

Target host: Argentine ant (AA) | *Linepithema humile* (Mayr)

## Laboratory Screening of Selected Synthetic and Organic Insecticides for Efficacy Against Argentine Ants When Incorporated Into Alginate Hydrogel Beads, 2021

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Section Editor: Michelle Brown

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The objective of this study was to assess the efficacy of nine different insecticides with previously demonstrated efficacy against *Linepithema humile* when delivered to ants via a novel delivery system, alginate hydrogel beads (HGBs). Insecticides mixed with 25% sucrose water were absorbed into HGBs upon which ants fed (see below for more details). The nine insecticides tested were: 1) Zylam L (dinotefuran), 2) Boracare (disodium octaborate tetrahydrate), 3) Mectinite (emamectin benzoate), 4) Taurus SC (fipronil), 5) Marrone Bio Innovations (MBI) 306 EP (94.5% heat-killed *Burkholderia rinojensis* cells and spent fermentation media), 6) Dominion 2L (imidacloprid), 7) Fuse (imidacloprid + fipronil), 8) Antixx Lab (Spinosad), and 9) Optigard Flex L (thiamethoxam); Tables 1 and 2). Spinosad and *B. rinojensis* are approved for organic use. All formulations screened in this study have water solubilities (Tables 1 and 2) that should enable uptake by HGBs when mixed with 25% sucrose solution.

All tests were conducted in the laboratory over a 5-mo period (Sep 2020 to Feb 2021) and used field-collected *L. humile* colonies that were maintained in plastic containers (see below for additional details). Efficacies of insecticide-sucrose water-infused HGBs were tested with three different concentrations of each insecticide examined (referred to as ‘low’, ‘medium’, and ‘high’ concentrations; Tables 1 and 2). Experimental concentrations identified for testing were selected based on a review of published scientific literature that assessed insecticide effects on *L. humile*. The total amount of AI in treatments differed across insecticide treatments. All experimental treatments were paired with sucrose water-treated HGB controls that lacked insecticides. The experimental design was a randomized complete block with 10 replications per treatment and blocking was based on time as five replicates/time of yr were prepared for each treatment ( $N = 360$ ; 5 replicates  $\times$  2 times of year [blocks]  $\times$  9 insecticides  $\times$  4 concentrations [untreated controls/low/medium/high]).

HGBs were made by mixing sodium alginate (Na-Alg) with a calcium chloride ( $\text{CaCl}_2$ ) solution. Briefly, 1 g of Na-Alg (medium-viscosity; Sigma Aldrich, St. Louis, MO) was added into 100 ml

of deionized water to obtain 10 g/liter of Na-Alg solution. The obtained solution was stirred for 30 min at 60°C to achieve a homogenous solution and left to cool to ambient temperature (~26°C). The cooled Na-Alg solution was then dispensed with a 5 ml syringe (BD Bioscience, San Jose, CA) dropwise into 10 g/liter of  $\text{CaCl}_2$  solution. The resulting HGBs were subsequently filtered from the  $\text{CaCl}_2$  solution after 5 min and washed with deionized water to remove excess  $\text{CaCl}_2$  from HGB surfaces. HGBs were then conditioned for 24 h in a 25% sucrose solution containing each one of the experimental insecticide concentrations. Control HGBs were made in an identical manner except the 25% sucrose solution in which beads were conditioned lacked test insecticides. Twenty-four hours later, HGBs were removed from the sucrose solution, dried with paper towels to remove excess moisture from the surface, and used immediately in experiments.

The efficacy of HGBs to deliver low/medium/high concentrations of each insecticide was tested with laboratory colonies of *L. humile*. Experimental ant colonies were kept in polyethylene containers (330  $\times$  190  $\times$  100 mm) the inner sides of which were coated with the polytetrafluoroethylene suspension (BioQuip, Rancho Dominguez, CA) to prevent ants escaping. A 100-mm Petri dish with the plaster of Paris nesting core with four equi-spaced 4-mm side holes served as the artificial nest site. Each AA colony had 300 workers and two queens collected from a citrus grove located at the University of California, Riverside, CA. *L. humile* colonies were acclimatized for 14 d before conducting experiments. Once per week, ants were provided with fresh water, a 25% sucrose solution, and fresh-killed cockroaches for protein. After this 2-wk period, experimental colonies were starved for 3 d prior to the introduction of either insecticide treated HGBs or control HGBs conditioned in sucrose solution that lacked toxicants. For insecticide and control treatments, three HGBs were placed on the bottom of each colony box. At 24 h post treatment, the experimental colonies were returned to their regular diet. The HGBs were not removed from the colony box. The number of

live queens and workers was recorded at 1, 3, 7, and 14 d post treatment. The percent reductions in the number of workers and queens were calculated and compared across treatments, including controls. Mortality rates were calculated using Abbott's correction formula.

Data were analyzed in SAS 9.4. Response variables were the percentage reductions of workers and queens. Mean response variables were compared within each evaluation date (DAT) using generalized linear mixed models (Proc GLIMMIX in SAS) assuming binomial distribution for percent mortality of workers and queens. Block was set as the random variable. Pesticide treatment differences in response variables (pooled across low/medium/high concentrations) were analyzed using a repeated measures model incorporating data from all DATs. When there were significant treatment effects, means separation using Tukey's HSD multiple comparisons tests were performed. Significance effects were set at  $P < 0.05$  for all tests.

All treatments reduced the number of AA workers and queens when compared with the untreated control on all posttreatment DATs and overall data (Tables 1 and 2). Zylam Liquid, Fuse, Antixx Lab, and Optigard Flex L caused significantly higher mortality of workers and queens than other treatments or the 25% sucrose-only HGB controls. There were no significant differences in worker and queen mortalities between the low, medium, and high concentrations of Zylam Liquid, Fuse, Antixx Lab, and Optigard Flex L on any DAT. Data analyses indicate that Zylam Liquid or Optigard Flex L (both represent the same mode of action; Tables 1 and 2), Fuse, and Antixx Lab, are efficacious against *L. humile*. Because these insecticides have different modes of action, they may collectively mitigate resistance development when used together in a rotation program for *L. humile* management. Field trials are needed to evaluate efficacy of best performing insecticides and rates when delivered to *L. humile* using HGBs.<sup>1</sup>

**Table 1.**

Treatment/Formulation (% AI)	IRAC MOA (water solubility w/v)	Concentration %/vol (‘concentration’)	Mean % mortality of AA workers at time (day) after exposure				
			1 DAT	3 DAT	7 DAT	14 DAT	All
Zylam Liquid (10% dinotefuran)	IRAC 4A (39.83 g/liter)	0.0001 (low)	51.73abcd	77.81abc	87.51a	94.87a	83.47a
		0.0005 (medium)	64.25a	85.37ab	92.46a	97.36a	
		0.001 (high)	73.1a	85.61a	94.1a	97.45a	
Bora-Care (40% disodium octaborate tetrahydrate)	IRAC 8D (223.65 g/liter)	0.25 (low)	28.43bcdefg	42.47defgh	50.01bc	59.48bc	48.56b
		0.5 (medium)	29.4cdefg	40.74defgh	54.73bc	63.52b	
		1 (high)	32.86bcdefg	56.58cdefgh	57.61b	66.86b	
Mectinita (4% emamectin benzoate)	IRAC 6 (320 mg/liter)	0.001 (low)	15.55g	23.5h	30.31c	37.59c	34.79b
		0.01 (medium)	17.13fg	33.55gh	39.92bc	50.02bc	
		0.1 (high)	24.96defg	40.18efgh	49.24bc	55.49bc	
MBI 306 EP (94.5% <i>Burkholderia rinojensis</i> )	N/A (N/A)	0.1 (low)	29.37cdef	51.59defgh	54.14bc	62.13b	44.39b
		1.1 (medium)	29.48cdef	43.83defgh	55.7b	67.39b	
		11 (high)	14.46g	29.61gh	43.51bc	51.42bc	
Dominion 2L (21.4% imidacloprid)	IRAC 4A (61 g/liter)	0.0001 (low)	22.78efg	33.24gh	40.53bc	51.66bc	38.56b
		0.0005 (medium)	15.77g	33.16gh	45.03bc	55.86bc	
		0.001 (high)	19.17efg	37.9fgh	48.33bc	59.18bc	
Taurus SC (9.1% fipronil)	IRAC 2B (3.78 mg/liter)	0.0001 (low)	45.01abcde	67.26abcde	84.43a	91.67a	80.24a
		0.0005 (medium)	61.44ab	73.15abc	86.06a	93.91a	
		0.001 (high)	73.48a	88.54a	98.13a	99.78a	
Fuse (21.4% imidacloprid + 6.6% fipronil)	IRAC 4A + 2B (61 g/liter + 3.78 mg/liter)	0.0001 (low)	46.06abcde	69.44abcde	85.34a	93.78a	76.7a
		0.0005 (medium)	52.74abcd	73.48abc	83.79a	90.12a	
		0.001 (high)	62.31a	77.17abc	89.72a	96.44a	
Antixx Lab (0.015% Spinosad)	IRAC 5 (10 g/liter)	0.00015 (low)	45.03abcde	66.83abcde	83.23a	93.14a	79.33a
		0.0005 (medium)	63.32a	80.26ab	89.79a	95.68a	
		0.0015 (high)	64.63a	84.36a	92.01a	97.69a	
Optigard Flex L (21.6% thiamethoxam)	IRAC 4A (4.1 g/liter)	0.0001 (low)	57.16abc	77.02abc	90.48a	96.46a	83.89a
		0.0005 (medium)	60.28a	84.31a	94.26a	97.72a	
		0.001 (high)	68.73a	87.87a	94.53a	97.91a	
Untreated Control	N/A	N/A	5.35h	7.26i	9.45d	10.73d	8.19c
<i>F</i>			25.47	38.96	66.29	87.51	13.43
<i>P</i>			<.0001	<.0001	<.0001	<.0001	<.0001

Means within columns followed by the same letter are not significantly different according to Tukey's HSD.

<sup>1</sup>This research was supported in part by the California Department of Pesticide Regulations grant No. 20-PMG-GR005 and by industry research funding and donations of test insecticides. The authors declare that they have no conflict of interest.

Table 2.

Treatment/Formulation (% AI)	IRAC MOA (water solubility w/v)	Concentration %/vol (‘concentration’)	Mean % mortality of AA queens at time (day) after exposure				
			1 DAT	3 DAT	7 DAT	14 DAT	All
Zylam Liquid (10% dinotefuran)	IRAC 4A (39.83 g/liter)	0.0001 (low)	40abc	50abc	55abcd	65abc	68.75a
		0.0005 (medium)	55ab	65ab	75ab	85ab	
		0.001 (high)	65a	85a	90a	95a	
Bora-Care (40% disodium octaborate tetrahydrate)	IRAC 8D (223.65 g/liter)	0.25 (low)	30abc	30abc	35cde	45cd	29.58b
		0.5 (medium)	15cd	20bcd	25de	35cd	
		1 (high)	25bcd	25bcd	30cde	40cd	
Mectinite (4% emamectin benzoate)	IRAC 6 (320 mg/liter)	0.001 (low)	10cd	10cd	20de	20de	17.92c
		0.01 (medium)	10cd	10cd	20de	20de	
		0.1 (high)	15cd	20cd	25de	35cd	
MBI 306 EP (94.5% <i>Burkholderia rinojensis</i> )	N/A (N/A)	0.1 (low)	15cd	20cd	50cd	50cd	20.42c
		1.1 (medium)	15cd	15cd	25de	30cd	
		11 (high)	5cd	5cd	5e	10e	
Dominion 2L (21.4% imidacloprid)	IRAC 4A (61 g/liter)	0.0001 (low)	15cd	15cd	30cde	35cd	23.75c
		0.0005 (medium)	10cd	15cd	30cde	45cd	
		0.001 (high)	10cd	10cd	35bcde	35cd	
Taurus SC (9.1% fipronil)	IRAC 2B (3.78 mg/liter)	0.0001 (low)	10cd	15cd	25def	35cd	31.25b
		0.0005 (medium)	5cd	20bcd	35bcde	55bc	
		0.001 (high)	10cd	20bcd	75ab	70ab	
Fuse (21.4% imidacloprid + 6.6% fipronil)	IRAC 4A + 2B (61 g/liter + 3.78 mg/liter)	0.0001 (low)	25bcd	40bc	60abcd	80ab	52.92a
		0.0005 (medium)	25bcd	35bcd	55bcd	75ab	
		0.001 (high)	40abc	60ab	60abcd	80ab	
Antixx Lab (0.015% Spinosad)	IRAC 5 (10 g/liter)	0.00015 (low)	15cd	55abc	80a	85a	62.5a
		0.0005 (medium)	40abc	55abc	70abc	70ab	
		0.0015 (high)	15cd	80a	85a	100a	
Optigard Flex L (21.6% thiamethoxam)	IRAC 4A (4.1 g/liter)	0.0001 (low)	20bcd	70ab	85a	95a	71.25a
		0.0005 (medium)	25bcd	75ab	90a	95a	
		0.001 (high)	30abc	80a	90a	100a	
Untreated Control	N/A	N/A	0.01e	0.01e	0.01f	0.02f	0.01e
F			5.98	15.08	19.57	26.37	7.62
P			<.0001	<.0001	<.0001	<.0001	<.0001

Means within columns followed by the same letter are not significantly different according to Tukey's HSD.