EVALUATION OF AN ARTIFICIAL DIET FOR THE SURVIVAL OF WORKERS IN LABORATORY OF *ACROMYRMEX LOBICORNIS* EMERY (INSECTA: HYMENOPTERA: FORMICIDAE)

Milton Ruiz Espíndola*, Alberto Pilati* and Estela M. Quirán*

* Faculty of Natural Sciences, UNLPam, Uruguay 151, CP LC6300CLB, Santa Rosa, La Pampa, ARGENTINA. E-mail: miltonespindolaruiz@gmail.com

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ABSTRACT: Cutting ants are social insects comprising two genera *Atta* and *Acromyrmex* (Insecta: Hymenoptera: Formicidae). They have the habit of cutting and transporting fragments of various vegetables, flowers and / or seeds to their underground nests. These habits have become pests of cultivated areas and natural pastures of South America, Central and North, especially eucalyptus and pine forest plantations because they cause damage to the productivity of plants. For their control are used agrochemicals and is currently initiating the study of plant extracts with insecticidal properties, which must be pre-tested. Therefore, the objective of this work was to obtain an artificial diet that meets the metabolic requirements of ants *A. lobicornis* E. in the laboratory, and ensure their survival while they last bioassays. Various diets ants were tested and concluded that SOL 24, LIQ 24, LIQ 48, 24 and SOL SOL 48 diets are suitable for the survival of *A. lobicornis* workers during toxicity bioassays.

KEY WORDS: Cutting ants, diets, survival, Argentina

Ants belonging to the family Formicidae the order Hymenoptera, further including wasps and bees. It has eleven subfamilies, 300 genera and almost no less than twenty thousand species (Hölldobler & Wilson, 1990). Within this family we are the Myrmicinae subfamily in which the tribe Attini containing all the ants cultivate fungus is located. This tribe contains two genera A*tta* and *Acromyrmex*, and are of great importance in agriculture due to the damage caused (Della Lucía, 1993).

The genera *Atta* and A*cromyrmex* are described by Hölldobler & Wilson (1990) as the dominant herbivores of the Neotropical Region, consuming many more plants than any other comparable group taxonomic diversity.

Cutting ants attack a large number of plants, being selective as to the plants foraged (Pilati et al., 1997).

Generally not forages for a long time on a single species, but this behavior depends a lot of plants available. The selectivity appears to be related to an amount of water and nutrients, as well as attractive compounds, repellent or plant deterrents (Diehl Fleig, 1995).

They are distributed in the neotropics from Texas to northern Argentina.

Acromyrmex genus contains 31 species that are distributed in South America, parts of Central America and some caribbean islands. They are commonly known as "leaf-cutter ants" within the genus *Acromyrmex,* the species *A. lobicornis* Emery, 1887 reached a significant geographic spread in Argentina from the north to the parallel 44° s, in Chubut (Franzel & Farji-Brener, 2000) and is the only species to harvest both monocots and dicots (Pilati et al., 1997).

These ants to cut and transport fragments of various vegetables, flowers and / or seeds to their underground nests, the pests have become cultivated areas and natural pastures of South America, Central and North (Della Lucía, 1993) especially eucalyptus and pine forest plantations because they cause damage to the productivity of plants. Because of this, countries spend huge amounts of agrochemicals to control them as ants (Marsaro & Della Lucía, 1997).

In the search for new active ingredients for pest control it has begun using plants, which was noted by the popular knowledge are indicated as they have a negative effect on insects in general and for the control of leaf-cutting ants in particular.

Undoubtedly, natural insecticides from plant extracts are an interesting alternative insect control, to replace synthetic pesticides, plus only very few plants have been evaluated in relation to the natural source that provides the planet (Chen et al., 1984, 1997; Vieira et al., 1997; Iannacone, 2003).

To perform the indicated bioassays (Hebling et al., 1993; Maroti et al., 1993) is required to have abundant biological material, in this case, workers of cutting ants, so it is essential to the survival of anthills in the laboratory, during throughout the year, and given that winter in the field, the insects are not active, you must have an artificial diet that meets the metabolic requirements of ants *A. lobicornis* E., during that season.

The survival of *A. lobicornis* laboratory for bioassays, can be achieved with a suitable artificial diet.

Studies on biology, ecology, behavior and control of ant species are made to field or laboratory ant stocked with fresh plant material. For this breeding systems of different types they are used but in all cases the environmental conditions must be perfectly controlled (Della Lucía, 1993).

The bioassays with insects in artificial conditions requires alternative feeding to keep them alive for long enough period of time to complete the necessary observations. Among the attempts to generate a suitable alternative diet, they may be mentioned Howard et al. (1988), *Atta cephalotes* on operatives of (L.), and Bueno *et al.* (1997) adult operatives *Atta sexdens rubropilosa* Forel, isolated artificial nests.

Hence the objective of this study is to evaluate artificial diets for workers isolated the anthills of field on ant *Acromyrmex lobicornis* of the Pampeana Region, allowing their survival in the laboratory, in order to use in bioassays of toxicity.

It is expected to achieve a suitable artificial diet for the survival of *A. lobicornis* in laboratory bioassays for toxicity.

MATERIALS AND METHODS

For this study they were used workers of *Acromyrmex lobicornis* ant isolated field. Four ant nests were collected, both located at the Faculty of Agronomy (La Pampa) and the other two in the city of Santa Rosa, La Pampa, Argentina. (Fig. 1).

They were placed in the brood chamber Invertebrate Biology II Professor, Department of Natural Sciences, Faculty of Natural Sciences, the UNLPam, artificial anthills, under controlled temperature, humidity and light.

While the experience lasted, ants were fed *Erodium cicutarum* (Brad) collected from the surrounding countryside to the brood chamber (Hall of Biology, UNLPam) and rose petals.

The composition of the diets was:

-Diet Liquid: 5% glucose, 0.1% yeast extract (Difco) and 1% of bacteriological peptone dissolved in 100 ml. of distilled water

-Diet Solid: liquid diet plus 1.5% bacteriological agar (Difco)

-Experience Control: single ant

They were applied in 6 treatments as follows: T1 (water: H2O), T2 (liquid diet every 24 Hs: LIQ24), T3 (liquid diet every 48 hours: LIQ48), T4 (solid diet every 24 Hs: SOL24) T5 (solid diet every 48 hours: SOL48) and T6 (only ants: Control), in Petri dishes with 10 ants were placed.

The tests were conducted under controlled conditions (24 +/- 2 ° C, 70% RH and 12 h photoperiod). 6 mentioned treatments with 8 repetitions were performed. The average maximum longevity was compared using ANOVA.

10 (ten) ants were placed with an average length of 6.5 to 7.5 mm in a Petri dish of 15 cm diameter for each of the treatments.

Data were analyzed using statistical program PAST *(Paleontologic Stadistic Software Package)*. While the trials lasted five days, the data on the third day of the experiment were analyzed performed because usually tests with chemical insecticides not last longer than 72 hours.

RESULTS

For each of the diets the following results:

Diet 1: (Table 1, Graphic 1)
Individuals exposed to the first diet showed a low rate of supervivenvia. This is seen from the second day of testing for most repetitions.

Diet 2: (Table 2, Graphic 2)
Diet 2 consisted only of water, with replacement every 24 hours. Suvervivencia rate was very low, although most individuals survived until the third day of the experiment.

Diet 3: (Table 3, Graphic 3)
For testing liquid diet replaced every 24 hours, the results showed a low rate of superviencia for the last day of the experiment.

Diet 4: (Table 4, Graphic 4)
Diet 4 (liquid diet replaced every 48 hours) showed a high survival rate, it remains constant during the five days of the trial.

Diet 5: (Table 5; Graphic 5)
Individuals exposed to solid foods with replacements every 24 hours (diet 5) had a high survival from the start to the end of the trial.

Diet 6: (Table 6, Graphic 6)
Finally, in June diet (solid diet replacements every 48 hours), it had a high rate of individuals who set survived the trials end.

Analyzing the five diets it is observed that except water fed ants, all showed a similarity in the survival rate of the working *Acromyrmex lobicornis* (Graphic 7).

DISCUSSION AND CONCLUSIONS

Diets showed similarity in the survival rate of workers of *Acromyrmex lobicornis*. Choosing the best diet may be affected by factors that influence it, such as: ease of preparation, the time required for handling during the replacement, and less pollution. Thus, the results agree with those reported by Bueno et al. (1997) for *A. sexdens rubropilosa*. The solid diet replacements every 24 hours (SOL 24) showed a higher survival rate of individuals in the 8 reps, so this diet may be the most advisable to use.

Diets: Liquid replaced every 24 hours (LIQ 24), fluid replaced every 48 hours (LIQ 48), solid replaced every 24 hours (SOL 24) and solid replaced every 48 hours (SOL 48) were shown to be suitable for laboratory testing . These four diets were similar in terms of the survival rate of worker *A. lobicornis*, so any of these diets can be used to maintain a stable population of ants for a specified short time, for laboratory toxicity bioassays.

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LITERATURE CITED

Bernard, F. 1968. Les fourmis d'Europe accidéntale et septentrionale. Faune eur. et Bass. Med. 3. Masson. París. 411 pp.

Bueno, O., Morini, M. S. C., Pagnocca, F., Hebling, M. & Silva, O. 1997. Sobrevivencia de operarias de *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) isoladas do formiguero e alimentadas con dietas artificiais. Annales de la Sociedad Entomologica de Brasil, 26 (1): 107-113.

Chen, T. K., Amico, L. A. & Speelman, D. J. 1984. Ant-repellent triterpenoids from *Cordia alliodora*. The Journal Organic Chemistry, 48 (20): 3525-3531.

Costa, C. G., Bueno, O. C., Hebling, M. J. A. & Pagnocca, F. C. 1997. Toxicidad de substancias presentes no gergelim (*Sesamum indicum*) sobre *Atta sexdens* Forel (Hym, Formicidae). *In:* Anais di VI Internacional Pest Ant Symposium & XIII Encontro de Mirmecologia, Ilheus, Bahia, Brasil, pp 134.

Della Lucia, T. 1993. *As formigas cortadeiras*. Ed. T. M. C. Della Lucia. Visosa. Brasil. 262 pp.

Diehl Fleig, L. 1995. *As formigas*. Unisinos, Brasil. 85 pp.

Franzel, C. & Farji-Brener, A. G. 2000. Oportunistas o selectivas Plasticidad en la dieta de la hormiga cortadora de hoja *Acromyrmex lobicornis* en el noroeste de la Patagonia. Ecoogial Austral, 10: 159-168.

Hebling, M. J. A., Maroti, P. S., Bueno, O. C., Silva, O. A. & Pagnocca, F. C. 1993. Efeito das folhas de *Ipomea batatas* (batata-doce) no desenvolvimiento de formigueiros de *Atta sexdens rubropilosa* Forel, 1908 em laboratorio. *In:* Resumos Congreso Brasileiro de Entomologia, 14, Piracicaba, pp. 230.

Hòlldobler, B. & Wilson, E. O. 1990. The Ants. The Belknap Press of Harvard University Press. Cambridge.

Howard, J. J., Green T. P. & Wiemer, D. F. 1988. Comparative deterrency of two terpenoids to two genera of attine ants. The Journal Organic Chemistry: 2279-2288.

Iannacone, O. J. 2003. Efecto insecticida de cuatro extractos botánicos y del cartap sobre la polilla de la papa *Phthorimaea opercullella* (Zeller) (Lepidoptera: Gelechiidae), en el Perú. Entomotrópica: Revista Internacional para el Estudio de la Entomología Tropical, 18 (2): 95-105.

Hebling, M. J. A., Maroti, P. S., Bueno, O. C., Silva, O. A. & Pagnocca, F. C. 1993. Tratamientos de formigueiros com folhas de *Ricinus communis* e *Ipomea batatas*: efeitos fisiológicos em operarias de *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae). *In:* Resumos Congreso Brasileiro De Entomologia, 14, Piracicaba, pp 18.

Marsaro Junior, A. L. & Della Lucia, T. M. C. 1997. Avaliação da nao-preferencia de *Acromyrmex laticeps nigrosetosus* Forel ao corte de *Eucapylptus*. *In:* Anais di VI Internacional Pest Ant Symposium & XIII Encontro de Mirmecologia, Ilheus, Bahia, Brasil; pp 117.

Oster, G. & Wilson, E. O. 1978. The Insect Societies. The Belknap Press of Harvard University Press. Cambridge, 548 pp.

Past (Paleontologic Stadistic Software Package). 2012.

Pilati, A., Quirán, E. M. & Estelrich, H. D. 1997. Actividad forrajera de *Acromyrmex lobicornis* Emery (Hymenoptera: Formicidae) en un pastizal natural semiárido de la provincia de La Pampa (Argentina). Ecologia Austral, 7: 49-56.

Vieira, P. C., Fernandez, J. B., Da Silva, M. F. G. F., Hebling, M. J. A., Bueno, O. C., Pagnocca, F. C. & Da Silva, O. A. 1997. A Utilizaçao de plantas inseticidas no controle de saúvas. *In:* Anais di VI Internacional Pest Ant Symposium & XIII Encontro de Mirmecologia, Ilheus, Bahia, Brasil; pp. 121.

Table 1. "Survival rate to control ants 2012/2013 trials".

Survival control										
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Mean	DS
Day 1	1	1	1	1	1	1	1	1	1	0
Day 2	0,9	0	0,5	0,4	0,7	0,3	0,7	0,6	0,5125	0,27998724
Day 3	0,2	0	0	0	0,4	0	0	0	0,075	0,14880476
Day 4	0,1	0	0	0	0,4	0	0	0	0,0625	0,1407886
Day 5	0,1	0	0	0	0,3	0	0	0	0,05	0,1069045

Table 2. "Survival rate ants fed water tests 2012/2013".

Survival H2o										
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Mean	DS
Day 1	1	1	1	1	1	1	1	1	1	0
Day 2	0,8	1	0,9	0,7	0,9	0,8	0,9	1	0,85714286	0,10350983
Day 3	0,5	0,9	0,8	0,6	0,7	0,5	0,8	0,7	0,68571429	0,1457738
Day 4	0,2	0,7	0,7	0,4	0,5	0,3	0,6	0,5	0,48571429	0,18077215
Day 5	0,1	0,5	0,7	0,1	0,5	0,3	0,5	0,5	0,38571429	0,21380899

Table 3. "Survival rate to a liquid diet ants replaced every 24 hours assays 2012/2013".

Survival LIQ 24										
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Mean	DS
Day 1	1	1	1	1	1	1	1	1	1	0
Day 2	1	1	0,7	1	0,9	0,9	1	0,7	0,9	0,12247449
Day 3	0,9	1	0,6	0,8	0,8	0,7	0,8	0,6	0,775	0,12990381
Day 4	0,7	1	0,6	0,8	0,6	0,6	0,6	0,6	0,6875	0,1363589
Day 5	0,7	1	0,6	0,7	0,6	0,6	0,6	0,5	0,6625	0,14086785

Table 4. "Survival rate ants replaced with liquid diet every 48 hours in trials of 2012/2013".

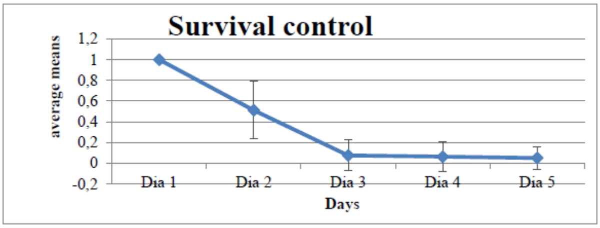
Survival LIQ 48										
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Mean	DS
Day 1	1	1	1	1	1	1	1	1	1	0
Day 2	0,8	1	1	1	0,9	1	0,9	1	0,95	0,07071068
Day 3	0,5	0,9	0,9	0,9	0,7	0,8	0,8	0,9	0,8	0,13228757
Day 4	0,5	0,8	0,9	0,8	0,7	0,8	0,8	0,9	0,775	0,11989579
Day 5	0,5	0,7	0,9	0,8	0,7	0,7	0,7	0,8	0,725	0,10897247

Table 5. "Survival rate to solid diet ants replaced every 24 hours assays 2012/2013".

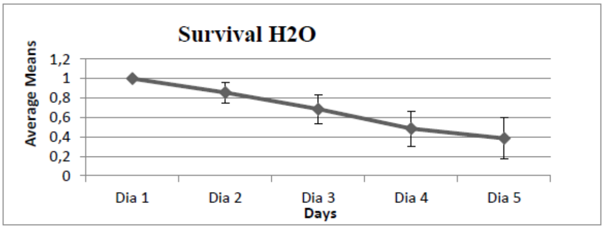
Survival SOL 24										
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Mean	DS
Day 1	1	1	1	1	1	1	1	1	1	0
Day 2	1	1	0,9	1	0,9	1	1	1	0,975	0,04330127
Day 3	1	0,9	0,8	1	0,9	1	0,9	0,9	0,925	0,06614378
Day 4	1	0,9	0,8	0,9	0,8	0,9	0,9	0,9	0,8875	0,05994789
Day 5	1	0,8	0,8	0,8	0,8	0,9	0,8	0,7	0,825	0,08291562

Table 6. "Survival rate to solid diet ants replaced every 48 hours assays 2012/2013".

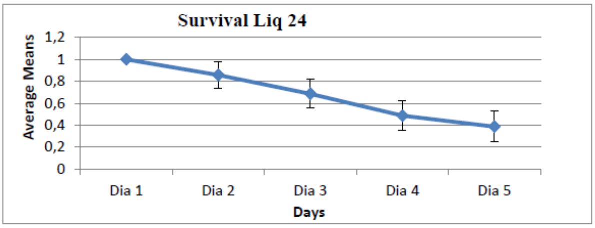
Survival SOL 48										
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Mean	DS
Day 1	1	1	1	1	1	1	1	1	1	0
Day 2	1	1	0,9	1	1	0,9	0,9	1	0,9625	0,04841229
Day 3	1	1	0,9	0,8	0,9	0,8	0,8	1	0,9	0,08660254
Day 4	1	1	0,7	0,8	0,8	0,7	0,7	1	0,8375	0,13169567
Day 5	1	0,8	0,6	0,8	0,7	0,5	0,7	0,8	0,7375	0,14086785



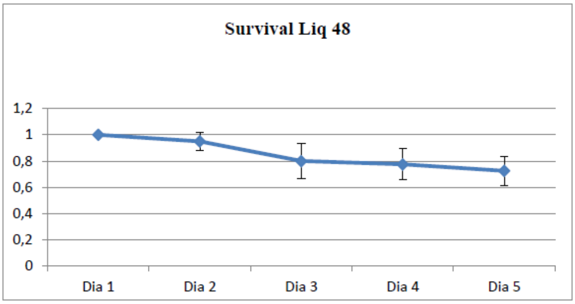
Graphic 1. "Evaluation of survival in control tests ants 2012/2013".



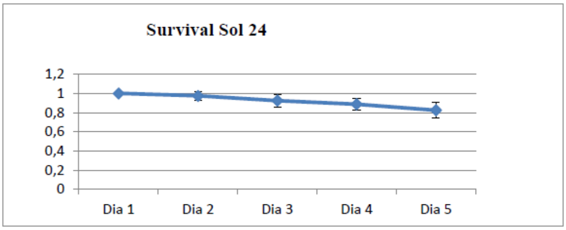
Graphic 2. "Evaluation of survival in ants fed water tests 2012/2013".



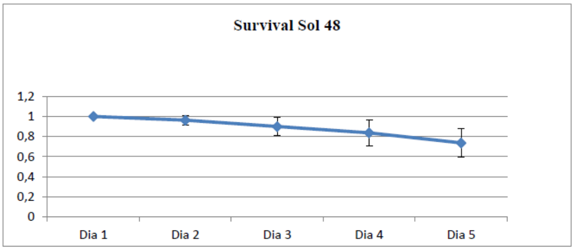
Graphic 3. "Evaluation of survival in liquid diet ants replaced every 24 hours in assays 2012/2013".



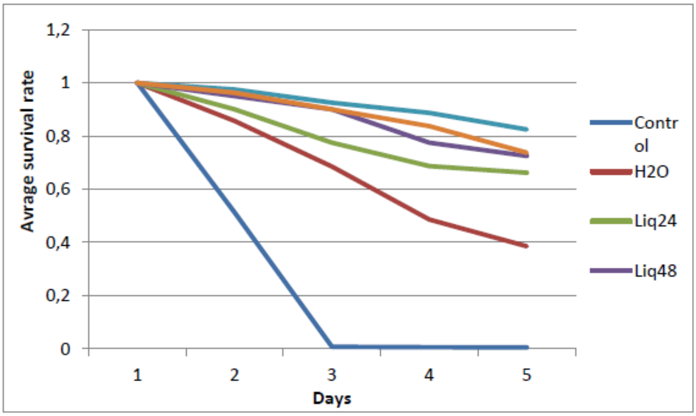
Graphic 4. "Evaluation of survival in liquid diet ants replaced every 48 hours assays 2012/2013".



Graphic 5. "Evaluation of survival in solid diet ants replaced every 24 hours assays 2012/2013".



Graphic 6. "Evaluation of survival in solid diet ants replaced every 48 hours assays 2012/2013".



Graphic 7. "Evaluation of the survival rate for five diets and control".

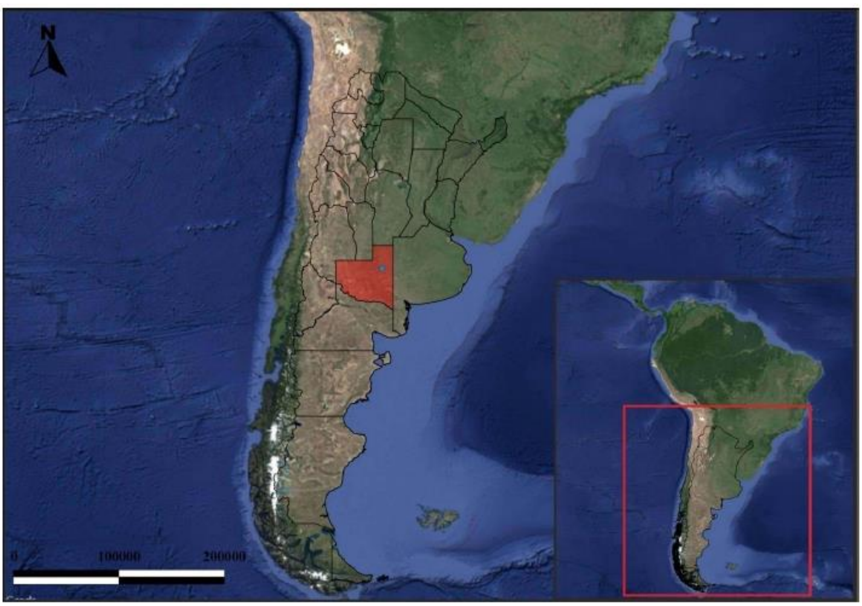


Figure 1. Geographical location of the study area.