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Potential of Water Extracts from *Reticulitermes Flavipes* on Control of *Solenopsis Gayi*

Joaquín Ipinza-Regla[1], Alejandra Olivares[1], Jaime Eduardo Araya[2, *]

[1]Laboratory of Zoology and Ethology, College of Science, Universidad Mayor, Santiago, Chile
[2]College of Agronomic Sciences, University of Chile, Santiago, Chile

Email address:
jaimearaya@yahoo.com (J. E. Araya)
[*]Corresponding author

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Abstract: Five nests each of *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae) and *Solenopsis gayi* Spinola (Hymenoptera: Formicidae) were collected in sites distanced at least 1 km in the Metropolitan Region, Santiago, Chile, to study the modification of hermeticism conducts of termites exposed to ant specimens on transparent plastic arenas lined with filter paper sprayed or bathed with a water extract of the termite, obtained by setting 20 of them on test tubes that were frozen, added acetone, and stirred in a vortex mixer. Finally the termites were eliminated and the acetone evaporated with gaseous nitrogen. Then, the extracts were dissolved in H_2O. A control group was used per nest. The behavior of *S. gayi* receiving *R. flavipes* treated or untreated was observed during 6 min, registering antennal exploration (AE), mandible opening (MO), biting (BI), abdomen flexed dorsally or ventrally (AFD and AFV, respectively), fighting (FI), backward movement (BM), transport of intruder (TI), and death (DE). The behavior of *R. flavipes* receiving treated or untreated termites was also observed during 6 min, registering the approach to the intruder and brief pursuit by one or more termites (API), mandible opening (MO), and biting (BI). The transference of ants treated to their original nests increased the frequency of recognition events (AE and MO), as well as BI, which occurred earlier, compared with control groups. In contrast, when treated ants were transferred to receiving termites, a decreased frequency and a delayed appearance occurred in API and MO. Intruder death (DE) did not occur in both transference bioassays. In summary, *S. gayi* treated were recognized as intruders and rejected by the ants of their nests. In contrast, the recipient *R. flavipes* termites recognized the extract-treated ants as members of their nests.

Keywords: Behavior Parameters, Epicuticle Extract, Fire Ant, Hermetism, Subterranean Termite

1. Introduction

The behavior of the ants is characterized by hermeticism, that is, the rejection of intrusive individuals, mediated by the identification of odors in their epicuticular wax [1]. Ants are the main natural enemies of termites [2], although few specialize in predating Isoptera [3]. Extracts of the ant *Pheidole megacephala* F. could be used to initiate the agonistic behavior of *Coptotermes formosanus* Shiraki soldiers towards termites of the same species [4]. In Chile, the fire ant *Solenopsis gayi* Spinola is distributed from Arica to Tierra del Fuego [5]. The agonistic effect between *S. gayi* and *R. flavipes* has beed reported in the laboratory [6]. Herein we evaluated the effect of the water extract of *R. flavipes* on the rejection behavior in nests of *S. gayi*. Also evaluated were individuals of this ant transferred to colonies of *R. flavipes*.

2. Materials and Methods

Five nests of *S. gayi* containing each at least 200 workers and varying numbers of eggs and larvae were collected in dry sites distanced at least 1 km in northern Santiago, Chile. Also, five colonies of *R. flavipes* were collected from infested houses in this area. These nests were taken to the Zoology and Ethology Laboratory, Universidad Mayor, Santiago, Chile, and were acclimated for 3 wk. The ants were kept in 34 x 26 x 14 cm transparent plastic boxes at 20±2°C, 45-50% RH, and 12 h fluorescent light, and fed apple pure mixed with honey and ground meat to ±provide them proteins. The termites were kept

in 29 x 19 x 12 cm transparent plastic boxes at 27°C and 90% RH, and fed pinewood and corrugate cardboard pieces.

The water extract of *R. flavipes* was obtained by setting 20 termites on test tubes that were frozen, added acetone, and stirred in a magnetic vortex mixer. Finally the termites were eliminated and the acetone evaporated with gaseous nitrogen. Then, H_2O was added to dissolve the extracts obtained. Controls treated only with H_2O sprayed or bathed were used per nest. The aggression and rejection conducts displayed by the termites exposed to ant specimens on the plastic arenas lined with filter paper sprayed or bathed (2 mL) with the water extract of the termite, were they remained 30 min before the transferences of at least 10 individuals to obtain statistically valid data [7]. The behavior of *S. gayi* receiving *R. flavipes* treated or untreated and left 3 h on the arenas before the transferences was observed in 9 x 9 x 8 cm plastic boxes during 6 min, registering with a video camera antennal exploration (AE), mandible opening (MO), biting (BI), abdomen flexed dorsally or ventrally (AFD and AFV, respectively), fighting (FI), backward movement (BM), transport of intruder (TI), and death (DE). The behavior of *R. flavipes* receiving treated or untreated termites was also observed during 6 min, registering the approach to the intruder and brief pursuit by one or more termites (API), mandible opening (MO), and biting (BI).

The data obtained were used to calculate behavior parameter means ± SD. Differences in parameter frequencies between treated and untreated ants were determined with the Fisher test; appearance times for each parameter were determined with the Student t test.

3. Results

The observations (n) and frequency of occurrence (%) of behavior parameters after transferences of *S. gayi* ants bathed on or sprayed previously with ant water extracts to untreated ants are presented in Table 1, and the results of transferences of untreated *S. gayi* to receiving ants bathed on or sprayed previously with ant water extracts in Table 2.

AE occurred in 90-92% of the ants treated by bath or untreated, respectively. MO occurred in 68 and 60% of the same ant treatments, respectively, also a non-significant difference. Also, only 2% of the bath treated or untreated ants presented AFV. However, BI occurred in 12 and 4% of the same ant treatments, respectively, this time a significant ($P \leq 0.05$) difference.

All the ants sprayed with the extract presented AE, and 86% in the control, a significant difference. MO occurred in 76% of the ants sprayed with the extract, and in 42% of those untreated, again a significant difference. BI occurred in 12% of the ants sprayed, and not at all in the corresponding controls, also a significant difference. Similarly AFV occurred in only 2% of the sprayed ants, but not in the untreated ones, not significantly different. AFD, FI, BM, and DE did not occur in any of the receiving nests.

Table 1. *Observations (n) and frequency of occurrence (%) of behavior parameters after transferences of S. gayi ants bathed on or sprayed previously with ant water extracts to untreated ants.*

Nests	Transferences	Behavior parameters							
		AE		MO		BI		AFV	
Ants bathed previously									
Nest 1	AE to AU	10	100	10	100	2	20	---	---
	AW to AU	10	100	6	60	---	---	---	---
Nest 2	AE to AU	8	80	1	10	---	---	---	---
	AW to AU	10	100	8	80	---	---	---	---
Nest 3	AE to AU	7	70	6	60	1	10	1	10
	AW to AU	9	90	3	30	---	---	---	---
Nest 4	AE to AU	10	100	8	80	---	---	---	---
	AW to AU	8	80	4	40	---	---	---	---
Nest 5	AE to AU	10	100	9	90	3	40	---	---
	AW to AU	9	90	9	90	---	---	1	10
N and freq. totals	AE to AU	45	90	34	68	6	12	1	2
	AW to AU	43	86	21	42	0	0	2	2
$p < 0.05$ (Fisher)		0.4028		0.1512		0.0326*		0.6894	
Ants sprayed previously									
Nest 1	AE to AU	10	100	8	80	---	---	---	---
	AW to AU	10	100	5	50	---	---	---	---
Nest 2	AE to AU	10	100	9	90	2	20	1	10
	AW to AU	10	100	7	70	---	---	---	---
Nest 3	AE to AU	10	100	7	70	2	20	---	---
	AW to AU	7	10	4	40	---	---	---	---
Nest 4	AE to AU	10	100	6	60	---	---	---	---
	AW to AU	---	---	---	---	---	---	---	---
Nest 5	AE to AU	10	100	8	80	2	20	---	---
	AW to AU	---	---	5	50	---	---	---	---
N and freq. totals	AE to AU	50	100	38	76	6	12	1	2
	AW to AU	43	86	21	42	0	0	0	0
$p < 0.05$ (Fisher)		<0.0001*		<0.0001*		0.0002*		0.2487	

AE: Antennal exploration; MO: Mandibles opening; BI: Biting; AFV: Abdomen flexed vertically. AE to AU: Ants treated with water extract transferred to ants untreated; AW to AU: Ants treated only with water transferred to ants untreated.

Table 2. *Observations (n) and frequency of occurrence (%) of behavior parameters after transferences of untreated S. gayi to receiving ants bathed on or sprayed previously with ant water extracts.*

Nests	Transferences	Behavior parameters							
		AE		MO		BI		AFV	
Receiving ants bathed previously									
Nest 1	AE to AU	10	100	10	100	---	---	1	10
	AW to AU	10	100	8	80	---	---	---	---
Nest 2	AE to AU	9	90	8	80	1	10	---	---
	AW to AU	10	100	8	80	---	---	---	---
Nest 3	AE to AU	10	100	10	100	1	10	1	10
	AW to AU	9	90	5	50	---	---	---	---
Nest 4	AE to AU	10	100	9	90	---	---	2	20
	AW to AU	10	100	8	80	1	10	2	20
Nest 5	AE to AU	10	100	9	90	1	10	---	---
	AW to AU	8	80	7	70	---	---	1	10
N and freq. totals	AE to AU	49	47	46	92	3	6	4	8
	AW to AU	98	94	36	72	1	2	3	6
$p < 0.05$ (Fisher)		0.1395		0.0002*		0.1395		0.914	
Receiving ants sprayed previously									
Nest 1	AE to AU	10	100	7	70	---	---	---	---
	AW to AU	10	100	10	100	---	---	---	---
Nest 2	AE to AU	10	100	8	80	1	10	---	---
	AW to AU	10	100	7	70	---	---	---	---
Nest 3	AE to AU	10	100	5	50	2	20	---	---
	AW to AU	6	60	3	30	---	---	---	---
Nest 4	AE to AU	10	100	5	50	---	---	---	---
	AW to AU	9	90	8	80	---	---	---	---
Nest 5	AE to AU	9	90	7	70	---	---	---	---
	AW to AU	9	90	9	90	1	10	---	---
N and freq. totals	AE to AU	49	98	32	64	3	6	---	---
	AW to AU	44	88	37	74	1	2	---	---
$p < 0.05$ (Fisher)		0.0050*		0.0843		0.1395		---	

AE: Antennal exploration; MO: Mandibles opening; BI: Biting; AFV: Abdomen flexed vertically. AE to AU: Ants treated with water extract transferred to ants untreated; AW to AU: Ants treated only with water transferred to ants untreated.

AE occurred in 98 and 94% of the ants sprayed and the controls, respectively, levels statistically similar. Also BI occurred in 6 and 2% of the ants sprayed and untreated, respectively, with not a significant difference. The same occurred with AFV, with 8 and 6% statistically similar levels, respectively. However, MO occurred statistically differently in 92 and 72% of the corresponding treatments, respectively. AFD, FI, BM, TI, and DE did not occur in the receiving nests.

Table 3. *Observations (n) and frequency of occurrence (%) of behavior parameters of R. flavipes after transferences to receiving termites of S. gayi ants bathed on or sprayed previously with ant water extracts.*

Nests	Transferences	Behavior parameters					
		AP		MO		BI	
Ants bathed previously							
Nest 1	AE to TE	10	0	7	70	1	10
	AW to TE	10	0	10	100	9	90
Nest 2	AE to TE	10	0	7	70	3	30
	AW to TE	9	90	7	70	2	20
Nest 3	AE to TE	8	80	6	60	2	20
	AW to TE	9	90	5	50	3	30
Nest 4	AE to TE	9	90	8	80	3	30
	AW to TE	10	0	9	90	4	40
Nest 5	AE to TE	10	0	6	60	2	20
	AW to TE	10	0	8	90	1	10
N and freq. totals	AE to TE	47	48	34	68	11	22
	AW to TE	94	96	9	78	19	38
$p<0.05$ (Fisher)		0.3738		0.0757		0.0101*	
Ants sprayed previously							
Nest 1	AE to TE	10	0	9	90	3	30
	AW to TE	9	90	8	80	8	80
Nest 2	AE to TE	10	0	8	80	1	10
	AW to TE	10	0	9	90	4	40
Nest 3	AE to TE	9	90	6	60	1	10
	AW to TE	10	0	4	40	---	---

Behavior parameters							
Nests	**Transferences**	**AP**		**MO**		**BI**	
Nest 4	AE to TE	7	70	9	90	---	---
	AW to TE	10	100	6	60	---	---
Nest 5	AE to TE	10	100	9	90	3	30
	AW to TE	10	100	7	70	3	30
N and freq. totals	AE to TE	46	92	41	82	8	16
	AW to TE	49	98	34	68	15	30
$p < 0.05$ (Fisher)		0.0503		0.0166*		0.0141*	

AP: Approach and short lasting close up of 1 or more termites to the intruder ant; MO: Mandibles opening and closing; BI: Biting; AFV: Abdomen flexed vertically. AE to TE: Ants treated with water extract transferred to *R. flavipes*; AW to TE: Ants treated only with water transferred to *R. flavipes*.

AE occurred in 98 and 88% of the ants sprayed and controls transferred into receiving nests, respectively, a significant difference. However, MO (64 and 74%, respectively, and BI (6 and 2%, respectively), were each statistically similar. Also, AFD, AFV, FI, BM, TI, and DE did not occur in any of these transferences.

When transferring *S. gayi* bathed in the extract to *R. flavipes*, 94 and 96% of the ants approached (AP) the receiving termites, respectively, statistically similar levels. Also similar were the levels of MO (68 and 78%, respectively) and BI (22 and 38%, respectively). When transferring *S. gayi* sprayed with the extract to *R. flavipes*, the corresponding AP, MO, and BI levels were 92 and 98%, 82 and 68%, and 16 and 30%, respectively, statistically similar values in each behavior parameter.

4. Discussion

Several studies have confirmed the role of the content of the postpharingeal gland as modifier of the agonistic behavior, as in the desert ant *Cataglyphis niger* (André) and the giant red ant *Manica rubida* (Latreille), where the application of the secretion of this gland from a resident to an intruder ant reduced the aggression to the alien, while the application of this secretion from an intruder to a resident increased the agonistic behavior in the nest workers [8]. Increased attacks have occurred when applying cuticle extracts onto dead ants [*Camponotus vagus* (Scopoli) [9] and *Cataglyphis cursor* Fonscolombe, respectively [10].

Coptotermes formosanus Shiraki termites sprayed with extracts from *Pheidole megacephala* F. ants, were attacked by their soldiers [4]. Increased recognition of *Camponotus morosus* Smith and decreased first appearance events towards *R. hesperus* have been observed [11], however with no increase in termite deaths.

Studies of ant hermeticism related with *R. flavipes* epicuticle extracts are important in the search of alternative management methods for the aggressive ant *S. gayi*, and possibly other Formicidae as well.

Our results were obtained during fall and winter, under not optimal temperature conditions that may have affected the behavior parameter registered. Further studies should eliminate this factor.

5. Conclusions

S. gayi ants sprayed or bathed with water extract from the cuticle of *R. flavipes* were similarly rejected aggressively by conspecific ants, but they were accepted in the termite colonies, thus could provide an alternative management for this aggressive ant. However, further studies are necessary to evaluate the possibility of using water extracts from *S. gayi* fire ants to control *R. flavipes*.

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