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MORTALITY TO THE GIANT AFRICAN SNAIL, *LISSACHATINA FULICA* (GASTROPODA: ACHATINIDAE), AND NON-TARGET SNAILS USING SELECT MOLLUSCICIDES

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ABSTRACT

Laboratory bioassays and caged field trials were conducted to compare the acute toxicities of molluscicide formulations on the neonate, juvenile, and adult development stages of giant African snail (GAS) *Lissachatina fulica* (Bowdich 1822) and 3 non-target snail species in Barbados. Nine commercially available molluscicides, diatomaceous earth, and a kaolin clay product (Surround WP) were evaluated. High levels of mortality to neonate GAS were seen in all the laboratory molluscicide bioassays except for Surround and diatomaceous earth. The highest mortality rates to neonate GAS were observed from Durham granules and Slugfest treatments tested during field trials. Deadline, Durham granules, Metarex, and Orcal pellets caused the highest rates of mortality to juvenile GAS in our field trials. For adult GAS several molluscicides including Blitzem, Deadline, Durham Granules, Mesuro 75W, Metarex, Orcal pellet, and Slugfest caused greater than 95 % mortality in laboratory bioassays. Field trials showed that Durham granules and Slugfest yielded the highest mortality rates. Sluggo pellet, touted as an environmentally safe molluscicide, did not cause high rates of mortality to juvenile and adult GAS in our field trials, but did cause higher rates of mortality to neonate GAS than the control. The majority of the molluscicides tested in our trials were equally or more lethal to 3 non-target snail species than GAS. Our results identify several effective commercially available molluscicides that can be used to control incipient populations of GAS. However, our results show that the potential impact on non-target snail species during control or eradication programs may be considerable, causing substantial mortality regardless of what brand, active ingredient, or formulation is used.

Key Words: diatomaceous earth, kaolin, non-target snail species, mortality, bioassay, Barbados

RESUMEN

Se realizaron bioensayos de laboratorio y pruebas de campo en jaulas para comparar la toxicidad aguda de formulaciones de moluscocidas en las etapas de desarrollo de neonatos, juveniles y adultos del caracol gigante africano (CGA) *Lissachatina fulica* (Bowdich 1822) y 3 especies de caracoles no-objetivos en Barbados. Nueve moluscocidas disponibles comercialmente, tierra diatomacea, y un producto de arcilla de caolin (WP Surround) fueron evaluados. Se observaron altos niveles de mortalidad en los neonatos de CGA en todos los bioensayos de moluscocidas en el laboratorio con la excepción de Surround y la tierra diatomaea. Se observaron las tasas más altas de mortalidad de los neonatos en los tratamientos de Slugfest y de los gránulos de Durham en las pruebas de campo. Deadline, los gránulos de Durham y los gránulos de Metarex Orcal causaron las tasas de mortalidad más altas en los juveniles de CGA en nuestras pruebas de campo. Para los adultos de CGA, varios de los moluscocidas incluyendo Blitzem, Deadline, gránulos de Durham, Mesuro 75W, Metarex, gránulos de Orcal y Slugfest causaron más de un 95% de mortalidad en bioensayos de laboratorio. Las pruebas de campo mostraron que los gránulos de Durham y Slugfest resultaron en las tasas

mas altas de mortalidad. Los gránulos de Sluggo, promocionado como un moluscocida ambientalmente seguro, no causó altas tasas de mortalidad en los juveniles y adultos de CGA en nuestras pruebas de campo, pero causó mayores tasas de mortalidad en los neonatos de CGA en el control. La mayoría de los moluscocidas probados en nuestros ensayos fueron igualmente o más letal a 3 especies de caracoles no-objetivos que el CGA. Nuestros resultados identifican varias moluscocidas eficaces disponibles en el mercado que se pueden usar para controlar las poblaciones incipientes de CGA. Sin embargo, nuestros resultados muestran que el impacto potencial sobre especies de caracoles no-objetivos durante los programas de control o erradicación puede ser considerable, causando una mortalidad considerable, independientemente de lo que marca, ingrediente activo o formulación que se utiliza.

Palabras Claves: tierra diatomacea, caolín, especies no-objetivos de caracoles, mortalidad, bioensayo, Barbados

The giant African snail (hereafter referred to as GAS), *Lissachatina fulica* (Bowdich 1822) (Gastropoda: Achatinidae), is probably one of the best-known snail pests in the world. Believed to have originated in coastal East Africa (Pilsbry 1904; Bequaert 1950), *L. fulica* has spread across southern and southeast Asia, and across the Indian Ocean and Pacific Basins, as a result of almost 2 centuries of human intentional introductions and accidental hitch-hiking. In the New World, *L. fulica* has become well established in the last 20 yr, and appears to be spreading virtually unimpeded. To date, only once has a population of the snail been successfully eradicated in the New World; this was a single relatively small population in southern Florida introduced in 1966 and only after a massive eradication and public education effort (Poucher 1975). In the Lesser Antilles, however, attempts to halt its progress have so far been in vain. After having been intentionally released by an unknown individual into a National Park in Guadeloupe in 1984, *L. fulica* has spread, by intentional and accidental means, to the French islands of Martinique in 1988, Marie-Galante in 1995, and Saint Martin in 1995 (Pollard et al. 2008). The giant African snail was reported from Barbados in 2000 and from St. Lucia in 2002, and their respective ministries of agriculture are currently making efforts to contain the species. GAS is also present in Anguilla (Connor 2006), Antigua, Dominica (Pollard et al. 2008) and Trinidad (Ministry of Agriculture, Land and Marine Resources, Trinidad and Tobago, 2009).

Lissachatina fulica is also established across Brazil (Thiengo et al. 2007) having been accidentally released into the environment, and there is no sign of any slowing of its spread. Currently, all states in Brazil with the exception of Rio Grande do Sul are considered infested (Thiengo, pers. com.). The snail was reported in Esmeraldas Province, Ecuador in 2005, and is now established in most of the coastal provinces of that country, as well as on the Amazonian side of the Ecuadorian Andes. A population was detected on Santa Cruz island in the Galapagos Islands in April 2010, but an aggressive eradication program there ap-

pears to be bringing that population under control (Correoso 2006). In November 2007, GAS was reported from the state of Aragua, Venezuela, and has since spread to the capital Caracas. In Misiones Province in Argentina, GAS was found in August 2010 in the town of Puerto Iguazú, and the governmental agricultural agency, SENASA Argentina, has conducted an aggressive and effective control program. The latest country in South America to confirm the presence of GAS was Paraguay in September 2012, in Misiones Province (SENAVE, Paraguay, 2012).

The expansion of the range of the giant African snail now threatens other islands in the West Indies, and is expected to reach Puerto Rico and the U.S. Virgin Islands. In September of 2011, an established population of *L. fulica* was found in Miami-Dade County (Capinera 2011), and significant organized efforts are currently underway by the Florida Department of Agriculture & Consumer Services, Division of Plant Industry and the USDA to eradicate the snail.

As well as being detritivorous and necrophagous, *Lissachatina fulica* is a voracious herbivore and is reported to feed on hundreds of different plant species. Raut & Barker (2002) compiled detailed lists of economically important plant species that have been reported as affected by the snail, including important agricultural crops as well as those of major horticultural and medicinal significance. In addition to its agricultural importance, the giant African snail is a vector of the rat lung worm, the parasitic nematode, *Angiostrongylus cantonensis* Chen 1935 (Nematoda: Metastrongylidae), has been implicated in the spread of human cerebral angiostrongyliasis (or eosinophilic meningitis) throughout the Pacific Basin (Bisseru 1971; Chen 1974; Carney et al. 1978; Mead 1979; Breuil & Coulanges 1982; Kliks et al. 1982; Kliks & Palumbo 1992). However, although *A. cantonensis* has been reported in the Greater Antilles (Aguiar et al. 1981; Jaume et al. 1981; Lindo et al. 2002; Orihel & Ash 1994), it was not associated with *L. fulica*. In the Lesser Antilles, cerebral angiostrongyliasis has been reported from Martinique (de Meuron 2005a, b), and more

recently the nematode *A. cantonensis* has been found in rats in Grenada (Chikweto et al. 2009). The recent finding of rat lung worm parasite, *A. cantonensis*, in Florida has elevated the level of urgency for eradication of GAS (FDACS 2012).

There are few published studies of replicated trials testing the efficacy of molluscicides against giant African snail and other land snails. Of those studies, several test a single metaldehyde based product for control of the snail, or test a few products under experimental laboratory conditions (summaries in Mead 1979; Raut & Ghose 1984; Srivastava 1992), however in most of these studies efficacy in natural environments was not demonstrated. The majority of the studies employ baits containing metaldehyde, calcium arsenate, or methiocarb as the principal toxicant. In addition, a few studies have tested naturally occurring plant based chemicals as molluscicides in laboratory studies (Panigrahi & Raut 1994; Singh & Singh 1977a, b; Rao & Singh 2000). The current study reports the efficacy data for 9 commercially available molluscicide formulations from laboratory bioassays as well as field trials for both *Lis-sachatina fulica*, and mortality to 3 nontarget snail species. The approach used in this study takes into consideration the differences in feeding behavior of the 3 different life stages of giant African snail and the nontarget species tested.

Three non-target gastropod species were selected to compare concomitant molluscicide effects on them as well as on *L. fulica*. These 3 species are widespread in Barbados and were sufficiently abundant to lend themselves to being included in the experiments, as well as living sympatrically with the giant African snail in synanthropic environments. *Pleurodonte isabella* Férussac 1821 (Pulmonata: Pleurodontidae) is a species endemic to Barbados. However, like many other pleurodontid species, it seems to thrive in disturbed as well as natural environments, and is known to damage the bracts of some ornamental heliconias and gingers. *Bulimulus guadalupensis* Bruguière 1789 (Bulimulidae) is sometimes considered native to Barbados, although Breure (1974) has suggested that it is a species that originated in the Windward Islands and that has spread throughout the West Indies and southern Florida. It is believed to be a detritivore as well as possibly grazing on algae and lichens encrusting tree trunks and branches. The third non-target species, *Zachrysia provisoria* Pfeiffer 1858 (Pleurodontidae) is a Cuban species that was first reported in Barbados by Chase & Robinson (2001), and is now considered by some horticulturalists as being as serious a pest as *L. fulica*, or perhaps even more damaging.

The 3 life stages of *L. fulica*; neonates, juveniles, and adult snails differ in their feeding biologies. Neonates primarily feed on detritus, juveniles are herbivorous, while the adult stage

is both detritivorous and herbivorous (Van Weel 1948/49; Olson 1973). This difference in feeding biologies may impact their susceptibility to molluscicides applied for their control. This paper describes laboratory and field trials testing the efficacy of select commercially available molluscicides on 3 life stages of *L. fulica*, and the potential for mortality effects on 3 non-target snail species with different feeding biologies.

MATERIALS AND METHODS

Laboratory Bioassays

Laboratory bioassays were conducted in March 2006 at the Entomology Laboratory of the Ministry of Agriculture, Food, Fisheries, and Water Resource Management, Graeme Hall, Christ Church, Barbados. Field-collected snails were held in sealed plastic 28 L Rubbermaid snap top containers (Rubbermaid Home Products, Fairlawn, Ohio), misted with distilled water (< 10 mL), and fed daily an artificial diet of a blended paste comprised principally of carrots, broccoli, beer and guinea corn flour, based on a diet described by Krull (2006). Approximately 15 mL of the prepared diet was applied to the bottom surface of a 9 cm Petri dish and inverted within each container. Mature, presumably adult, *Bulimulus*, *Zachrysia*, and *Pleurodonte* were selected from the holding containers for use in the bioassays. The neonate (7-20 mm shell length), immature (20-45 mm), and adult (> 45 mm) GAS stages were held separately in snap top containers and provided ripe breadfruit as a food source. All snails were held for at least 24 h prior to beginning the study, and all dead or moribund individuals were discarded.

In each bioassay, 10 individuals of each snail taxa were confined in 11.4 L plastic Rubbermaid snap top containers (Rubbermaid Home Products, Fairlawn, Ohio) along with 5 g of the pellet or granule molluscicide being tested (Table 1). The bioassays tested 9 commercially available molluscicides used for snail control, as well as 2 compounds that are sometimes used for insect control and are known to be nontoxic to humans. In the case of the liquid and wettable powder (WP) formulations, a solution was prepared and transferred to a 0.75 L plastic spray bottle (Contract Filling LLC, New York, New York 10003). Approximately 15 mL of the liquid formulation was applied by misting one-half of the bottom surface of the snap top container and was allowed to dry prior to placing snails into the containers. Treatment controls were established by misting one-half of the bottom surface of the container with 15 mL of distilled water only. Temperature and relative humidity were recorded during the study by placing a Hobo data recorder (Onset Computer Corp., Bourne, Massachusetts) into one of the snap top containers.

TABLE 1. LIST OF MOLLUSCICIDES, THEIR ACTIVE INGREDIENTS, AND APPLICATION RATES TESTED IN LABORATORY BIOASSAYS AND FIELD TRIALS.

Product name (formulation ¹)	Active Ingredient	Rate per 1.0 m ²	Manufacturer
Blitzem (P)	Metaldehyde 4%	4.9 g	Yates, Orica Australia Pty Ltd.
Deadline® MPs (P)	Metaldehyde 4%	4.9 g	Amvac Chem. Corp.
Diatomaceous earth (WP)	Sedimentary mineral	30.0 g	Golden Harvest Organics, LLC
Durham® Granules (G)	Metaldehyde 7.5%	1.9 g	Amvac Chem. Corp
Mesuro® 75W (WP)	Methiocarb 75%	10.1 mL	Gowan Co. LLC
Mesuro® Pro (P)	Methiocarb 2%	5.0 g	Bayer Crop Science
Metarex® (P)	Metaldehyde 4%	4.9 g	Liphatech, Inc.
Orcal® Pellet (P)	Metaldehyde 3.25%	4.9 g	Or-Cal Inc.
Slugfest® (L)	Metaldehyde 25%	15.0 mL	Or-Cal Inc.
Sluggo® Pellet (P)	Iron Phosphate 1%	5.0 g	Western Farm Services, Inc.
Surround® (WP)	Kaolin clay	28.5 g	Engelhard Corp.

¹Formulation description: G = granule, L = liquid, P = pellet, WP = wettable powder. The mode of action for pellet (P) and granular formulations is presumably through ingestion, whereas the wettable powder (WP) and liquid (L) formulations is through direct contact.

The bioassay was conducted over a 24 h period at which time mortality was recorded. Mortality was determined by holding the snail and prodding the foot with a dissecting probe for approximately 15 seconds, a lack of motor response was considered evidence of mortality. Moribund individuals, or those showing motor response reactions, were not considered dead. All snails were discarded at the end of each bioassay, and new snails from the holding containers were used in each subsequent bioassay. Each snail taxa and molluscicide treatment was replicated 5 times. Percent mortality was calculated as the number of dead individuals divided by 10, then multiplied by 100.

Field Trials

Molluscicide field trials were conducted in St. Thomas Parish, Barbados in October 2006. Sixty field cages were constructed in an open grassy field of approximately 1 ha in size. The grass vegetation was 7.5 to 10 cm tall in the field cages, and was not removed in order to provide the snails a vegetation and detritus food source. Cages, 0.6 m width × 0.6 m depth × 0.3 m height, were constructed by hammering 4 wooden stakes 18 cm into the ground. The stakes were covered with 30% Dewitt knitted shade cloth (Hummert International, Earth City, Missouri) to form the outer surfaces of the cage, and staked to the ground surface using wire ground stakes (Hummert International, Earth City, Missouri). A 15 cm long opening was cut into the top surface to allow entry to the cage in order to apply molluscicides and introduce snail cohorts. Molluscicides were applied using the same methods as described in the laboratory bioassays. The opening was stitched shut with 24 gauge copper wire to prevent snails from escaping the experimental arena.

Snails used in the experiment were collected and handled in the same fashion as those in the laboratory bioassays. Table 1 lists the molluscicides tested; however, Surround WP and diatomaceous earth treatments were not included in the field trials because they did not cause significant mortality to GAS in the laboratory bioassays. Application and quantities of molluscicides were the same as used in the laboratory bioassays. A total of 60 field cages were setup to accommodate a single field cage for testing each of the snail taxa and molluscicide treatment combinations (3 GAS life stages + 3 non-target snails × 10 treatments). Each of the snail taxa by molluscicide combinations were setup as in a complete randomized design and replicated a total of 5 times, over time during a 14 day period. Molluscicides were applied only once to each cage. In each field cage, 10 individuals of each snail taxa were introduced to the cage after the molluscicide treatment was applied. Mortalities were scored at both 24 h and 48 h post treatment following the same scoring methods used in the laboratory bioassays. Dead individuals were removed from the experimental arena and discarded at 24 h, with all remaining individuals removed and discarded at 48 h. There were a few occurrences of escaped snails during the trial. In this case, percent mortality was calculated as the number of dead individuals divided by the number of remaining live individuals, multiplied by 100.

Percentage data from both the laboratory bioassays and field trials were arc sine transformed prior to one-way analysis of variance tests. ANOVAs were run using JMP statistical analysis software (JMP 2007) among the molluscicide treatments by snail taxa, and means were separated using Tukey-Kramer's HSD at the 0.05 level. Only the mean percentage data are reported in Tables 2-7.

TABLE 2. MEAN (\pm SE) PERCENTAGE MORTALITY TO GIANT AFRICAN SNAIL GROWTH STAGES FROM LABORATORY BIOASSAYS TESTING THE EFFICACY OF DIFFERENT MOLLUSCICIDES.

Treatment	Growth Stage		
	Neonate	Juvenile	Adult
Control	2.5 \pm 2.5 d ¹	2.5 \pm 2.5 b	2.5 \pm 2.5 c
Blitzem	85.0 \pm 9.6 ab	100.0 \pm 0.0 a	100.0 \pm 0.0 a
Deadline	97.5 \pm 2.5 a	100.0 \pm 0.0 a	100.0 \pm 0.0 a
Diatomaceous Earth	0.0 \pm 0.0 d	2.5 \pm 2.5 b	0.0 \pm 0.0 c
Durham Granules	97.5 \pm 2.5 a	97.5 \pm 2.5 a	100.0 \pm 0.0 a
Mesuro 75W	100.0 \pm 0.0 a	100.0 \pm 0.0 a	95.0 \pm 5.5 a
Mesuro Pellet	70.0 \pm 12.2 bc	80.0 \pm 16.8 a	52.5 \pm 18.4 b
Metarex	95.0 \pm 2.9 a	92.5 \pm 7.5 a	100.0 \pm 0.0 a
Orcal Pellet	97.5 \pm 2.5 a	95.0 \pm 5.0 a	95.0 \pm 2.9 a
Slugfest	100.0 \pm 0.0 a	100.0 \pm 0.0 a	100.0 \pm 0.0 a
Sluggo Pellet	60.0 \pm 4.1 c	35.0 \pm 15.5 b	0.0 \pm 0.0 c
Surround	0.0 \pm 0.0 d	5.0 \pm 5.0 b	0.0 \pm 0.0 c
	<i>F</i> = 72.09	33.69	70.66
	<i>P</i> = 0.0001	0.0001	0.0001

¹Means in a column followed by the same letter are not significantly different using Tukey's HSD ($P < 0.05$), $n = 5$ and $df = 11$, 38 for all ANOVA's.

RESULTS

Temperature ranged from a low of about 22 °C to a high of 27 °C, and relative humidity ranged from 95 to 99 % in the laboratory bioassays (Fig. 1.). High levels of mortality to neonate GAS were seen in all the molluscicide treatments except for Surround, Diatomaceous earth, and Sluggo Pellet (Table 2). All of the treatments, with the exception of Surround and Diatomaceous earth, caused significantly greater mortality to neonate GAS than the control ($F = 72.09$, $P = 0.0001$). The highest

levels of mortality to neonate GAS were found in the Mesuro 75W and Slugfest treatments, both of which are liquid formulations. The highest rates of mortality to juvenile GAS occurred in the Blitzem, Deadline, Mesuro 75 W, and Slugfest treatments. All treatments with the exception of Diatomaceous earth, Sluggo pellet, and Surround caused significantly greater mortality to juvenile GAS than the control ($F = 33.69$, $P = 0.0001$). For adult GAS, 100 % mortality occurred in the Blitzem, Deadline, Durham granules, Metarex, and Slugfest treatments. All the treatments, with the

TABLE 3. MEAN (\pm SE) PERCENTAGE MORTALITY TO NON-TARGET SNAIL SPECIES FROM LABORATORY BIOASSAYS TESTING THE EFFICACY OF DIFFERENT MOLLUSCICIDES.

Treatment	<i>Bulimulus</i>	<i>Zachrysia</i>	<i>Pleurodonte</i>
Control	10.0 \pm 4.1 c ¹	0.0 \pm 0.0 d	0.0 \pm 0.0 c
Blitzem	90.0 \pm 4.1 a	80.0 \pm 5.7 ab	100.0 \pm 0.0 a
Deadline	100.0 \pm 0.0 a	70.0 \pm 9.1 ab	57.5 \pm 11.1 abc
Diatomaceous earth	72.5 \pm 12.5 ab	7.5 \pm 4.8 cd	35.0 \pm 16.6 bc
Durham Granules	100.0 \pm 0.0 a	80.0 \pm 11.5 ab	85.0 \pm 9.6 ab
Mesuro 75W	92.5 \pm 2.5 a	95.0 \pm 5.0 a	80.0 \pm 16.8 ab
Mesuro Pellet	72.5 \pm 17.0 ab	67.5 \pm 4.8 ab	75.0 \pm 10.4 ab
Metarex	100.0 \pm 0.0 a	82.5 \pm 11.1 a	87.5 \pm 9.5 ab
Orcal Pellet	95.0 \pm 2.9 a	82.5 \pm 4.8 a	90.0 \pm 7.1 ab
Slugfest	100.0 \pm 0.0 a	97.5 \pm 2.5 a	100.0 \pm 0.0 a
Sluggo Pellet	87.5 \pm 4.8 ab	52.5 \pm 16.5 abc	40.0 \pm 17.8 abc
Surround	45.0 \pm 21.0 bc	30.0 \pm 23.8 bcd	32.5 \pm 21.4 bc
	<i>F</i> = 9.53	9.86	6.79
	<i>P</i> = 0.0001	0.0001	0.0001

¹Means in a column followed by the same letter are not significantly different using Tukey's HSD ($P < 0.05$), $n = 5$ and $df = 11, 38$ for all ANOVAs.

TABLE 4. MEAN (\pm SE) PERCENTAGE MORTALITY AT 24 H TO GIANT AFRICAN SNAIL GROWTH STAGES FROM FIELD TRIALS TESTING EFFICACY OF DIFFERENT MOLLUSCICIDES.

Treatment	Growth Stage		
	Neonate	Juvenile	Adult
Control	0.0 \pm 3.9 c	0.0 \pm 0.0 b	0.0 \pm 0.0 a
Blitzem	6.9 \pm 2.6 c	5.0 \pm 5.0 b	2.5 \pm 2.5 a
Deadline	15.3 \pm 8.4 bc	28.9 \pm 9.8 ab	0.0 \pm 0.0 a
Durham Granules	47.4 \pm 4.9 ab	37.5 \pm 15.5 ab	42.5 \pm 20.2 a
Mesurool 75W	9.5 \pm 6.9 c	0.0 \pm 0.0 b	5.0 \pm 2.9 a
Mesurool Pellet	1.3 \pm 3.8 c	0.0 \pm 0.0 b	5.0 \pm 2.9 a
Metarex	8.6 \pm 3.5 c	45.1 \pm 9.0 a	15.0 \pm 11.9 a
Orcal Pellet	18.0 \pm 3.4 bc	20.0 \pm 12.2 ab	22.5 \pm 13.1 a
Slugfest	64.5 \pm 12.0 a	8.1 \pm 5.3 ab	37.5 \pm 14.4 a
Sluggo Pellet	33.4 \pm 13.7 abc	7.5 \pm 7.5 ab	5.0 \pm 0.0 a
	<i>F</i> = 7.65	4.13	2.51
	<i>P</i> = 0.0001	0.001	0.028

¹Means in a column followed by the same letter are not significantly different using Tukey's HSD ($P < 0.05$), $n = 5$ and $df = 9,40$ for all ANOVAs.

exception of Diatomaceous earth, Sluggo pellet, and Surround, caused significantly greater mortality to adult GAS than the control.

Three different snail species were tested to determine the non-target effects of the molluscicide treatments in laboratory bioassays (Table 3). Deadline, Durham granules, Metarex, and Slugfest caused the highest mortality to *Bulimulus*. All of the molluscicide treatments, excluding Surround, caused significantly greater mortality to *Bulimulus* than the control. For *Zachrysis*, highly significant mortality rates occurred in all the molluscicide formulations tested with the exception of Surround and Diatomaceous earth ($F = 9.86$, $P = 0.0001$). All of the molluscicide formulations,

with the exception of Deadline, Diatomaceous earth, Sluggo pellet, and Surround caused significantly greater mortality to *Pleurodonte* than the control ($F = 6.79$, $P = 0.0001$). The highest rates of mortality to *Pleurodonte* occurred in the Blitzem and Slugfest treatments.

Our field trials excluded testing Surround and Diatomaceous earth because of the limited efficacy observed in most of the laboratory bioassays. Low rates of mortality were observed for neonate GAS in the 24 h field trials; however, Slugfest caused about 64% mortality at that development stage (Table 4). Low mortality rates were observed for both the juvenile and adult stages of GAS at 24 h for all the molluscicides tested, less than 50 %

TABLE 5. MEAN (\pm SE) PERCENTAGE MORTALITY AT 48 H TO GIANT AFRICAN SNAIL GROWTH STAGES FROM FIELD TRIALS TESTING EFFICACY OF DIFFERENT MOLLUSCICIDES.

Treatment	GROWTH STAGE		
	Neonate	Juvenile	Adult
Control	0.0 \pm 2.9 d	2.5 \pm 2.5 b	0.0 \pm 0.0 b
Blitzem	24.4 \pm 5.2 cd	46.1 \pm 18.7 ab	33.1 \pm 9.9 ab
Deadline	43.9 \pm 9.8 bc	85.0 \pm 6.5 a	60.0 \pm 20.4 ab
Durham Granules	84.7 \pm 4.2 a	85.0 \pm 8.7 a	75.0 \pm 14.4 a
Mesurool 75W	27.8 \pm 5.3 cd	2.5 \pm 2.5 b	20.6 \pm 8.2 ab
Mesurool Pellet	1.3 \pm 2.7 d	15.0 \pm 9.6 b	15.0 \pm 11.9 ab
Metarex	55.5 \pm 6.5 abc	82.8 \pm 6.1 a	55.0 \pm 18.9 ab
Orcal Pellet	43.6 \pm 5.0 bc	84.2 \pm 7.1 a	47.5 \pm 10.3 ab
Slugfest	84.0 \pm 11.4 a	25.2 \pm 6.3 b	72.5 \pm 7.5 a
Sluggo Pellet	74.3 \pm 11.9 ab	30.8 \pm 13.3 b	23.5 \pm 16.5 ab
	<i>F</i> = 16.54	14.31	3.79
	<i>P</i> = < 0.0001	< 0.0001	0.003

¹Means in a column followed by the same letter are not significantly different using Tukey's HSD ($P < 0.05$), $n = 5$ and $df = 9,40$ for all ANOVAs.

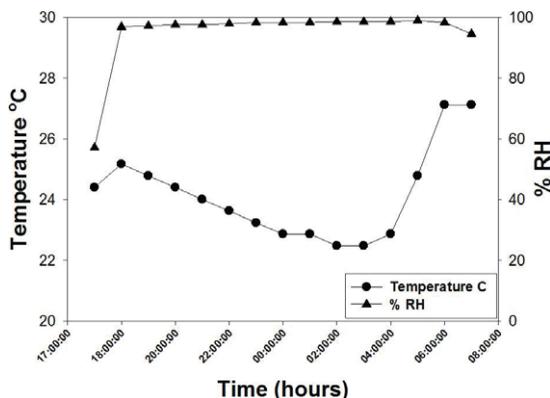


Fig. 1. Temperature ($^{\circ}\text{C}$) and percent relative humidity in Rubbermaid snap top containers during the laboratory bioassays.

in all cases (Table 4). Metarex caused the highest mortality rate of all formulations tested, and it was significantly greater than the control ($F = 4.13$, $P = 0.001$). At 24 h none of the molluscicides caused significantly greater mortality to adult GAS than the control. At 48 h, only Durham granules and Slugfest caused significantly greater mortality to adult GAS than the controls. Overall, higher mortality rates were observed for juvenile GAS than the adult growth stage at 48 h (Table 5). Deadline, Durham granules, Metarex, and Orcal pellet caused significantly greater mortality to juvenile GAS at 48 h than the controls ($F = 14.31$, $P < 0.0001$). Two of the products tested, Durham granules and Slugfest, contained greater than 4% metaldehyde. They did not cause significantly greater mortality to GAS lifestages than those products that only contained 4% metaldehyde. Similarly, Mesurol 75W, which contains a

higher concentration of methiocarb, did not cause significantly greater mortality to GAS life stages than the lower concentration in the Mesurol pellet formulation.

Deadline, Durham granules, and Orcal pellets caused the highest amount of mortality to *Bulimulus* at 24 h (Table 6). Only these 3 molluscicides caused significantly greater mortality than the control ($F = 7.02$, $P = 0.001$). The amount of mortality to *Bulimulus* in the control at 48 h was more than double that observed in the first 24 h. At 48 h, only Blitzem caused significantly greater mortality than the control (Table 7). Metarex caused the greatest amount of mortality to *Zachrysia* at 48 h, while all of the molluscicides, with the exception of Blitzem and Mesurol pellet, caused significantly greater mortality to *Zachrysia* than the control. The greatest amount of mortality to *Pleurodonte* at 24 h occurred in the Blitzem and Slugfest treatments, whereas Mesurol 75 W, Mesurol pellet, and Sluggo pellet were not significantly different than the control. All of the molluscicides, with the exception of Mesurol 75W and Mesurol pellet, caused significantly greater mortality to *Pleurodonte* than the control at 48 h (Table 7).

DISCUSSION

Overall, the laboratory bioassays showed greater levels of mortality to the molluscicide treatments than the field cage trials for all of the snail taxa tested. This was an expected outcome as the laboratory experimental arenas lacked the heterogeneous environment of the field cages. Although we provided an artificial diet in the bioassays, while field cages contained an abundance of detritus and plant material, snails in the bioassays most likely had a higher chance of coming in

TABLE 6. MEAN (\pm SE) PERCENTAGE MORTALITY AT 24 H TO NON-TARGET SNAIL SPECIES FROM FIELD TRIALS TESTING EFFICACY OF DIFFERENT MOLLUSCICIDES.

Treatment	<i>Bulimulus</i>	<i>Zachrysia</i>	<i>Pleurodonte</i>
Control	12.5 \pm 9.5 bc ¹	0.0 \pm 0.0 b	5.3 \pm 3.1 c
Blitzem	41.9 \pm 6.4 abc	11.9 \pm 8.9 b	62.5 \pm 13.1 a
Deadline	61.4 \pm 6.5 a	25.6 \pm 9.3 ab	45.0 \pm 13.2 ab
Durham Granules	60.5 \pm 0.0 a	35.8 \pm 4.8 ab	52.5 \pm 22.9 ab
Mesurol 75W	6.3 \pm 6.3 c	0.0 \pm 0.0 b	2.5 \pm 2.5 c
Mesurol Pellet	15.6 \pm 5.2 bc	3.1 \pm 3.1 b	20.0 \pm 13.5 abc
Metarex	45.5 \pm 5.0 ab	67.5 \pm 13.8 a	43.6 \pm 11.6 ab
Orcal Pellet	52.5 \pm 7.5 a	30.0 \pm 19.6 ab	40.0 \pm 8.2 ab
Slugfest	42.5 \pm 0.0 abc	28.8 \pm 10.9 ab	65.0 \pm 16.6 a
Sluggo Pellet	28.8 \pm 12.3 abc	8.8 \pm 5.9 b	37.8 \pm 3.1 abc
$F =$	7.02	4.78	2.73
$P =$	0.001	0.001	0.01

¹Means in a column followed by the same letter are not significantly different using Tukey's HSD ($P < 0.05$), $n = 5$ and $df = 9$, 40 for all ANOVAs.

TABLE 7. MEAN (\pm SE) PERCENTAGE MORTALITY AT 48 H TO NON-TARGET SNAIL SPECIES FROM FIELD TRIALS TESTING EFFICACY OF DIFFERENT MOLLUSCICIDES.

Treatment	<i>Bulimulus</i>	<i>Zachrysia</i>	<i>Pleurodonte</i>
Control	35.8 \pm 19.3 b	0.0 \pm 0.0 d	20.0 \pm 9.1 b
Blitzem	97.5 \pm 2.5 a	32.5 \pm 13.8 bcd	90.0 \pm 7.1 a
Deadline	95.0 \pm 5.0 ab	67.5 \pm 8.5 abc	90.0 \pm 7.1 a
Durham Granules	90.0 \pm 4.1 ab	82.5 \pm 10.3 ab	92.5 \pm 2.5 a
Mesurool 75W	42.9 \pm 20.3 ab	26.9 \pm 16.9 cd	18.3 \pm 8.0 b
Mesurool Pellet	35.5 \pm 11.7 ab	35.6 \pm 16.3 abcd	48.0 \pm 20.3 b
Metarex	90.0 \pm 7.1 ab	90.0 \pm 10.0 a	95.0 \pm 5.0 a
Orcal Pellet	90.0 \pm 5.8 ab	60.0 \pm 14.7 abc	82.5 \pm 11.1 a
Slugfest	82.5 \pm 11.8 ab	57.5 \pm 7.5 abc	82.5 \pm 8.5 a
Sluggo Pellet	65.0 \pm 18.5 ab	75.0 \pm 2.0 abc	72.2 \pm 14.2 a
	<i>F</i> = 4.19	6.19	8.17
	<i>P</i> = 0.001	< 0.001	< 0.001

¹Means in a column followed by the same letter are not significantly different using Tukey's HSD ($P < 0.05$), $n = 5$ and $df = 9,40$ for all ANOVAs.

contact with or ingesting lethal doses of the molluscicide formulation being tested. The bioassays were conducted over a 24 h time period, and 100% mortality rates were seen in numerous molluscicide treatments. The laboratory bioassays served as good preliminary trials for testing the efficacy of the molluscicides and provided us data to justify the elimination of a few products from field testing. Both Surround WP, a kaolin clay based product, and diatomaceous earth, an abrasive powder used in insect control, were eliminated from field trials because of the low rates of mortality to GAS. In our field trials we recorded mortality at both 24 and 48 h after treatment. None of the treatments reached 100 % mortality at 24 h, whereas several treatments exceeded 95% mortality at 48 h. These results suggest that a 48 h time interval may be sufficient for future molluscicide field trials.

Three development stages of GAS were used in both the laboratory bioassays and field trials. High levels of mortality to neonate GAS were seen in all the molluscicide treatments in the laboratory bioassays except for Surround, and Diatomaceous Earth. In field trials, the highest mortality rates to neonate GAS were recorded in the Durham granules and Slugfest liquid treatments. The neonate life stage of GAS predominantly feeds on detritus. Our field trials suggest that pelletized formulations of molluscicides were less effective, and possibly less attractive, on this stage than liquid or granular formulations. The highest rates of mortality to juvenile GAS occurred in the laboratory bioassays in the Blitzem, Deadline, Mesurool 75W and Slugfest treatments. In field trials, Deadline, Durham granules, Metarex, and Orcal pellets caused 100% mortality to juvenile GAS, whereas the liquid formulations, Mesurool 75W and Slugfest, caused significantly less mor-

tality. Juvenile GAS are predominantly herbivores, at times causing significant damage to foliage and fruits of agricultural crops. Results from our field trials indicate that pellet formulations caused higher mortality rates than liquid formulations, presumably they were more attractive to the juvenile snails because of the bran or cereal grains used as an attractant in the formulation of the pellets. For adult GAS several molluscicides including Blitzem, Deadline, Durham Granules, Mesurool 75W, Metarex, Orcal pellet, and Slugfest caused greater than 95% mortality in laboratory bioassays. Field trials showed that Durham granules and Slugfest yielded the highest mortality rates for adult GAS. These 2 products contained the highest concentrations of metaldehyde; however, the differences in mortality rates were not significantly greater than the other molluscicide products containing only 4% metaldehyde. The 2 methiocarb based products tested showed the lowest mortality rates to both adult and juvenile GAS, contraindicating their use in control programs for the snail. The adult stage of GAS is both herbivorous and detritivorous, presumably making it vulnerable to either liquid or pellet formulations of molluscicides. In our field trials liquid and granular formulations caused greater mortality to adult GAS than pellet formulations. Sluggo pellet (iron phosphate 1%), touted as an environmentally safe molluscicide, did not cause high rates of mortality to juvenile and adult GAS in our field trials. However, both the laboratory bioassays and field trials showed that Sluggo pellets caused greater neonate GAS mortality than the control. Collectively, these observations suggest that census data assessing the number of individuals in a particular development stage, in a specific area or environment requiring treatment, may be necessary in order to selectively treat the

predominant development stage with the most appropriate molluscicide or formulation. Other factors may influence the choice of using either a liquid, granular or pellet formulation such as potential rainfall, vegetation coverage, potential nontarget effects, or toxicity risks associated with the presence of pets and children. Pellet formulations generally have a greater persistence or resistance to breakdown from rainfall, often making them the preferred formulation for mollusc control programs.

Three non-target gastropod species were included in our bioassays and field trials in order to compare the molluscicide effects on them as well as GAS. *Bulimulus guadalupensis* is detritivorous as well as a micro-grazer known to feed on algae and lichens. High rates of mortality were observed in field trials of the metaldehyde based pellet formulations and lower mortality rates were seen in the methiocarb based formulations. Several metaldehyde based products caused moderate to high rates of mortality to *Zachrysis provisoria*. This information may be useful to nurseries and producers of landscape ornamentals where *Z. provisoria* at times reach high numbers and cause extensive feeding damage. *Pleurodonte isabella* is a herbivorous mollusc that occasionally damages the floral bracts of ornamental plants. *P. isabella* showed high rates of mortality from the metaldehyde based pellet formulations, and much lower mortality from the methiocarb based formulations. The majority of the molluscicides tested in our trials were equally or more lethal to the non-target snails than GAS. This was certainly the case with Sluggo pellet, which caused greater mortality to all three non-target snails than GAS. On the other hand, exposure to Mesurrol 75W and Mesurrol pellets resulted in lower mortality in all three non-target snails than in GAS. Our results may not be indicative of what might occur in natural environments simply because in our trials snails were confined in the presence of the molluscicide and could not leave the treated area. However, the observed mortality can be used as a relative measure of the efficacy of the different products that were tested. Our trials suggest that the potential impact on non-target snail species during control or eradication programs may be substantial, causing high rates of mortality regardless of what brand, active ingredient, or formulation is used. The results of this study indicate that molluscicide formulations with higher concentrations of either metaldehyde or methiocarb did not cause greater mortality to giant African snail life stages, suggesting that selection of molluscicides with no more than 4% metaldehyde is sufficient for control programs and this may help limit adverse impacts on the environment.

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REFERENCES CITED

- AGUIAR, P. H., MORERA, P., AND PASCUAL, J. 1981. First record of *Angiostrongylus cantonensis* in Cuba. *Am. J. Trop. Med. Hyg.* 30: 963-965.
- BEQUAERT, J. 1950. Studies on the Achatinidae, a group of African land snails. *Bull. Mus. Comp. Zool., Harvard* 105: 1-216.
- BISSERU, B. 1971. The prevalence of *Angiostrongylus cantonensis* larvae collected from the giant African snail, *Achatina fulica* in west Malaysia and Singapore. *Southeast Asian J. of Trop. Med. Publ. Health.* 2: 523-526.
- BREUIL, J., AND COULANGES, P. 1982. Note sur *Angiostrongylus cantonensis* a Madagascar. *Archives de l'Institut Pasteur de Madagascar* 50: 35-38.
- BREURE, A. S. H. 1974. Caribbean land mollusks: Bulimulidae I. *Bulimulus*. *Studies of the Fauna of Curaçao and other Caribbean Islands* 45: 1-80.
- CAPINERA, J. L. 2011. Giant African land snail in Florida. *Entomol. & Nematol. Dept., Florida Coop. Ext. Serv., Univ. Florida, ENY-512 (IN904)*: 3 pp.
- CARNEY, W. P., STAFFORD, E. E., PURNOMO, AND TANUDJAJA, S. 1978. *Angiostrongyliasis* in Indonesia: additional geographic and host occurrence records. *Southeast Asian J. Trop. Med. & Publ. Health* 9: 516-519.
- CHASE, R., AND ROBINSON, D. G. 2001. The uncertain history of land snails on Barbados: implications for conservation. *Malacologia* 43: 33-57.
- CHEN, S. N. 1974. Molluscan hosts of *Angiostrongylus cantonensis* in Taiwan. *Bull. Malacol. Soc. China.* 1: 105-108.
- CHIKWETO, A., BHAIYAT, M. I., MACPHERSON, C. N. L., DEALLIE, C., PINCKNEY, R. D., RICHARDS, C., AND SHERMA, R. N. 2009. Existence of *Angiostrongylus cantonensis* in rats (*Rattus norvegicus*) in Grenada, West Indies. *Vet. Parasitol.* 162: 160-162.
- CONNOR, R. A. 2006. Distribution, habitat association, species abundance, and perceptions of residents towards *Achatina fulica* in Anguilla. M.Sc. Thesis, Univ. Exeter, Devon, UK, 41 pp.
- CORREOSO, M. 2006. Estrategia preliminar para evaluar y erradicar *Achatina fulica* (Gastropoda: Achatineaceae) en Ecuador. *Boletín Técnico IASA, Serie Zoológica* 2: 45-52.
- DE MEURON, K. 2005a. *Angiostrongylose* en Martinique: à propos de quatre cas pédiatriques. Ph.D thesis. Faculté de Médecine, Univ. Bourgogne, France. 134 pp.

- DE MEURON, K. 2005b. Angiostrongylose en Martinique. II Congrès de la Société Antillo-Guyanaise de Pédiatrie, 10-12 Nov 2005.
- FDACS (FLORIDA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES). 2012. Rat lungworm confirmed in giant African land snail sample collected in Miami-Dade County. Press release 12 Oct 2012.
- JAUME, M. L., PERERA DE PUGA, G., AND AGUIAR PRIETO, P. H. 1981. *Bradybaena similaris* (Férussac): hospedero intermediario de *Angiostrongylus cantonensis* en Cuba. Rev. Cubana Med. Trop. 33: 207-209.
- JMP. 2007. JMP software, version 7. SAS Institute Inc., Cary, NC. 1989-2007.
- KLIKS, M. M., KROENKE, K., AND HARDMAN, J. M. 1982. Eosinophilic radiculomyeloencephalitis: an angiostrongyliasis outbreak in American Samoa related to the ingestion of *Achatina fulica* snails. Am. J. Trop. Med. & Hyg. 31: 1114-1122.
- KLIKS, M. M., AND PALUMBO, N. E. 1992. Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis caused by *Angiostrongylus cantonensis*, the nematode lungworm of rats. Soc. Sci. & Med. 34: 199-212.
- KRULL, P. 2006. Rearing tropical arboreal snails in the laboratory. Tentacle 14: 3-5.
- LINDO, J. F., WAUGH, C. A., HALL, J. J., CUNNINGHAM-MYRIE, C., ASHLEY, D., EBERHARD, M., SULLIVAN, J. J., BISHOP, H. S. ROBINSON, D. G., HOLTZ, T., AND ROBINSON, R. D. 2002. Assessment of enzootic *Angiostrongylus cantonensis* in Jamaican rats and snails following an outbreak of human eosinophilic meningitis. Emerg. Infect. Dis. J. 8: 324-326.
- MEAD, A. R. 1979. *Pulmonates*. Volume 2B. Economic malacology with particular reference to *Achatina fulica*. Academic Press, London. 150 pp.
- MINISTRY OF AGRICULTURE, LAND, AND MARINE RESOURCES, TRINIDAD AND TOBAGO. 2009. Joint press release; giant African snail advisory. Press release 14Aug. 2009. 2 pp.
- OLSON, F. J. 1973. The screening of candidate molluscicides against the giant African snail, *Achatina fulica* Bowdich (Stylommatophora: Achatinidae). Thesis. Univ. Hawaii. 98 pp.
- ORIHIEL, T. C., AND ASH, L. R. 1994. Parasites in Human Tissue. ASCP Press, Chicago. 386 pp.
- PANIGRAHI, A., AND RAUT, S. K. 1994. *Thevetia peruviana* (Family: Apocynaceae) in the control of slug and snail pests. Memorias do Instituto Oswaldo Cruz 89: 247-250.
- PILSBRY, H. A. 1904. Manual of Conchology, Structural and Systematic. With illustrations of the species. Second Series: Pulmonata. Volume XVII. African Achatinidae. Acad. Nat. Sci. Phil. 232 pp.
- POLLARD, G. V., FIELDS, A., AND TAYLOR, B. 2008. Giant African snail in the Caribbean Sub-Region. Proc. Caribbean Food Crops Soc. 44: 126-134.
- POUCHER, C. 1975. Eradication of the giant African snail in Florida. Proc. Florida State Horticultural Society 1975: 523-524.
- RAUT, S. K., AND BARKER, G. M. 2002. *Achatina fulica* Bowdich and other Achatinidae as pests in tropical agriculture, p. 55-114 In G. M. Barker [ed.], Molluscs as Crop Pests. CABI Publ., Wallingford, UK and New York, USA.
- RAUT, S. K., AND GHOSE, K. C. 1984. Pestiferous land snails of India. Zoological Survey of India No. 11, Bani Press, Calcutta, 151 pp.
- RAO, I. G., AND SINGH, D. K. 2000. Effect of single and binary combinations of plant-derived molluscicides on reproduction and survival of the snail *Achatina fulica*. Arch. Environ. Contam. Toxicol. 39: 486-493.
- SENAVE, P. 2012. Notificación de Plaga - *Lissachatina fulica* (Caracol Gigante Africano). Press release Oct 2012. 2 pp.
- SINGH, K., AND SINGH, D. K. 1997a. Molluscicidal activity of plant derived molluscicides. J. Herbs Spices Med. Plants 5: 66-73.
- SINGH, K., AND SINGH, D. K. 1997b. Molluscicidal activity of *Nerium indicum* leaf. Fitoterapia 68: 545-546.
- THIENGO, S. C., FARACO, F. A., SALGADO, N. C., COWIE, R. H., AND FERNANDEZ, M. A. 2007. Rapid spread of an invasive snail in South America: the giant African snail, *Achatina fulica*, in Brasil. Biol. Invasions 9: 693-702.
- SRIVASTAVA, P. D. 1992. Problem of land snail pests in agriculture: a study of the giant African snail. Concept Publ. Co., New Delhi, 234 pp.
- THIENGO, S. C., FARACO, F. A., SALGADO, N. C., COWIE, R. H., AND FERNANDEZ, M. A. 2007. Rapid spread of an invasive snail in South America: the giant African snail, *Achatina fulica*, in Brasil. Biol. Invasions 9: 693-702.
- VAN WEEL, P. B. 1948/49. Some notes on the African giant snail, *Achatina fulica* Fer. I. On its spread in the Asiatic tropics. II. On its economic significance. III. On its biological balance and means of destruction. Chronica Naturae 104: 241-243, 278-280, 335-336.