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THE ROLE OF BEHAVIOR IN THE SURVIVAL OF *BIOMPHALARIA GLABRATA* IN BIOASSAYS WITH THE PLANT MOLLUSCICIDE *PHYTOLACCA DODECANDRA*

P. JURBERG, J. V. BARBOSA & L. ROTENBERG

Instituto Oswaldo Cruz, Departamento de Biologia, Laboratório de Comportamento Animal, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

This work examines the role of behavior in the survival of Biomphalaria glabrata *exposed to 25, 50, 75 and 100 mg* l^{-1} *of* Phytolacca dodecandra. *Time-lapse cinematography was used to quantify accurately the following parameters: (a) frequency of exits from the solution, (b) time spent out of the solution and (c) time elapsed until the first exit from the solution. These behavior patterns were statistically compared between surviving snails and those which later died. The proportion of surviving snails leaving the liquid medium was significantly higher than that of dying snails. In addition, the surviving group spent significantly more time out of the solution than the group which died, except for the 100 mg* l^{-1} *concentration. However, no significant difference was detected in the time elapsed until the first exit from the solution. It can be concluded that both the tendency to leave the* P. dodecandra *solutions, and the time spent out of them, contributed significantly to snail survival. Molluscicide bioassays should take into account the possibility that some behavior patterns of planorbids might contribute to the protection of the snails.*

Key words: behavioral bioassay – *Biomphalaria glabrata* – *Phytolacca dodecandra* – schistosomiasis control – molluscicide

The protective behavior patterns of snail hosts of schistosomiasis are of epidemiological importance (W. H. O., 1965) since survival of a few individuals can impair the effectiveness of eradication campaign (Pieri & Jurberg, 1981a); the planorbid snail hosts are very prolific, hermaphroditic and capable of self-fertilization (Brumpt, 1941; Paraense, 1955). In a survey of the protective behavioral patterns of planorbids, Pieri & Jurberg (1981a) pointed out those that (a) reduce the probability of an individual being exposed when the product is applied and (b) decrease the toxic effects in individuals already reached.

With respect to the response of *Biomphalaria glabrata* to the presence of toxic substances, the following patterns have been reported: exit from the water (Nolan et al., 1953); the distress syndrome (Harry & Aldrich, 1963); withdawing into the shell (Cheng & Sullivan, 1973) and the ability of the snail to avoid high doses of the product when exposed to a concentration gradient (Etges, 1963; Etges & Gilbertson, 1966). Although the occurrence of these patterns may be associated with protection of the animal against the toxic product, our knowledge on whether behavior actually contributes to snail survival remains scanty.

The present study aims to evaluate the role of behavior in the survival of *B. glabrata* in bioassays with *Phytolacca dodecandra.* Concentrations were used that caused death of a proportion of the snail sample being tested so that a true comparison could be made between the surviving and the dead animals in terms of their behavior throughout the bioassays. This enabled to distinguish between the lethal effect of the molluscicide, as revealed by the snails that never left the solution and the lethality observed in the snails that spent varying periods of time out of the liquid medium.

MATERIAL AND METHODS

Melanic specimens of *Biomphalaria glabrata* (12-14 mm) produced by individuals from Touros, State of Rio Grande do Norte (Northeastern Brazil) were used.

The bioassays were conducted in acrylic containers measuring 51.0 x 22.5 x 2.5 cm

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(Fig. 1) with 10 individual interconnected compartments permitting simultaneous recording of the separate activity of each snail. Two side ledges were attached to the anterior and posterior surfaces of the container at an angle of 150°. The nylon net covering the container was placed on the side ledges to allow the snail to remain out of the solution or to return to the liquid medium. The container was adapted from the model described by Rotenberg et al. (in press). Snail behavior, during exposure to the product, was recorded by time-lapse cinematography (Pieri et al., 1980).

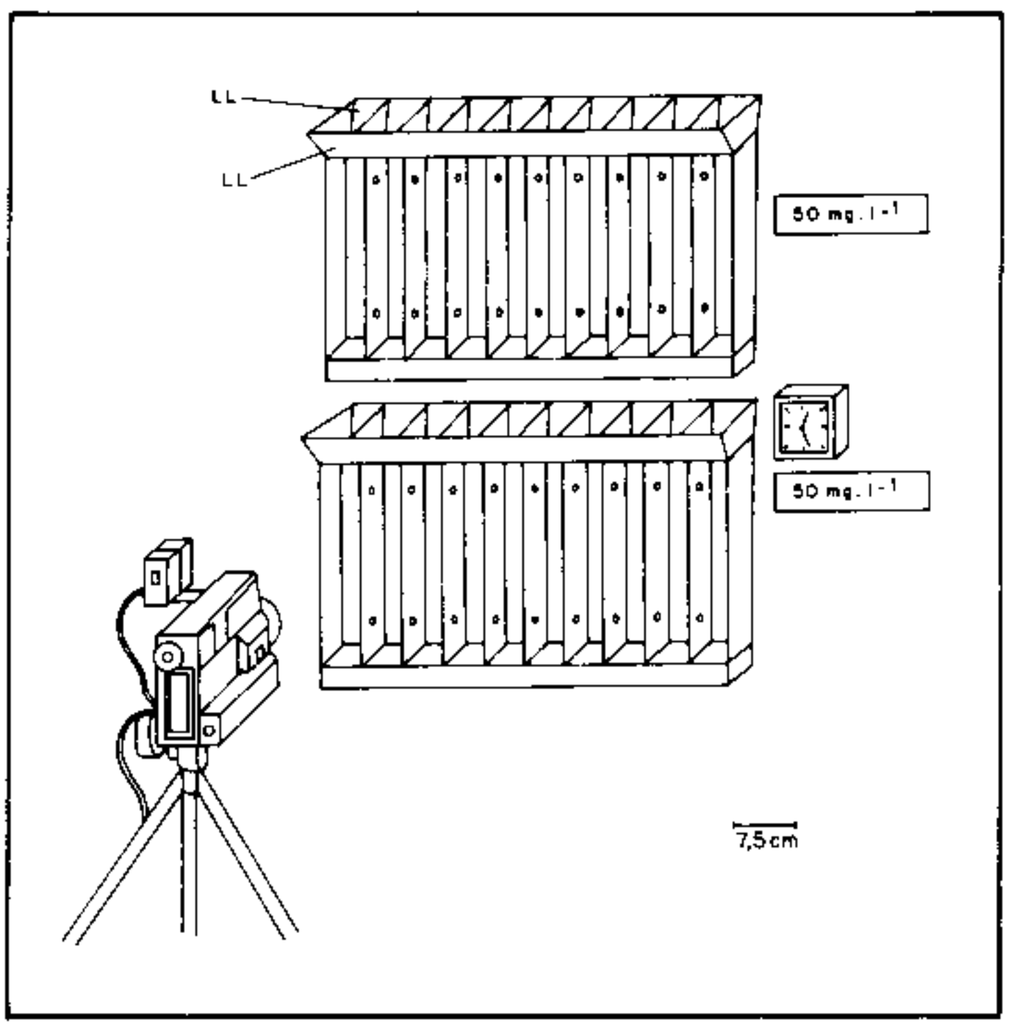

Fig. 1: Schematic diagram of the experimental situation for studying protective behavior patterns of *Biomphalaria glabrata* exposed to molluscicides. Test containers with lateral ledges (LL) and with individual interconnected compartments. A super-8 movie camara synchronized to an electronic flash is also shown.

During preliminary tests with *Phytolacca dodecandra* (Endod) it was observed that some snails emerged from the solutions. In addition, Endod is considered to be one of the most promising of plant molluscicides (Kloos & McCullough, 1982; Hostettmann, 1984). Solutions of 25, 50, 75 and 100 mg l^{-1} of *Phytolacca dodecandra* were prepared by a procedure adapted from Lemma & Heyneman (1979) as follows: *P. dodecandra* seeds were ground and passed through a 0.42 mm mesh sieve. The resulting powder was weighed in quantities of 62.5 mg; 125.0 mg; 187.5 mg and 250.0 mg, each of which was then mixed with 150 ml dechlorinated water. Each mixture was boiled for 5 minutes and filtered through hydrophilic cotton wool. The filtered liquid was then topped up to 2,500 ml. This corresponded to 25, 50, 75 and 100 mg l^{-1}, respectively. Independent groups of 30 snails were each exposed to a concentration of the molluscicide for 23 hours; the control group was placed in dechlorinated water. At the end of this period each snail was carefully removed from its compartment and the testing containers were washed several times with Extran (Merk) and distilled water. Each exposed snail was rinsed three times with 500 ml of dechlorinated water and placed in its respective recovery container in 150 ml of dechlorinated water. When poor handling was suspected during this phase (due to crushing or dropping, for example), the specimen was excluded from the set of observations. The period of recovery lasted 48 hours, with food (lettuce) supplied to the animals at the beginning of the period and 24 hours later. At the end of this period, the surviving and dead specimens were identified for each concentration tested. The control snails were similarly handled. Two groups of ten snails were tested simultaneously; therefore the whole experiment lasted nine days.

With the aid of a super-8 movie editor, the following parameters were quantified:

- Frequency of exits (total emergence) from the solution;
- Time elapsed until the first exit from the solution;
- Time spent out of the solution, with a calculation of all the exits from the solution.

In order to be more certain in attributing the survival of a given snail to the behavior shown during the assays, it was decided to reduce the probability of the occurrence of type I error using an "α" value of 1% in all the statistical analyses, which was of two types:

- Data relative to the time spent outside the solution and the time elapsed until the first exit from the solution. To test the hypothesis that the survival of the snails which had remained out of the solution for a long time tends to be favored as compared to the others, the mean value for surviving and for dead snails at each concentration (25, 50, 75 and 100 mg l^{-1}), as well as the ratio between them, was calculated. This ratio corresponds to our original observation. With the use of a computer programme a sampled

randomized test (Sokal & Rohlf, 1979) was performed. Thus based on raw data, 2 random samples were taken, their means and the ratio between them were calculated. This procedure was repeated 1000 times so that a distribution of ratios was obtained in which the significance of our original observation could be decided. Similar comparisons were made for the parameter "time elapsed until the first exit from the solution" for which we verified if the mean value for the surviving snails was lower than that for the dead ones.

– Data relative to the frequency of exits from the solution. The proportion of surviving and dead snails exposed to the molluscicide was compared by a contingency table with respect to leaving or not emerging from the solution ("no exit" x "at least one exit"). The "G" test for independence was used since only total sample size (n) was fixed a priori (Sokal & Rohlf, 1979).

The exit index was computed as corresponding to the percentage of snails encountered outside the solutions at the end of the period of exposure (Jurberg et al., 1985); the percentage for the control group and for the set of snails in the experimental group were calculated.

RESULTS

The numbers of surviving and dead snails were respectively 29 an 1, 19 and 9, 10 and 20, 13 and 15, 9 and 21, and in that order for the control group and for the groups exposed to 25, 50, 75 and 100 mg l^{-1}.

With regard to the time spent out of the solution, it was observed that the surviving group exhibited higher mean values than the group which died, at the 25, 50 and 75 mg l^{-1} concentrations. However, for the snails exposed to 100 mg l^{-1} of the product the mean value for the surviving group was not statistically different from the mean value for the group which died (Fig. 2). As shown in Fig. 3, the time elapsed until the first exit from the solution did not vary statistically between surviving and dead snails.

The data in Table show the numbers of surviving and dead snails leaving or not the media containing molluscicide. The hypothesis that survival does not depend on leaving the liquid medium was rejected.

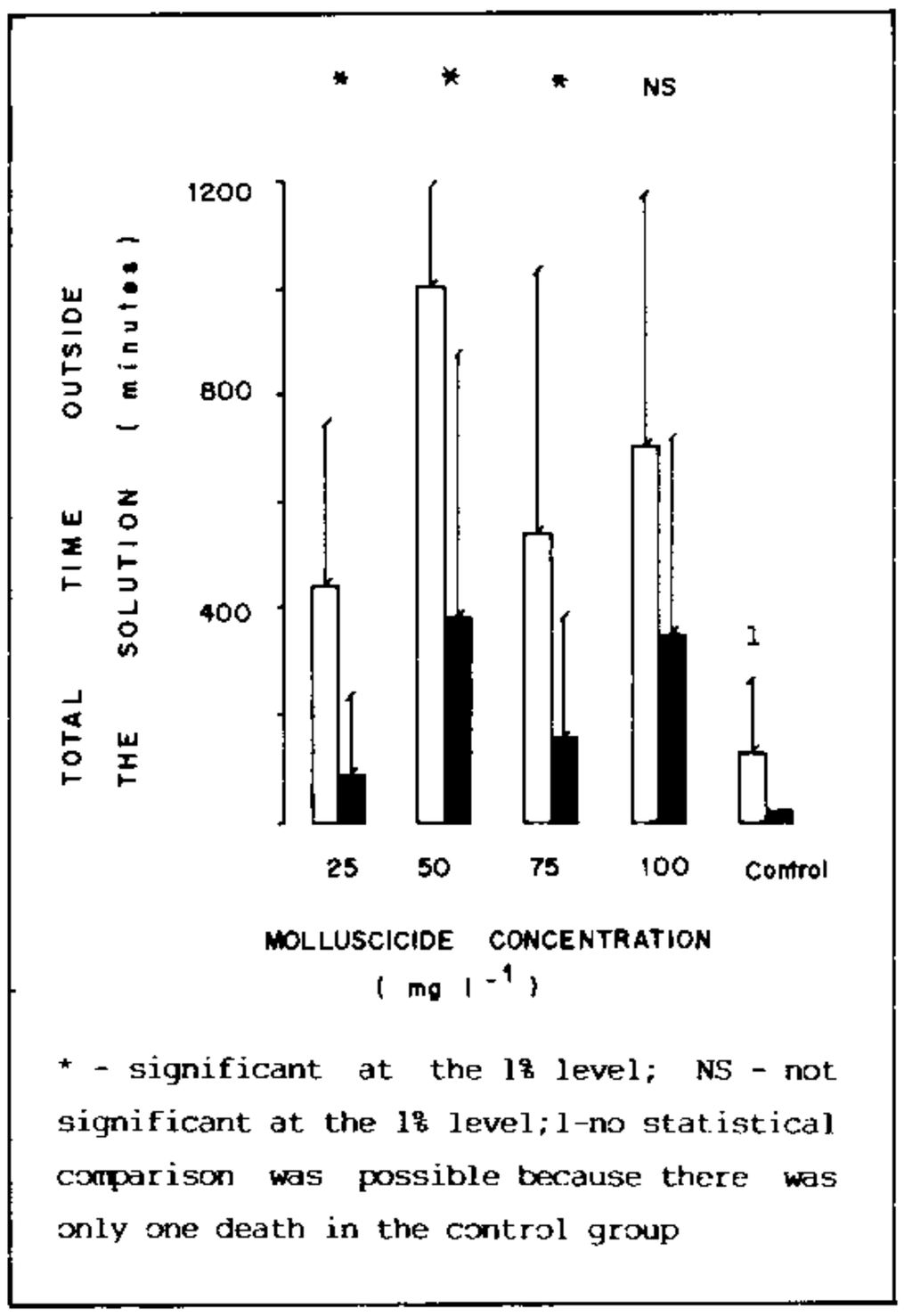

Fig. 2: Total time outside the solution for *Biomphalaria glabrata* specimens exposed to concentrations of 25, 50, 75 and 100 mg l^{-1} of the plant molluscicide *Phytolacca dodecandra*. Mean and standard desviation relative to surviving ▭ and dead snails ▬ at each concentration.

The exit index was 7% in the control group and 40% in the experimental group.

TABLE

Relationship between exit from te solution and survival of *Biomphalaria glabrata* for the total number of snails exposed to *Phytolacca dodecandra*

	At least one exit	No exit	Total
Dead snails	46	20	66
Surviving snails	47	3	50
Total	93	23	116§
G = 10.06*			

* significant at the 1% level.
§ four snails were excluded due to mishandling.

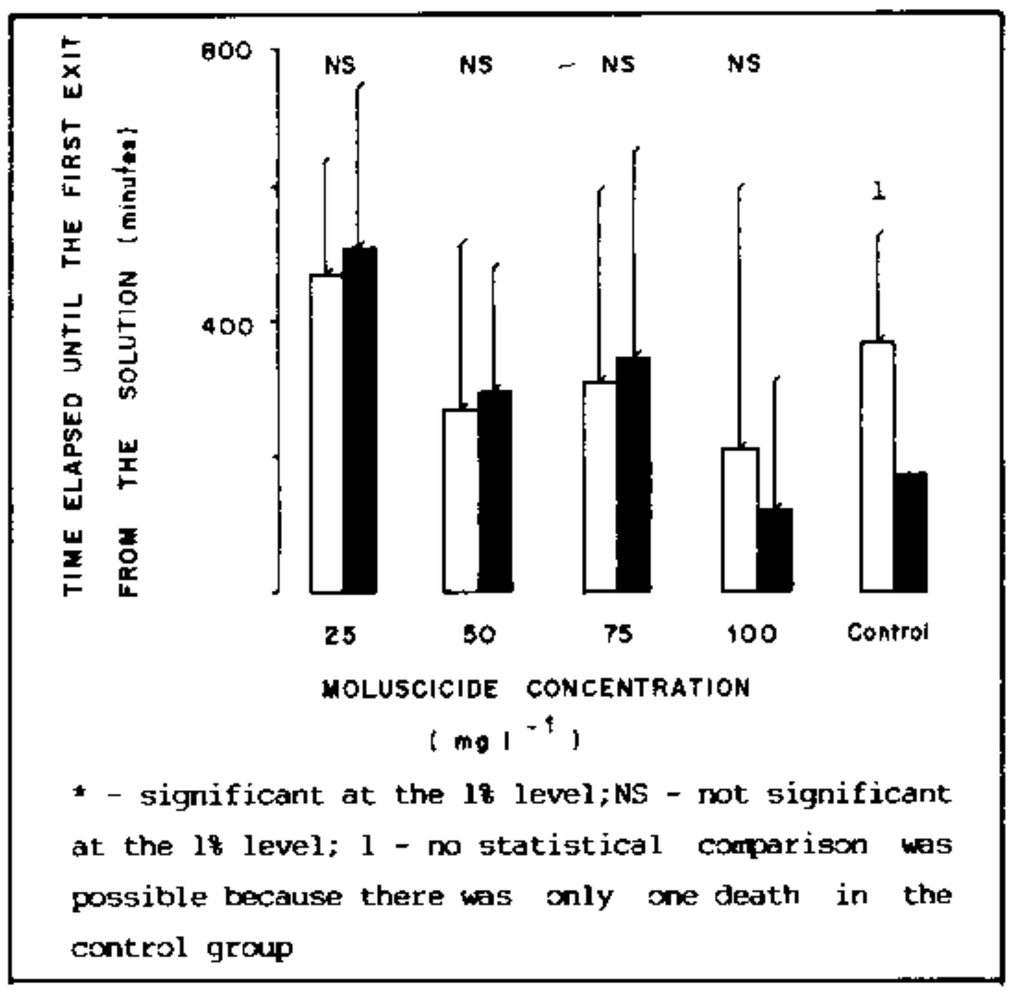

Fig. 3: Time elapsed until first exit from the solution for *Biomphalaria glabrata* specimens exposed to concentrations of 25, 50, 75 and 100 mg l^{-1} of the plant molluscicide *Phytolacca dodecandra*. Mean and standard desviation relative to surviving □ and dead snails ■ at each concentration.

DISCUSSION

Behavior and survival aspects – The results clearly reveal that behavior can significantly contribute to the survival of *B. glabrata* in bioassays using *P. dodecandra*. The records showed that leaving the liquid medium enhanced the survival chances of the snails exposed to the product. The time spent out of the solution has also contributed to snail survival, except for the snails submitted to the 100 mg l^{-1} concentration. At this concentration, survival was not related to the time spent by the snail outside the solution, probably because the toxic effect of the product may have been high enough to kill the snails even though they had spent long time out of the solution. Furthermore, the data concerning time elapsed until the first exit from the solution did not vary statistically between the survivors and the dead snails. Thus a planorbid's prompt exit from the toxic solution may not be sufficient for survival if it returned quickly to the liquid medium.

The experimental design here described allowed the distinction between lethality resulting from the toxic effect of the product and lethality observed when the snails left the solution once or more. Thus, only 13% (3 out of 23) of survival was observed among the snails which never left the solutions, whereas 50.5% (47 out of 93) of survival occurred among the snails that left the liquid medium at least once. It was observed that in all groups some snails (3% to 25%) never left the liquid medium throughout the test, whereas others left repeatedely. The high variability among *B. glabrata* snails, as previously noted by Corr et al. (1984), Bair & Etges (1973), Henriksen & Jelnes (1980) and by Mulvey & Vrijenhoeck (1981) among others, makes it difficult to predict how many animals will leave the liquid medium or how long they will remain out of it. Nevertheless, the present data show that a proportion of the sample may leave the solution and stay out of it for a long time and that this favours the probability of their survival.

Since *B. glabrata* has a wide distribution, certain behavioral patterns in a population may result from adaptation to local environmental factors (Paraense, 1972) such as a tendency to show diapause during the dry season in planorbids from Northeastern Brazil (Paraense, 1957). In this respect, Etges & Gilbertson (1966) in a study on a Surinam strain, noted that this strain was less susceptible to molluscicides because of its natural tendency to spend considerable periods out of water. Thus, variations in susceptibility to molluscicides among different strains may be related, amongst other things, to natural protective behavioral patterns in a given population.

In the present study it was also noted thay the control snails showed a tendency to return rapidly to the liquid medium, leaving it and returning to it several times throughout the test. In contrast, the snails exposed to higher concentrations (50, 75 and 100 mg l^{-1}) of the product, when they left the solution did so few times and remained for long periods outside the liquid medium. This observation shows that this pattern does not depend on the presence of the toxic product in the water. Similar results had reported by Pieri & Jurberg (1981b) with respect to sublethal doses of copper sulfate. Thus, even though leaving the liquid medium does not correspond to a response to the molluscicide, once outside the solution the snail has the ability to avoid new contact with the toxic agent.

Procedural aspects – The method here described was based on snail identification, so that the behavioral patterns of surviving snails and those that later died could be compared. The time-lapse cinematography proved to be ad-

equate for obtaining accurate measurements of different aspects of snail behavior during exposure to molluscicides, as had been reported by Pieri & Jurberg (1981b). This technique, together with systems of close-circuit television (Miller et al., 1982) demonstrated the feasibility of incorporating behavioral parameters into toxicological tests with aquatic organisms.

Covering the containers at a certain distance from the water surface and using containers with side ledges allowed an animal to remain out of contact with the molluscicide when it left the water or to return to the test solution. Problems due to the placing of a cover either at or above the water surface during molluscicide bioassays have been evaluated by Souza & Paulini (1967). These authors, studying the mortality curves of *B. glabrata* under different doses of sodium pentachlorophenate, observed that the snail could escape contact with the molluscicide when the cover was placed above the water surface. When the other conditions were kept constant, this apparently small modification caused a 3-fold increase in the LC 50 value. Considering the role of behavior in toxicological tests involving *B. glabrata,* Jurberg et al. (1985), in tests with *Euphorbia tirucalli,* defined the exit index, which provides important response level for the evaluation of the molluscicide and is easily carried out: the same recommendations by W. H. O. (1965) for bioassays with molluscicides are followed, although without the cover that prevents the snails from leaving the solution. On the basis of the high exit index observed in the present study for the experimental groups – 40%, we suggest that this index is a good indicator of a behavior pattern closely related to the protection of the snails. The conditions used for these experiments do not permit extrapolation of the results to field conditions; among the factors contributing to artificialization of the tests is the confinement of the snails, which may have favored their exit from the liquid medium. In any case, the present study represents a starting point for a better understanding of the behavioral aspects of *B. glabrata* submitted to the action of molluscicides. We emphasize the need for studies under natural conditions in order to investigate the behavior of snails exposed to toxic products under an ecological approach since part of the snail population may contact sublethal doses of the molluscicides at the time of application of the product.

On the basis of these considerations, we conclude that lethality assays should be carried out in containers with side ledges and without a cover at the water surface so as not to mask snail behavior. Such a procedure would permit the determination of not only the exit index but also a more valid LC 50 in the case of a linear relationship between mortality rate and doses of the product. The cinematography technique as described in this paper should be employed whenever behavior is suspected of playing a role in the survival of the test snails as indicated by a high exit index or a non-linearity between mortality and doses.

RESUMO

O papel do comportamento na sobrevivência de *Biomphalaria glabrata* submetida a bioensaios com o moluscicida vegetal *Phytolacca dodecandra* – Este trabalho investiga o papel do comportamento na sobrevivência de *Biomphalaria glabrata* exposta a 25, 50, 75 e 100 mg l^{-1} de *Phytolacca dodecandra*. Foi utilizada a técnica de cinematografia com lapso de tempo para quantificar acuradamente os seguintes parâmetros (a) freqüência de saídas da solução, (b) tempo de permanência fora da solução e (c) tempo decorrido até a primeira saída da solução. Estes padrões comportamentais foram estatisticamente comparados no que se refere aos caramujos sobreviventes e aos que vieram a morrer. Dentre os caramujos que abandonaram o meio líquido, a proporção de sobreviventes foi significativamente maior que a de mortos. Além disso, um tempo significativamente maior de permanência fora da solução foi observado no grupo que sobreviveu, em relação ao grupo que veio a morrer, exceto no que diz respeito à concentração de 100 mg l^{-1}. No entanto, nenhuma diferença significativa foi detectada no tempo decorrido até a primeira saída da solução. Conclui-se que a tendência a abandonar as soluções de *P. dodecandra* e o tempo de permanência fora delas favorecem a sobrevivência dos caramujos. Bioensaios com moluscicidas deveriam levar em conta a possibilidade de que alguns padrões comportamentais dos planorbídeos venham a contribuir para a sua sobrevivência.

Palavras-chave: bioensaio comportamental – *Biomphalaria glabrata* – *Phytolacca dodecandra* – controle da xistosomose – moluscicida

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