxico que Ortho Bug-Geta Plus en pruebas de opción. Aunque Niban tenían una tasa de mortalidad muy alta en las pruebas de no opción, cuando tenian una opción la mortalidad disminuyó considerablemente lo que indica que este producto no es muy atractivo para CGA. De los 2 productos a base de hierro, Sluggo y Ferroxx, el Ferroxx fue menos eficaz en 35.8% de mortalidad en las pruebas de opción. Mientras que sólo tiene una tasa de mortalidad moderada, se consideró que el Sluggo es suficientemente eficaz para incorporar en el programa de erradicación de la Florida.

Palabras Clave: Niban, Sluggo, Ferroxx, Ortho-Geta Bug Plus, molusquicida

The control of the giant African snail (GAS), Lissachatina fulica Bowdich (Gastropoda: Achatinidae) has been the subject of much debate for decades. GAS is a notorious generalist that consumes over 500 plant species and ranks consistently among the world's most invasive pests (Lowe et al. 2000). This species has been intercepted or introduced all over the world, and aggressive tactics have been implemented to mitigate the risk of dispersal via trade routes through international quarantines and surveillance protocols (USDA-APHIS 2007). Additionally, GAS serves as a vector in the transmission of several vertebrate parasites (Civeyrel & Simberloff 1996; Thiengo et al. 2007).

In Sep 2011, an established population of L. fulica was discovered in Miami, Florida. This population was discovered in an urban neighborhood with moderately sized homes on relatively small lots (< 0.5 acres). An abundant supply of host material was available to the snails in this area consisting of ornamental plants, subtropical fruit trees and an abundant supply of detritus. This part of Florida is known for its high rainfall, mild winters and high humidity making it an ideal habitat for GAS establishment. Within days of this first find, an eradication program was initiated by the Florida Department of Agriculture and Consumer Services in conjunction with the United States Department of Agriculture. Small populations of this pest have been eradicated in Australia (Colman 1977) and once before in Florida in 1975 (Poucher 1975; Mead 1979; Simberloff 2003). Successful eradication efforts rely heavily on molluscicides (Simberloff 2003) particularly metaldehyde and carbamate formulations, supplemented with mechanical collection.

Use of metaldehyde as a molluscicide was developed in the late 1930s and carbamate in the 1950s. Molluscicides formulated with varying proportions of these compounds have been highly efficacious over the years (Kemp & Newell 1985; Sparks et al. 1996; Ebenso et al. 2005). However, concerns have been raised regarding the negative environmental impacts of those formulations, particularly non-target effects on vertebrates (USDA-APHIS 2007). Additionally, molluscicides are typically formulated with bran to increase palatability and act as an attractant for slugs and snails (Howlett et al. 2008). Unfortunately, these bait formulations may be highly attractive to domesticated animals (e.g., dogs). Several alternative compounds have been developed and incorporated in molluscicide formulations. These include calcium arsenate, calcium cyanamid, and DDT, all highly toxic and of limited use or no longer available. Other products are formulated with iron sulphate, boric acid and iron phosphate, which are considered less toxic and often promoted as more environmentally "friendly."

The current GAS eradication program in South Florida is concentrated in a densely populated urban area. This poses unique challenges as it may be necessary to exclude or limit the use of several restricted use molluscicides with proven efficacy due to potential non-target impacts. It is, therefore, prudent to evaluate the efficacy of less toxic alternative molluscicides relative to the known efficacy of metaldehyde-carbamate formulations. The purpose of this experiment was to explore the efficacy of several safer alternative molluscicides. It was hypothesized that at least one of the safer alternatives would have effects comparable to that of a metaldehyde-carbamate-based product. Therefore, the effect of each molluscicide on the mortality, mobility and food consumption of GAS was tested.

MATERIALS AND METHODS

This study used choice and no-choice tests to evaluate and compare the efficacy of the metal-dehyde/carbamate molluscicide Ortho Bug-Geta Plus, relative to 3 alternatives Sluggo (a.i. = iron phosphate), Niban (a.i. = orthoboric acid) and Ferroxx (a.i. = sodium ferric EDTA) to induce mortality in GAS.

Molluscicide trials were conducted in the Florida Department of Agriculture and Consumer Services' Florida Biological Control Laboratory (FB-CL) quarantine facility, at the Division of Plant Industry, Gainesville, Florida. Approximately 1,500 GAS were hand-collected from Miami-Dade County, Florida and established in quarantine. The snails were provided organic romaine lettuce (Lactuca sativa L.; Asterales: Asteraceae) as the main food source along with cuttlebone as a source of calcium. The snails were organized into 3 classes, based on their developmental stage: neonates (7 to 20 mm), juveniles (20-45 mm) and adults (45-110 mm) (Ciomperlik et al. 2013). Only snails close to the median measurement for each respective size class were used in the experiment. This was done in an effort to minimize the possibility of snails developing into a new size class during the experiment.

A treatment consisted of 10 snails (one snail per container) of each life stage being exposed to a molluscicide along with 10 snails of each same

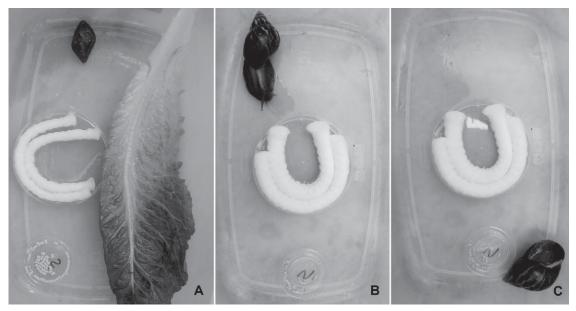


Fig. 1. Layout of molluscicide efficacy choice and no-choice tests: A) Choice test with juvenile snail, B) No-choice test with live adult snail and C) No-choice test with dead adult snail 24 hrs after the initiation of the experiment.

life stage with no molluscicide acting as a control (Fig. 1). Four molluscicides were tested, resulting in 4 replications using 150 snails in each replication (Table 1). Consequently, 1,200 GAS were used in choice and no-choice tests.

The entire experiment was divided into 2 test types: choice and no-choice. In the no-choice tests, only bait and water were provided in the first 48 h. After the initial 48 h, food was provided to prevent starvation from obscuring the results. In the choice tests, bait and food were provided from beginning to end. Each vented container (18 \times 28 \times 10 cm) was supplied with water via moistened braided cotton wicks in a Petri dish (Fig. 1). After the first 48 h, lettuce was replaced every other day or as needed.

Initial doses of the molluscicide baits were provided at the maximum labeled rates based on the surface area of the testing arena for each individual unit. Subsequent applications were made every 4 days so that bait was always available to

the snails (Table 2). The bait was replaced sooner if fully consumed, mildewed or fouled by the snail.

Mortality, mobility and consumption (feeding or not feeding) were documented daily for 15 days. A snail was considered to be immobile if it was completely withdrawn into its shell. Snails that were unresponsive to vigorous, mechanical stimulation (probing) were considered dead.

Percent mortality was analyzed using an analysis of variance (ANOVA) of percent dead at the end of 15 days by treatment type, with multiple mean comparison via Tukey-Kramer's (HSD) test ($\alpha=0.05$).

Results

The provision of a food source as an option within the first 48 hours of the experiment proved to significantly influence molluscicide-induced mortality in the snails. Without a food source in

Table 1. Number of Lissachatina fulica per life stage used in each of 4 replications.

Treatment	Life Stage		
	Neonate	Juvenile	Adult
Sluggo	10	10	10
Niban	10	10	10
Ortho Bug-Geta Plus	10	10	10
Ferroxx	10	10	10
Control	10	10	10
Total	50	50	50

Commercial Product	Formulation	Active Ingredient	Application Rate
Ortho Bug-Geta Plus	Granule	Carbaryl (5%) and Metaldehyde (2%)	1 lb/2000 sq. ft (453.6 g/185.6 m²)
Niban	Granule	Orthoboric acid (5%)	6 oz/100 sq. ft (170.1 g/9.29 m ²)
Sluggo	Granule	Iron phosphate (1%)	1 lb/1000 sq. ft (453.5 g/92.9 m ²)
Ferroxx	Granule	Sodium Ferric EDTA (5%)	0.44 lb/1000 sq. ft (200 g/92 9 m ²)

Table 2. Commercial product name, formulation, active ingredient dose and application rate of molluscicides evaluated in the laboratory.

the first 48 hrs, both Ferroxx and Niban demonstrated a much greater mortality when compared to their performance in the choice test. In the nochoice test, Niban caused the highest mortality and was not statistically different from the Ortho Bug-Geta Plus (74.2% and 71.7% mortality, respectively); Ferroxx caused 50.8% mortality, which was also not statistically different from Ortho Bug-Geta Plus (Table 3).

In the choice test, however, Ortho Bug-Geta Plus caused the greatest mortality (69.2%), followed by Sluggo and Niban with 49.2% and 48.3%, respectively. Ferroxx did not perform well, causing only 35.8% mortality. There was less than 3% average mortality in the controls for both choice and no-choice tests, and all treatments were different from the control (Table 3).

In choice and no-choice tests, adults and neonates exhibited greater susceptibility to the molluscicides compared to juveniles (Figs. 2 and 3). For all 4 molluscicides, the juveniles averaged 32.5% mortality in the choice tests, compared to 60.6% in adults and 58.8% in neonates (F=5.90, df = 2, 45, P=0.0053). Similar results were observed in the no-choice experiment where juveniles averaged 45.6% mortality compared to 76.3% in adults and 70.0% in neonates (F=7.55, df = 2, 45, P=0.0015).

The amount of time required to induce snail mortality varied over time with each molluscicide, in both the choice and no-choice tests. The percent mortality from OrthoBug-Geta Plus gradually increased over time. In Sluggo and Ferroxx, the mortality peaked very early, often within the first 2 to 5 days; however, Niban-induced mortality peaked later in the study (approximately 10 days after the initial contact with the product) (Figs. 2 and 3).

DISCUSSION

Results from this study clearly demonstrated that while select alternatives to metaldehyde/carbamate-based molluscicides can elicit mortality in populations of GAS they simply are not as effective as traditional metaldehyde based products. It was determined that the results of the choice tests were more instructive as GAS are unlikely to encounter molluscicides in a no-choice scenario under field conditions. This decision was reinforced by the high mortality induced by Niban in the no-choice tests (74.2%) compared to its relatively poor performance in the choice tests (48.3% mortality).

Results from the choice tests demonstrate that the metaldehyde/carbamate formulation (OrthoBug-Geta Plus) induced the highest percent mortality (69.2%) of all the molluscicides evaluated. This would suggest that metaldehyde/carbamate formulations remain one of the most useful tools for chemical control of pest molluscs outside of environmentally sensitive areas. Sluggo and Niban both had a lower percent mortality by comparison at approximately

Table 3. Mean percent mortality of all 3 life stages of Lissachatina fulica treated with 4 molluscicides, initially at labeled rates then subsequently at $\frac{1}{4}$ the rate, in choice and no-choice tests evaluated at the end of 15 days.

Product	Mortality (%) after 15 days (Mean \pm S.E.)*		
	Choice Test	No-Choice Test	
Ortho Bug-Geta Plus	69.2 ± 5.5 a	71.7 ± 8.7 a	
Niban	$48.3 \pm 8.7 \text{ ab}$	74.2 ± 8.3 a	
Sluggo	$49.2 \pm 9.5 \text{ ab}$	59.2± 5.8 a	
Ferroxx	$35.8 \pm 5.5 \text{ b}$	$50.8 \pm 5.3 \text{ a}$	
Control	$2.5 \pm 1.6 c$	$2.5 \pm 1.6 \text{ b}$	

^{*}Means within a column with different letters are significantly different according to Tukey-Kramer' (HSD) Comparison test. Overall mean of means calculated for N = 30 snails in each of 4 replicates. Choice $F_{4,15}$ = 13.297, P < 0.0001; No-Choice $F_{4,15}$ = 20.191, P < 0.00001.

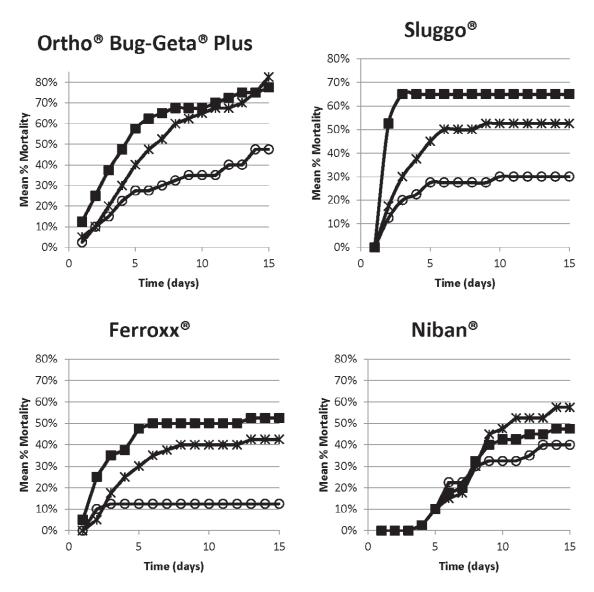


Fig. 2. Choice Test: Mortality of $Lissachatina\ fulica$ treated with molluscicides over 15 days. (Square = Adults, Circle = Juveniles, X = Neonates). Overall mean of means calculated for N=10 snails per life stage in each of 4 replicates.

49% and 48%, respectively; however, these molluscicides may be suitable candidates for inclusion in circumstances that preclude the use of more efficacious, but potentially toxic molluscicides.

The molluscicides Niban, Sluggo and Ferroxx are formulated with active ingredients (orthoboric acid, iron phosphate and sodium ferric EDTA, respectively), which initially act as antifeedants, then alter specific vital biochemical pathways necessary for proper functioning of organ systems (Iglesias & Speiser 2001; Speiser & Kistler 2002; David Moore, personal communication 2011).

This disruption eventually results in death. While the maximum efficacy of Ferroxx and Sluggo was achieved within the 15 day experimental period it is probable that additional mortality may have been observed beyond this period with Niban and Ortho Bug-Geta Plus.

It was observed that in all treatments, a majority of the treated snails that died would cease eating and moving, and became moribund prior to expiring. This behavior was particularly evident in snails treated with Niban. Cochran (1995) reported that boric acid destroyed the foregut cells of treated German cockroach (Blattella germanica)

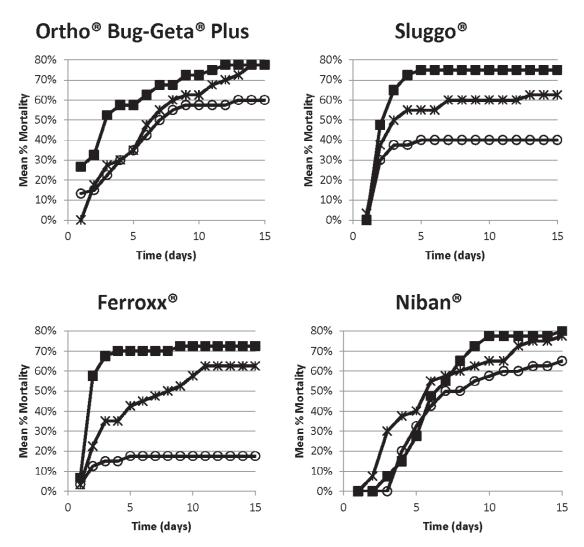


Fig. 3. No-Choice Test: Mortality of $Lissachatina\ fulica$ treated with molluscicides over 15 days. (Square = Adults, Circle = Juveniles, X = Neonates). Overall mean of means calculated for N = 10 snails per life stage in each of 4 replicates.

and caused excessive bloating that inhibited feeding and led to delayed mortality. In this study, mortality of Niban-treated GAS peaked approximately 10 days after initial exposure suggesting starvation as the eventual cause of death. OrthoBug-Geta Plus showed similar results with a peak mortality from 7 to 10 days. In contrast, most of the snail mortality occurred within the first 2 to 5 days when treated with Ferroxx and Sluggo.

In this study, adult snails and neonates exhibited greater susceptibility to all molluscicide treatments compared to juveniles. Bailey (2002) also noted age-determined differential susceptibility of gastropods to different molluscicides. Godan (1983) reported similar results when limacid species were provided with Isolan

(carbamate-based bait), and Frain & Newell (1982) reported the same for *Deroceras reticulatum*, where the juveniles were less likely to consume the molluscicide baits and were less susceptible than the hatchlings and the reproductive adults.

It remains unclear why juveniles are such difficult molluscicide targets, but it is possible that juveniles are at a dispersal stage and invest more resources into wandering, so ingestible baits are not as attractive. It has also been noted that GAS food preferences change during the lifetime of the snail. Neonates seem to be detritivores switching to a more herbivorous diet in the juvenile stage and then reverting to a more mixed diet as adults utilizing both detritus and living plant matter (Ciom-

perlik et al. 2013). Therefore, it is possible that the attractant used in most snail baits may not be as attractive to the more herbivorous juveniles. Further testing should be carried out to determine the type of products that could target juvenile terrestrial gastropods. This information would aid in the development of more effective molluscicide baits and eradication programs.

It was evident that all the molluscicides evaluated induced mortality to varying degrees; however, the most efficacious product was the metaldehyde-carbamate-based product (OrthoBug-Geta Plus). Sluggo and Niban baits produced moderate results and are safer alternatives to OrthoBug-Geta Plus in sensitive areas. More field testing of each of these products is needed, applied exclusively or in rotation, along with stronger molluscicides that may be applied in a more focused manner even in sensitive areas. As is the case in Florida's eradication program an integrated approach to controlling and eradicating this pest must be utilized. This may include the establishment of quarantine zones to limit the movement of plant material out of the affected area, debris removal in urban landscapes, mechanical collection of snails and trapping, in addition to chemical treatments.

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