# Asymmetrical Behavioral Response Towards Two Boron Toxicants Depends on the Ant Species (Hymenoptera: Formicidae)

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**ABSTRACT** Urban ants are a worldwide critical household pests, and efforts to control them usually involve the use of alimentary baits containing slow-acting insecticides. A common toxicant used is boron, either as borax or boric acid. However, the presence of these compounds can affect the consumption of baits by reducing their acceptance and ingestion. Moreover, as feeding motivation varies widely, according not only to food properties but also to colony conditions, bait consumption might be diminished further in certain situations. In this study, we compared the feeding response of ants toward two boron toxic baits (boric acid and borax) in low motivation situations that enhance any possible phago-deterrence the baits may produce. Most studies investigating bait ingestion evaluate whole nests or groups of ants; here, we analyzed the individual ingestion behavior and mortality of the Argentine ant, *Linepithema humile* (Mayr), and the carpenter ant, *Camponotus mus* (Roger), for two boron baits, to detect which compound generates a higher rejection in each of these species. Although these two species have similar feeding habits, our results showed that ants under low motivation conditions reduced the acceptance and consumption of the toxic baits asymmetrically. While *L. humile* mostly rejected the borax, *C. mus* rejected the boric acid. These results denote the importance of considering the preference of each species when developing a pest management strategy.

**KEY WORDS** ant, boric acid, sodium borate, feeding, bait

Several ant species interact with human societies by affecting individuals and their resources. In urban areas, ant control strategies focus on the introduction of the toxic agent into the nest by the ants themselves, which is frequently difficult to locate or inaccessible, therefore minimizing the distribution of toxicants. In social insects, the only individuals that regularly leave the nest are a small fraction of the infertile workers, the foragers. Foragers bring resources back to the nest, where the food is shared with nest-mates, queen (or queens), and the brood. As the baits reach most colony members, they are the most effective way to introduce a toxic agent into a nest. For many ant species, sugar solutions can work as effective baits because carbohydrates constitute a major part of their diet (Baker et al. 1985, Hölldobler and Wilson 1990).

Field records have shown conspicuous seasonal variations in the consumption of sucrose solutions (*Formica lugubris,* Sudd and Sudd 1985; *Linepithema humile,* Rust et al. 2000; *Camponotus pennsylvanicus,* Tripp et al. 2000). This variation could be because of changes in the number of workers and/or brood, as this determines the balance of protein and carbohydrate requirements (Howard and Tschinkel 1980, Dussutour and Simpson 2008). In fact, foragers do not collect resources for their own individual needs, but for those of the whole colony (Cassill and Tschinkel 1999). In addition, changes in the availability of alternative natural sources could affect the intake of any offered sugar-based bait (Sudd and Sudd 1985, Kay 2002, Vega and Rust 2003, Daane et al. 2006, Silverman and Brightwell 2008). Therefore, toxic bait acceptance recorded in a given situation does not necessarily represent its acceptance when used in a control program. One of the most difficult problems has been formulating toxicants into consistently acceptable baits (Baker et al. 1985, Silverman and Roulston 2001, Rust et al. 2004). Water evaporation from any toxic bait also poses a problem for certain toxicants because such baits could become too toxic, or the toxic agent may become detectable to the ant, causing a phago-deterrent effect, and begin to interfere with acceptance and/or recruitment (Klotz et al. 2000, 2004).

Processes related to foraging on sugar solutions depend on the balance between the incoming sugar flow to the nest and the colony requirements. The former is related to the availability of sugar sources (e.g., number, distance to the nest), and to their productivity (sugar concentration, flow rate, etc.); whereas the latter determines the motivation to forage. There-

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fore, in laboratory colonies, foraging and recruitment can be partially manipulated by the supply or deprivation of carbohydrates to the colony (Howard and Tschinkel 1980, Josens and Roces 2000, Mailleux et al. 2006, Falibene and Josens 2008, Falibene et al. 2009). Thus, either in nature or laboratory controlled conditions, a certain sugar bait might be accepted, ingested, and transported back to the nest and also may trigger recruitment in some situations but not in others (Josens and Roces 2000; Mailleux et al. 2000, 2005, 2006, 2011). The internal state of a forager affects the assessment of a given resource (external stimulus) that, in turn, affects the individual motivation to feed (McFarland 1971). This affects the behavioral responses to an appetitive stimulus (Mangel 1993). Therefore, the individual motivation of each forager may determine if a toxic bait will be accepted, how much of it will be consumed, and whether or not it will trigger recruitment.

Boric acid ($H_3BO_3$) and Borax (sodium borate, $Na_2B_4O_7$) have been used as an insecticide in baits for ants since the late 1800s (Riley 1889, Rust 1986). Their use fell in the 1940s, but reappeared in the last decades as they have some advantages over other toxicants; for example, low toxicity for mammals in small dosages (Quarles 1992), solubility in water and delayed toxicity, which is essential for social insects (Klotz and Moss 1996). For boron-based toxicants, acceptance, consumption, or mortality have been comprehensively studied on different ant species both in laboratory and field assays (Klotz et al. 1996, 1997, 1998, 2000; Rust et al. 2004; O'Brien and Hooper–Bui 2005; Nelson and Daane 2007; Stanley and Robinson 2007; Daane et al. 2008; Nyamukondiwa and Addison 2011). In general, most studies on ants using toxic baits were performed on the whole colony or groups of ants, but very few on individuals (Hooper–Bùi and Rust 2001, O'Brien and Hooper–Bùi 2005).

Regarding the active ingredient concentration, many studies suggest using <1% wt:vol of boron toxicants (Klotz and Williams 1996, Hooper–Bui and Rust 2000), even though several commercial baits contain 5% wt:vol (Klotz et al. 2000). Preliminary results with *Camponotus mus* (Roger) showed that increasing the concentration of boric acid led to a higher rejection and a lower volume ingested per ant, suggesting some degree of phagodeterrence (Fernandez 2001). Conversely, very low concentrations of boron toxicants can be more easily accepted but they could be less effective for control, as sharing the bait by trophallaxis among a large population can readily dilute a toxicant to a sublethal dose (Rust et al. 2004, Greenberg et al. 2006).

Regarding the sucrose concentration of the bait, several studies apply 10% wt:wt sucrose baits for ants (Klotz and Moss 1996; Klotz et al. 1996, 1997). However, in field studies, the Argentine ant showed more recruitment toward higher sucrose concentrations (Klotz et al. 1998). In addition, it is worth considering that even without toxicants sugar solutions can be rejected if the concentration is not high enough. For example, under certain conditions, the carpenter ant *C. mus* ingests solutions of concentrations below 20% wt:wt in lower volumes, for less time and with more interruptions than solutions above 20% (Josens et al. 1998).

The objective of this study was to compare the acceptance of two boron baits (boric acid and borax) in a situation that enhances the possibility of phagodeterrence in two species of nectivorous ants, the carpenter ant *C. mus* and the Argentine ant, *L. humile.* This situation was achieved under low-motivation conditions for foraging. For *L. humile,* we enhanced a possible phagodeterrence by selecting a suboptimal sucrose concentration, and therefore we reduced the attractiveness of the bait. For *C. mus*, this was accomplished by varying the supply of carbohydrate to the colonies in two different feeding motivation states. For both species, we used a high toxic bait concentration to intensify any possible repellent effect, thus allowing us to detect if one toxicant could be accepted even when ants showed low motivation.

## Materials and Methods

**Insects.** Experiments were performed on colonies of *C. mus* and *L. humile* captured in Argentina, their native range. Two colonies of *C. mus* (≈3,000 workers and one or more queens) were collected in the city of Buenos Aires, and kept for at least 1 yr under laboratory conditions (Josens et al. 1998, Provecho and Josens 2009). Four colonies of *L. humile* were captured at the Campus of the University of Buenos Aires, and kept in the laboratory for at least 2 mo. The number of workers was estimated to be around 4,000–5,000 per nest.

Colonies of both species were kept in artificial nests consisting of large plastic boxes (30 × 50 × 30 cm) with plaster bottoms and sides painted with fluon to prevent the ants from escaping. Nests were maintained in a temperature-controlled environment (25 ± 3°C) under a natural light–dark cycle. Ants were fed daily with honey-water, and three times a week with fresh cockroaches (*Blaptica dubia* Serville) and tinned meat; water was provided ad libitum.

**Feeding Behavior.** *L. humile.* Ants were collected each day from one of the laboratory nests in groups of 40 individuals and allowed to settle for 15 min before the experiment started. As *L. humile* workers are monomorphic, all the experimental ants were of a similar size. One at a time each ant was allowed access to a bridge (2 × 50 mm) in which end a drop (10 $\mu$l) of sucrose solution was offered. This volume constituted an ad libitum source for this species as their crop load is between 0.1–0.5 $\mu$l (F. J. Sola, personal observation). To measure individual crop load, we filmed the ants in lateral view, while they were drinking, using a camera-fitted stereomicroscope (Leica MZ8−25× magnification, with a Leica ICA camera). The amount of solution ingested was then estimated by the differences in gaster volume before and after feeding (Mailleux et al. 2000). The narrowness of the bridge allowed us to film the ants from the side, and to keep them perpendicular to the shooting angle and at the focal length of the microscope while drinking. As the camera was placed laterally, we could measure the maximal length and maximal height of the gaster directly. The width of the abdomen could not be seen on these lateral images. To estimate the relationship between this axis and the height in the lateral view, we performed preliminary measurements on 40 ants fed in similar conditions, but filmed laterally and from above. We found that the relationship between width: height was 1:1.1 for both empty and filled gasters. Therefore, we approximated the abdomen to be an ellipsoid to calculate the volume of the gaster of each forager before (Vi) and after (Vf) drinking, and assessed the volume of solution ingested ($\mu$l) by the difference (V = Vf - Vi). Feeding time (s) was also obtained from the videos, and was defined as the time during which the mandibles of the ant were in contact with the solution.

Acceptance (%) was the percentage of ants that drank from the total number of ants that made contact with the solution with their antennae or mouth-parts (whether ingesting the solution or not). This variable was not measured during the first days of data recording; therefore, the sample size for this variable was smaller than for the rest of the recorded variables. After the video recordings, the ants were kept separate from the rest of the colony.

*C. mus.* Ants used in the experiments were of similar sizes to avoid any distortion of the variables measured caused by the ant size. We applied a widely used protocol for this species (Josens et al. 1998, Josens and Roces 2000, Falibene and Josens 2008, Falibene et al. 2009). Briefly, each experimental forager was allowed individual access to a bridge (10 × 2 cm) that ended in a recording arena (a platform of 2 × 3 cm) where a sucrose solution was offered from an Eppendorf tube (0.5 ml). This constituted an ad libitum food source for a single forager. Once in the arena, the ant was not disturbed to avoid interference with acceptance or feeding behavior.

Individuals (gently placed in a small plastic tube) were weighed before and after feeding (Mettler Toledo balance, to the nearest 0.01 mg) to obtain the initial weight (Wi, ant mass) and final weight (Wf, ant mass plus crop load mass), respectively. Crop load (mg) was obtained from the difference between Wf and Wi. Volume of solution ingested ($\mu$l) was calculated by dividing the crop load by the density of the solution (obtained by weighing 1 ml of each solution and averaging five measurements). Acceptance (%), feeding time (s), and intake rate ($\mu$l/s) were calculated as described for *L. humile.* After the data recordings, the ants were kept separate from the rest of the colony.

**Experimental Series.** The goal of these experiments was to compare acceptance and feeding behavior of individual ants to toxicants commonly used in commercial baits in a low feeding motivation state, to enhance a possible deterrent effect of the toxicants. To lower the motivation to feed, high toxicant concentrations were used (5% wt:vol) to make the baits less palatable. We also made use of two factors that affect feeding motivation: sucrose concentration (for *L. humile*) and the sugar requirements of the colony (for *C. mus*), both of which affect individual feeding behavior (Josens and Roces 2000, Falibene and Josens 2008, Falibene et al. 2009). As it was not possible to carry out all replicates of each treatment on the same day, data within each experiment were gathered on different days. Colonies were previously subjected to a starvation period of 48–72 h. On each data-recording day, all the treatments (control and toxic baits treatments) were registered evenly, throughout daylight hours, and this was repeated on several days to achieve a large sample size. In all the experiments, each individual ant was provided with only one solution and tested only once.

***L. humile.*** *Sucrose Concentration in Borax Solutions.* In this experiment, we tested whether low sucrose-concentration baits were less attractive than higher sucrose concentration baits. We analyzed the behavioral variables for each individual worker when feeding on a sucrose solution of 5, 10, or 20% wt:wt, all with 5% wt:vol borax. After being tested, 30 ants per treatment were maintained in groups of 15, according to the treatment received, for 14 d. The flasks (7 cm diameter) containing the ants were covered with perforated plastic lids to maintain humidity within the flask, while allowing air exchange. During this period, ants had access to water and honey-water ad libitum but no more toxicant was offered. Mortality was recorded as the cumulative number of dead ants per day.

*Toxicant Comparison.* To discourage *L. humile* from feeding, we used a low sucrose concentration (5% wt:wt) to evaluate a possible deterrent effect on feeding while comparing boric acid and borax. We offered each ant one of three 5% wt:wt sucrose solutions: without toxicant (*control treatment*), with 5% wt:vol boric acid (*boric acid treatment*) and 5% wt:vol borax (*borax treatment*). Then, ants were kept in groups of 15 per flask, and mortality rate was recorded daily as described above.

*Toxicant Comparison and Sucrose Concentration.* In this experiment, we reproduced (in another year with other colonies) all the series previously described. However, while in the former experiments each series was independent of the other, in this experiment sucrose concentration and toxicant were evaluated in parallel and simultaneously. We offered each individual ant one of nine treatments; one factor to be analyzed was the sucrose concentrations (5, 10, and 20% wt:wt) and the other was the toxicant: control (without toxicant), boric acid, and borax (5% wt:vol). Mortality was measured as described above.

***C. mus.*** *Toxicant Comparison.* We offered each ant one of three 30% wt:wt sucrose solutions: without toxicant (*control treatment*), with 5% wt:vol boric acid (*boric acid treatment*), and 5% wt:vol borax (*borax treatment*). On each recording day the three treatments were recorded. As motivation to feed varied among recording days, two groups were defined according to their acceptance of the control solution: *Total acceptance group* (recording days on which no ant rejected the control solution) and *Partial accep-*

*tance group* (recording days on which at least one ant rejected the control solution). Bait acceptance and feeding behavior were compared within each group for the three treatments. After tested, the experimental ants were grouped in sets of five and kept with ad libitum honey-water and water for 14 d to register mortality as described above.

**Statistical Analyses.** Feeding variables were analyzed using one-way analysis of variance (ANOVA). In cases of significant differences, post hoc Tukey's for pair-wise multiple comparisons were applied. Acceptance for different solutions was compared using G-tests. Mortality was compared by means of Kaplan Meier Survival Curves with corrected alpha when performing pair-wise comparisons. For *L. humile* in the experiment *Toxicant comparison and sucrose concentration,* two-way ANOVA were performed for the feeding variables, and then, if there were no significant interaction, Neumal–Keuls post hoc comparisons were applied. The general significance level used was 5% in all experiments.

## Results

***L. humile.*** *Sucrose Concentration in Borax Solutions. L. humile* displayed differences in feeding behavior according to the sucrose concentration of the borax bait. The lower the sucrose concentration, the less solution was ingested (ANOVA; $F_{2,87} = 27.38$; $P < 0.0001$; Fig. 1a). Mortality was consistent with the behavior described (Fig. 1b); ants that drank from the 5% sucrose bait had lower mortality than the other groups (Kaplan–Meier; $\chi^2 = 12.78$; $P = 0.0017$). As they ingested less solution, they consumed less toxicant because all the baits had the same borax concentration (5% wt:vol). For 10 and 20% wt:wt sucrose baits, 50% of the ants died (LT50) between the second and the third day after treatment, but for the 5% wt:wt sucrose bait, 50% of the ants died by the 11th day. As during the period of posttreatment all ants had free access to honey-water, differences in mortality can only be a consequence of larger volumes of toxicant ingested for the more concentrated solutions.

*Toxicant Comparison.* When offering Argentine ants a bait of low sugar concentration and high toxicant concentration, different behavior was observed for boric acid (5% wt:vol) and borax (5% wt:vol) (Fig. 2). Ants showed a more pronounced rejection of borax than boric acid (G-test; $P = 0.035$; Fig. 2a). This result was also reinforced by the volume ingested (ANOVA; $F_{2,129} = 19.76$; $P < 0.0001$; Fig. 2b) and feeding time (ANOVA; $F_{2,129} = 25.12$; $P < 0.0001$; Fig. 2c). The control showed the longest ingestion time and highest crop load, which were similar to the boric acid bait but significantly different than the borax bait.

Mortality of these three groups showed differences which were consistent with the behavioral variables (Kaplan–Meier; $\chi^2 = 42.18$; $P < 0.00001$; Fig. 2d). Ants of the borax group presented a much lower mortality than the boric acid group and mortality comparable to the control group. The boric acid group had 50% mortality between the first and second day after treatment, while borax and control groups did not reach 20% during the 14 d recorded.

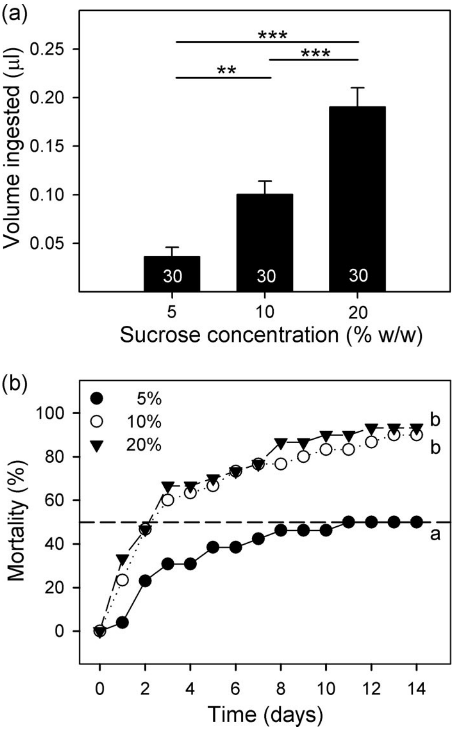


**Fig. 1.** Argentine ant ingestion of borax solution of different sucrose concentration. (a) Volume ingested (mean ± SE) by individual ants for sucrose solutions of 5, 10, and 20% wt:wt all with 5% wt:vol of borax. Horizontal lines indicate significant differences (Tukey post hoc comparisons: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). The numbers inside the *bars* represent the number of experimental ants. (b) Mortality as the accumulative number of ants that died each day after treatment (day 0). Curves that do not share letters are significantly different (Kaplan–Meier; $P < 0.05$). Sample sizes as in (a).

*Toxicant Comparison and Sucrose Concentration.* Volume ingested varied both with sucrose concentration and with toxicant treatment (two-way ANOVA, concentration*toxicant: $F_{4,261} = 0.91$; $P = 0.46$; conc: $F_{2,261} = 10.33$; $P < 0.0001$; toxicant: $F_{2,261} = 8.12$; $P < 0.001$; Fig. 3). There were differences between the toxicants, but as sucrose concentration rose, these differences disappeared. While for 5% sucrose baits, the borax was significantly lower than the control and the boric acid (Fig. 3a), for 10%, the borax tended to be lower than the control (Fig. 3b), and for 20% there were no significant differences among treatments (Fig. 3c). Only when the richness of the bait is low can differences between toxicants be detected as they disappeared with the increment of the bait richness. In the borax treatment, the more the sucrose concentration, the more volume ingested. Reduced ingestion at lower sucrose concentrations was significant for the borax (Neuman–Keuls, 5 vs. 10: $P > 0.1$; 10 vs. 20: $P = 0.0696$, 5 vs. 20: $P < 0.001$) but not for boric acid or the control treatment. These results indicate that in cer-

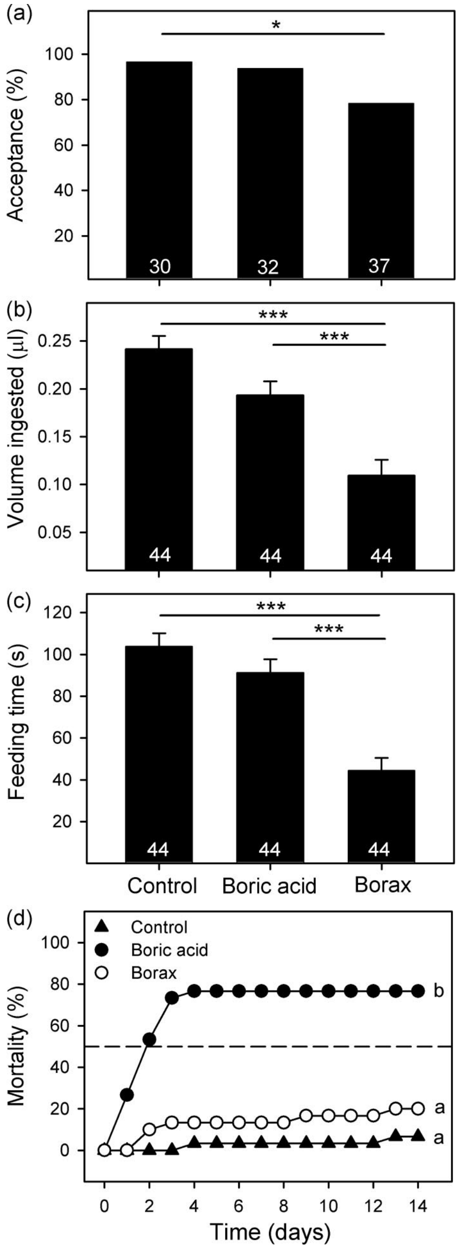


**Fig. 2.** Ingestion variables for individual Argentine ants for 5% wt:wt sucrose solution without toxicant (control) or containing boric acid (5% wt:vol) or borax (5% wt:vol). (a) Acceptance (percentage of ants that ingested the solution in relation to the number of ants that made contact with the solution), (b) volume ingested (mean ± SE), and (c) feeding time (mean ± SE). Horizontal lines indicate significant differences (Tukey post hoc comparisons: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). The numbers inside the *bars* represent the number of experimental ants. (d) Daily mortality (accumulated) after receiving one of the three treatments: control, boric acid, or and borax. Each curve represents 30 ants. Curves that do not share letters are significantly different (Kaplan–Meier; $P < 0.0001$).

tain circumstances borax affected intake more negatively than boric acid.

It is worth mentioning that in a previous experiment (not mentioned in Materials and Methods) with the same protocol using only sucrose solution (5, 10, and 20%), the tendency to ingest less with the decrease of concentration was more pronounced (ANOVA; $F_{2,111} = 6.00$; $P < 0.004$; data not shown). This fact would indicate that even with the same protocol, other factors can affect feeding motivation (e.g., season, time, weather, etc.).

Mortality of the control, boric acid, and borax groups differed for each of the three sucrose concentrations: 5% sucrose (Kaplan–Meier; $\chi^2 = 49.2$; $P < 0.00001$), 10% sucrose (Kaplan–Meier; $\chi^2 = 44.97$; $P < 0.00001$), and 20% sucrose (Kaplan–Meier; $\chi^2 = 45.17$; $P < 0.00001$; Fig. 3 right panels). In all cases, the control group had a low mortality, never reaching 50% over 14 d. Regarding the toxicants, they presented no differences in mortality for the higher sucrose concentrations (10 and 20%; Fig. 3b and c right panels). However, when the sucrose was low (5%), the boric acid bait reached a mortality rate significantly higher than the borax group (Fig. 3a right panel).

***C. mus.*** *Toxicant Comparison.* In this species we separated the data recorded in days of *Total acceptance* of the control solution from days of *Partial acceptance* of the control solution, that is, days when at least one ant rejected the control solution. The latter averaged 80% of acceptance of the control solution. This separation allowed us to display the differences in days with high and low motivation for feeding. During *Total acceptance* days, no differences were found in the acceptance among control, boric acid, and borax solutions (G-test; $P = 0.33$; Fig. 4a).

The other behavioral responses were similar; in *Total acceptance* days no differences were found among the treatments in volume ingested (ANOVA; $F_{2,88} = 0.57$; $P = 0.57$; Fig. 4b), feeding time (ANOVA; $F_{2,88} = 0.60$; $P = 0.55$; Fig. 4c), and intake rate (ANOVA; $F_{2,88} = 0.35$; $P = 0.71$; Fig. 4d). However, during *Partial acceptance* days, acceptance of boric acid differed significantly from the control, while the borax did not (G-test; $P = 0.001$; *control versus borax:* $P = 0.68$; *control versus boric acid:* $P = 0.001$; Fig. 4e). Moreover, less boric acid bait was ingested than control and borax baits (ANOVA; $F_{2,72} = 13.62$; $P < 0.0001$; Fig. 4f). The same result was obtained for feeding time (ANOVA; $F_{2,72} = 4.59$; $P = 0.013$; Fig. 4g) and intake rate (ANOVA; $F_{2,72} = 7.31$; $P = 0.001$; Fig. 4h), where boric acid baits reached values significantly lower than those of the control baits.

Mortality reflected the results of the behavioral variables. In *Total acceptance* situations, mortality differed among the three groups (Kaplan–Meier; $\chi^2 = 25.13$; $P < 0.00001$; Fig. 5a). Both toxic baits showed similar mortality curves reaching 50% of ants dead on the third day after treatment; while for the control group the curve passed this value on the 14 d (Fig. 5a). However, in *Partial acceptance*, the mortality was significantly different for the two toxic baits. The borax group reached 50% of mortality on the second day,

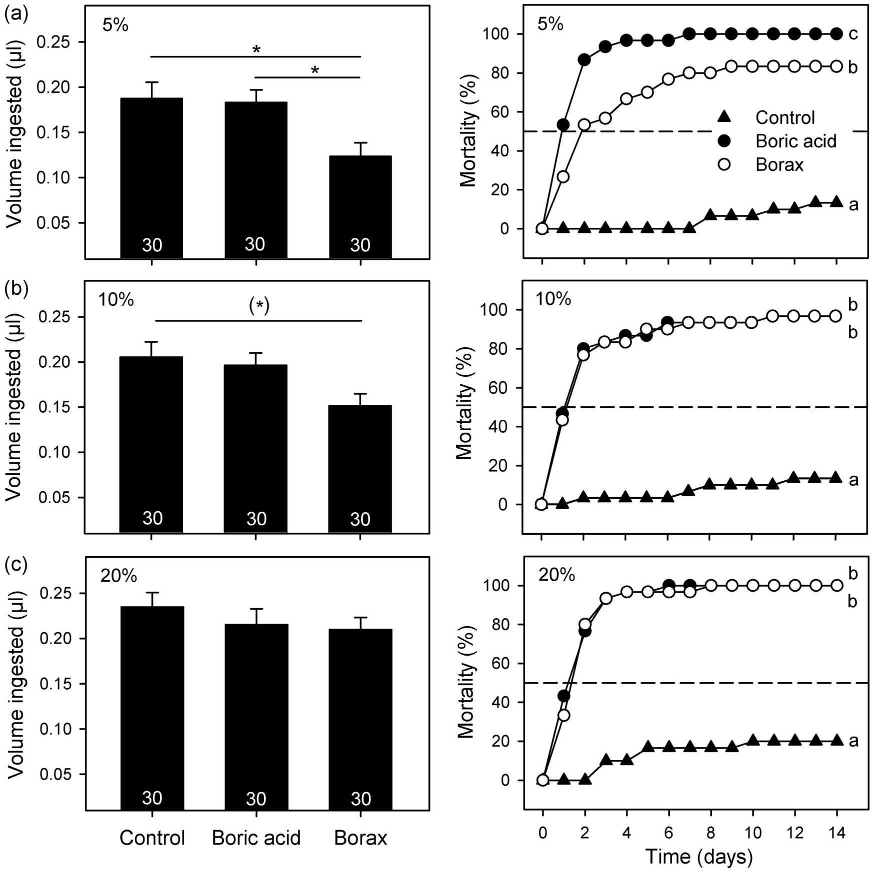


**Fig. 3.** Volume ingested (mean ± SE; left-hand panels) and daily mortality (accumulated; right-hand panels) of Argentine ants after receiving one of three treatments: control (no toxicant), boric acid (5% wt:vol), and borax (5% wt:vol) in (a): a 5% wt:wt sucrose solution, (b): a 10% wt:wt sucrose solution, and (c): a 20% wt:wt sucrose solution. Horizontal lines in left panels indicate significant differences (Tukey post hoc comparisons: (*) $0.05 < P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Each bar corresponds to each curve in mortality graphs; $N = 30$. Within each panel, different letters indicate curves that are significantly different (Kaplan–Meier; $P < 0.05$).

while the boric acid group reached this value on the fifth day and the control group on the 11th day (Kaplan–Meier; $\chi^2 = 38.58$; $P < 0.00001$; Fig. 5b).

## Discussion

We demonstrate that ants responded asymmetrically toward two boron toxicants, depending on the species and the feeding motivation. Therefore, the potential effectiveness in toxic bait application for ant control bears a close relationship with the species and the current conditions. Both species evaluated in our study are nectivorous ants from the same region, with similar distributions in their native range; they are both tolerant of disturbed environments and urban landscapes. Both species primarily consume sugary solutions from extra floral nectaries and from hemipterans (Baker et al. 1985, Hölldobler and Wilson 1990). However, in this study carpenter ants and Argentine ants presented opposed deterrence when offered sucrose solutions with borax or boric acid; *C. mus* more readily accepted the borax baits, while *L. humile* more readily accepted the boric acid baits. Therefore, attention should be paid when making assumptions or extrapolating conclusions from experiments about species with similar feeding habits and diets. Furthermore, many commercial baits are recommended for different ant species based only on their feeding habits (i.e., nectivorous, leaf-cutting ants), with no consideration for possible differential deterrence inside each group. Our results suggest that the choice of the toxicant bait to be used is even more complex.

The asymmetrical response toward the two boron toxicants appeared only when motivation to feed was low, regardless of how this motivation was reduced. In Argentine ants, we observed a reduction in the acceptance and consumption of borax baits for low sucrose concentrations. However, these differences were absent when sucrose concentration was higher, which would make the bait more attractive for ants. In this scenario, as ants ingested more toxicant, their mortality per day rose accordingly. Argentine ants ingested a volume of boric acid bait similar to the control bait for all sucrose concentrations. Therefore,

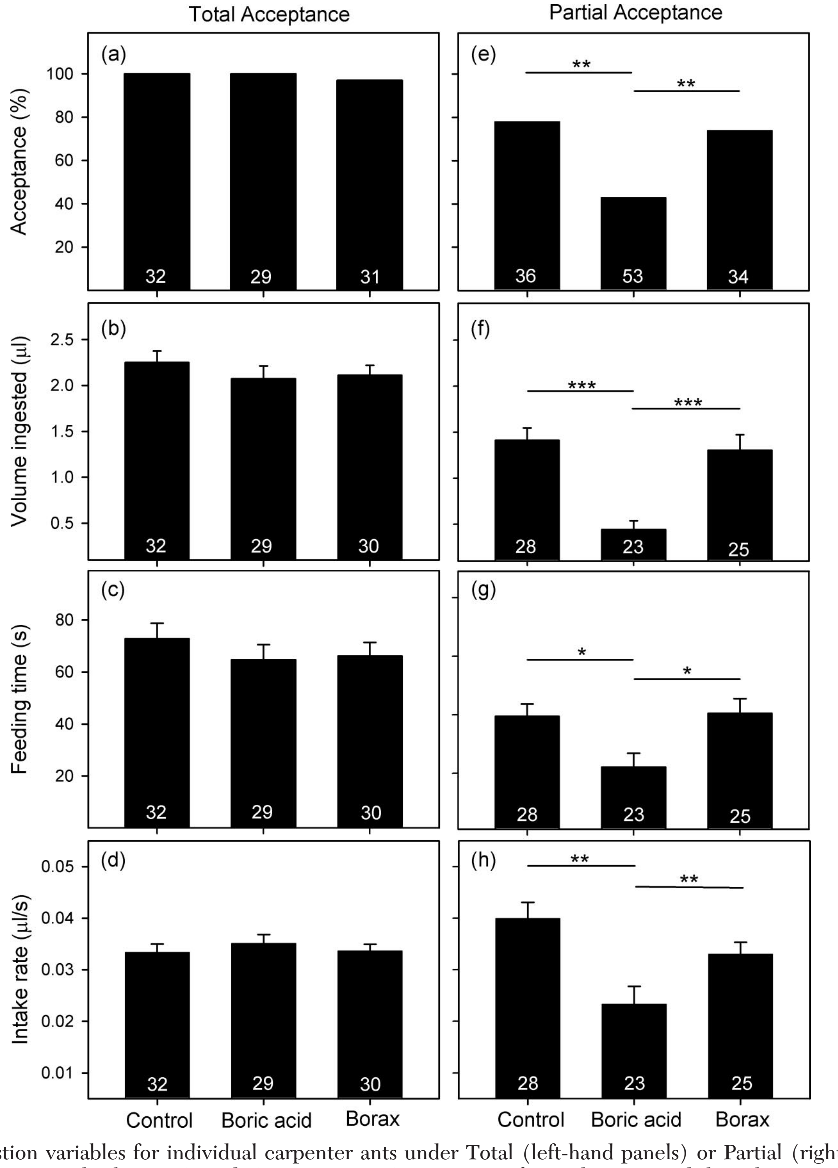


**Fig. 4.** Ingestion variables for individual carpenter ants under Total (left-hand panels) or Partial (right-hand panels) acceptance of the control solution. (a and e) Acceptance (percentage of ants that ingested the solution in relation to the number of ants that made contact with the solution), (b and f) volume ingested (mean ± SE), (c and g) feeding time (mean ± SE), and (d and h) intake rate (mean ± SE) for each of the three solutions offered: control (30% wt:wt sucrose), boric acid (30% wt:wt sucrose and 5% wt:vol boric acid), and borax (30% wt:wt sucrose and 5% wt:vol borax). Horizontal lines indicate significant differences (Tukey post hoc comparisons: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). The numbers inside the *bars* represent the number of experimental ants.

no differences in the boric acid mortality appeared, as was observed in the borax baits. These results demonstrated that borax is more likely to be more deterrent to Argentine ants than boric acid.

The nondeterrence generated by low concentrations of boric acid when formulated into sugar baits for the Argentine ant has been shown for laboratory colonies (Hooper–Biu and Rust 2000). Our results on individual performances support this also for higher concentrations of boric acid.

Alternatively, carpenter ants with high carbohydrate requirements displayed a high acceptance, and ingested large volumes of both boron baits, equal to the control bait. Differences in acceptance and volumes ingested among the offered baits emerged only when motivation for feeding was lower. Contrary to *L. humile, C. mus* showed a stronger deterrent effect for the boric acid bait rather than for the borax bait.

Mortality rates were a consequence of the amount of toxic solution ingested in all of the experiments performed in this study. As more toxicant was ingested, less time was required for the ants to reach 50% mortality (LT50). Mortality was the result of a single ingestion of the toxic bait on day 0, as the following

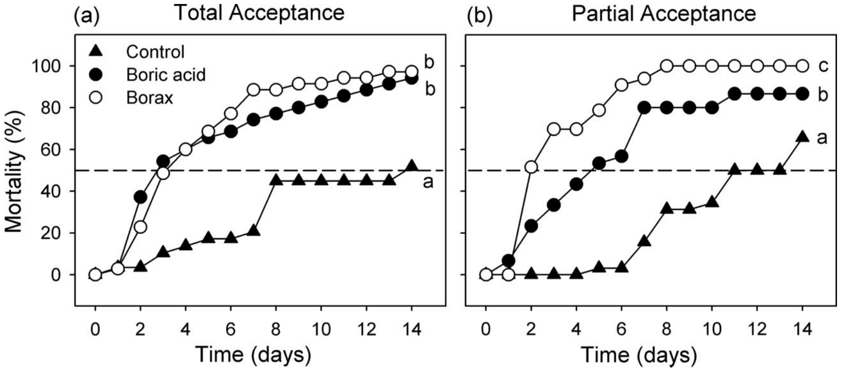


**Fig. 5.** Daily mortality (accumulated) of carpenter ants after receiving one of the three treatments: control (without toxicant), boric acid (5% wt:vol), and borax (5% wt:vol) all in 30% wt:wt sucrose. (a) Total Acceptance group: control ($N$ = 29), boric acid ($N$ = 35), and borax ($N$ = 35). (b) Partial Acceptance group: control ($N$ = 32), boric acid ($N$ = 30), and borax ($N$ = 33). Curves that do not share letters are significantly different (Kaplan–Meier; $P < 0.05$).

days no more toxicant was administered and ants had access to water and sugar solution ad libitum.

We emphasize the importance of the prevailing conditions associated with all of these results. Although some research indicates that concentrations of borates >1% wt:vol could be repellent (Klotz and Williams 1996, Hooper–Bui and Rust 2000), our study shows that when conditions lead to a high motivation, even 5% wt:vol boron baits are well accepted.

However, under different conditions, when motivation could be even lower than in our experiments, deterrent effects might be observed for both toxicants. Argentine ants can reject boric acid baits in certain situations, for example, 5% wt:vol boric acid appeared to be poorly accepted as it caused low mortality (Knight and Rust 1991), and these ants also ingested a smaller volume of solutions with a lower concentration (2 and 4% wt:vol boric acid) than a control solution (Klotz et al. 2000). However, in other situations borax could be well accepted by Argentine ants as reported for a commercial bait with borax (>5%) (Mathieson et al. 2012), or both boron toxicants could be equally consumed (Klotz et al. 2000). According to our results, these cases would reflect a high feeding motivation.

Ingestion experiments typically use starvation methods to promote feeding in the test subjects (Josens and Roces 2000; Mailleux et al. 2000, 2005, 2006; Falibene and Josens 2008; Falibene et al. 2009). Although this is a useful tool, it should be implemented carefully as prolonged starvation can have a detrimental effect on the amount of information one can retrieve from a given experiment. We have shown that distinct differences under certain conditions can be masked or lost if the motivation to feed is heightened, either by affecting the resource or the requirements. An animal with a high motivation to feed will have lower rejection thresholds, and possibly accept a resource that may be deterrent under natural conditions. Longer starvation periods on laboratory colonies caused more toxic bait consumption and higher mortality (Hooper–Bui and Rust 2000, Mathieson et al. 2012).

While most studies on this subject focus on the performance of groups or colonies (Klotz et al. 1997, 1998, 2000; Daane et al. 2006, 2008), we instead focus on the performance of individuals. In social insects, decision making in individuals will lead to the foraging success of the group as a whole (Seeley 1989, Mailleux et al. 2003, Hölldobler and Wilson 2008). As foraging involves sharing information with one another, these interactions and the individual decisions contribute to the coordination and regulation of the emergent foraging process. Therefore, our results, although based on individual performance, are relevant at the colony level.

The effectiveness of optimized ant baits is challenged by remarkable constraints as high-quality natural food is abundant, in the form of honeydew, extrafloral nectar and prey items or any accessible food in urban areas. This is an important consideration as under natural conditions available resources can vary greatly, effectively competing against baits used to control ants. Therefore, the type of toxicant to be used for a bait solution, and the concentrations of sucrose and toxicant are essential to maximize the effectiveness of control programs.

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