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Laboratory and field insights into the dynamics and behavior of Argentine ants, *Linepithema humile,* feeding from hydrogels

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ABSTRACT

BACKGROUND: Hydrogels that have absorbed a liquid containing a toxicant are a novel bait-delivery form for ant control. Here, we study the abilities of Argentine ant (*L. humile*) workers to imbibe liquid from hydrogels. We quantified feeding behavior with 1) hydrogels containing different sucrose concentrations (20, 30, 40, and 50%w/w), 2) hydrogels versus liquid droplets and 3) hydrogel age (time of air exposure). We also performed a field assay to assess visits of *L. humile* and other ant species to hydrogels.

RESULTS: Ingested volume and feeding time decreased with increasing sucrose concentrations, but the number and duration of pauses were similar. Feeding from hydrogels was slower than from a liquid droplet and ants imbibed less liquid and fed for shorter times from hydrogels. Feeding time increased with hydrogel age, whereas ingested volume decreased and approached zero after 120 minutes under laboratory conditions. In the field, ants attended the hydrogels during the full 120-minute period. When *L. humile* workers found a hydrogel, they monopolized it to the exclusion of other ant species. *L. humile* occupied and dominated hydrogels predominantly in shaded locations.

CONCLUSION: Hydrogels with sucrose concentrations no greater than 30% appear best for liquid uptake for *L. humile*. Hydrogels not in direct sunlight will have greater attendance by *L. humile* and, therefore, less attendance by non-target ant species. Shady and humid places may prolong the longevity of hydrogels, which would imply higher intakes.

Keywords: Ant control; Argentine ant; Bait delivery; Feeding behavior; Foraging; Water-storing crystals

1. INTRODUCTION

Several ant species are globally prominent for being pestiferous in urban, agricultural, and natural environments [1, 2], and resultantly much effort and money are spent on their management.[3, 4] Broadscale chemical control of ants is primarily conducted using sprays or granular baits. Both are effective but also have severe limitations. Sprays in particular are not focused on the target species and have excessive non-target effects [5, 6], and therefore are not an acceptable treatment option in natural environments. Granular products have a low performance on species that prefer aqueous sugar over solid matrices (e.g. corn grit, fishmeal).[7, 8] Both treatment forms are susceptible to environmental moisture (rain, dew, moist leaf litter) that would wash the toxicants into the soil.

In 2012, a novel ant bait form was used for the first time, being a water-storing matrix that had absorbed a sucrose solution containing a toxicant.[9] Polyacrylamide hydrogels are superabsorbent polymers that can absorb up to around 350 times their weight in water and are used in soil applications to maintain moisture. The use of hydrogels for ant control was ingenious in that it provided a liquid food source that can be imbibed by ants, but it is in a solid form which allows for ground or aerial dispersal. Even though it is not yet a commercial product, toxic bait delivered in polyacrylamide hydrogels has proven to be highly efficacious against the Argentine ant, *Linepithema humile,* in numerous locations in both laboratory and field settings [9-13], as well as against numerous other invasive ant species.[14, 15] This bait delivery form is likely to have a great future role to play for ant management, especially within eradication programs.

In the effort to determine how best to use this new tool, there have been multiple laboratory studies investigating hydrogel dehydration rates [16], feeding preference based on hydrogel dehydration state [10], and efficacy rates with different toxicant concentrations.[12] But no study to date has manipulated the sucrose concentration to quantify uptake, with all published works using a standard 25% sucrose solution. Sucrose concentration may be important because it affects liquid viscosity and potentially the ability of ants to extract the liquid from the hydrogels. Understanding and maximizing bait uptake is important for ant management because only a small percentage of the population forage to bring back food resources to be shared with the other colony members, so presumably maximizing bait volume that can be ingested by foragers will assist maximizing treatment efficacy.

In this study, we assessed the ability of individual *L. humile* workers to imbibe liquid sucrose solution from hydrogels (hereafter referred to feeding), in order to evaluate the potentiality and limitations of this matrix for bait delivering to be used in control strategies applied to this invasive species. To analyze to what extent the hydrogel matrix imposes restrictions for feeding and affects individual ingestion performance, we compared the feeding behavior for the following scenarios: 1) different sucrose concentrations, 2) hydrogels versus liquid droplet and 3) hydrogel age.

We additionally conducted a field trial in *L. humile*'s native range, i.e. Argentina (where this ant coexists with other species) to characterize its foraging activity on sucrose solution delivered by hydrogels. Knowing that *L. humile* ants tend to outcompete other ant species in the case of valuable resources, we assessed the dominance on the offered hydrogels by comparing the visits to the foraging stations of both *L. humile* and other ants for 2 hours. Considering the importance of *L. humile* as an invasive species at a global level and taking

into account the few studies carried out in its native range, we analyzed different factors that could affect the dominance of the offered hydrogels with the aim of seeking insight for improving the specificity of this tool for the Argentine ant control.

2. MATERIALS AND METHODS

2.1 *Laboratory Experiments*

Experiments were performed using two *L. humile* colonies that had been collected at the Campus of the University of Buenos Aires, Argentina (34°32”48’S; 58°26”21’W), which is within this species’ native range. Each colony contained approximately 4000-5000 workers and had more than one queen. The colonies were housed in the laboratory for at least two months prior to use. The colonies were kept in plastic boxes (30cm x 50cm x 30cm, width x length x height) with the sides painted with fluon to prevent escapes. The colonies were maintained in a temperature-controlled environment (25 ± 2°C) under a natural light-dark cycle. Ants were fed daily with honey-water and three times a week with fresh cockroaches (*Blaptica dubia*) or minced beef. Water was provided *ad libitum*. No starvation was required for the experiments.

For all experiments, trials were performed over 5-10 days to achieve the required replication. In all cases, trials were conducted one at a time, with trials assigned to treatments randomly. Ants were utilized only once (i.e. within only one trial, for one treatment) and were discarded after use. All experiments were conducted in laboratory conditions at 26°C (±1°C) and relative humidity of 50-62%.

2.1.1. Experimental design

For each trial, an ant was gently taken from a colony and placed onto a toothpick (2 mm x 50 mm) partly inserted into the lateral wall of a plastic cup (Fig. 1). The ant could move freely on the toothpick and the cup's wall was painted with fluon to restrict the ant to the toothpick. The cup had a small hole, just above where the toothpick was inserted, where a food (hydrogel cube or liquid droplet depending upon the experiment) was accessible to the ant (Fig. 1). For the hydrogel cube, only the non-cut face was accessible to the ant, because this is the surface accessible to ants in real-use field conditions. The food was set on a flat piece of plastic that had been glued onto the toothpick so that the wood would not absorb any liquid. Each ant was filmed from the lateral view, using a camera-fitted stereomicroscope (Leica MZ8-25x magnification, with a Leica ICA camera), which was connected to an analog-to-digital signal converter to store the video on a computer (Fig. 1). The video was turned on before putting the ant on the toothpick and turned off (and the trial ended) when the ant finished feeding (typically < 5 minutes). After each trial, the food and ant were discarded, and the plastic surface was cleaned with clean moist cotton and dried. To ensure no bias of time of day etc, trials using different sucrose concentrations were conducted randomly and trials of all treatments were tested every day (from 10 am to 4 pm).

From each video, feeding time was measured as the total time that the ant spent feeding on the food. If the ant stopped feeding for any reason (e.g. walked along the toothpick, remained immobile, or tried to climb the cup's wall), this time was considered as *pause time*, but only if the ant fed again. Pause time, therefore, was the time between multiple feeding events.

The volume of solution ingested was estimated as the difference in gaster volume before and after feeding. [17] We approximated the gaster to be an ellipsoid in order to calculate its volume. We measured the length and height of the gaster directly from the videos, and the width of the gaster (not visible from side view) was estimated from the relationship width:length=1.0:1.1 determined for *L. humile*. [18, 19]

2.1.2. Hydrogels

Polyacrylamide hydrogels (Magic Water Beads, NFL Enterprises. Miami, Florida. Clear, dehydrated diameter 2-2.5 mm) were soaked in one of four sucrose concentrations (20%, 30%, 40%, or 50% w/w) in a flask in a fridge set at 8°C for 48h hours. These sucrose solutions were prepared just before the hydrogel's immersion, and we used a volume that would cover completely the beads even after they have been hydrated. Just prior to a trial, a cube (3mm x 3mm x 3mm) was cut from a hydrated hydrogel, allowed to sit for 2 minutes to reach room temperature, and then placed on the feeding arena (Fig. 1). A new cube was used for each trial. A hydrated hydrogel could be cut into about 15 cubes. A hydrogel was only used for trials on a single day.

2.1.3. Experiments

Sucrose Concentration: We compared feeding variables (feeding time, pause time, volume of solution ingested, and intake rate) among hydrogels containing the four sucrose concentrations. Hydrogels were always freshly removed from the hydrating solution just prior to use so that there were no environmental evaporation effects. Thirty trials were conducted for each of the four sucrose solutions (n=120).

Hydrogel vs liquid droplet: We compared feeding variables of 20% w/w sucrose solutions offered as a liquid droplet or in a hydrogel. We used 20%ww as this had the greatest uptake among the concentrations in the sucrose concentration experiment. Thirty trials were conducted for both treatments (n=60).

Hydrogel lifespan: We compared feeding variables among hydrogels containing 20%w/w sucrose solution exposed to room air (T=26±1°C and RH=56±6%) for 0, 30, 60, 90, and 120 minutes. Eighteen trials were conducted for each of the five treatments (n=90).

2.2. Field Experiment

2.2.1. Study site

This study was conducted on the campus of the University of Buenos Aires (34°32´45´´S; 58°26´20´´W) in the central-eastern region of Argentina. The weather is temperate with a monthly precipitation that varies between 58 and 140 mm. Minimal and maximal temperatures are 20°C and 30°C respectively in January (summer) and 7°C and 15°C in July (winter) (National Meteorological Service, Argentina). Our study was conducted in late October (middle Spring). On the days of the experiment, temperatures were between 16 and 24 degrees, and the RH between 30% and 71%.

The campus, University City, is a ~50 ha property on the banks of the Parana River. *L. humile* abounds in a patchy distribution on the campus [20, 21], with sufficient abundance that it requires constant chemical control within buildings. Fifty-six other ant species are known to occur on the campus [20, 22], but *L. humile* is the only species that must be regularly controlled. Of the 50 ha, 15 ha is sport fields; 4 ha is for parking, 5 ha is covered by buildings and the rest is landscaped green space, alternating grassy areas with wooded areas (values estimated with Google Earth Pro).

2.2.2. Experimental design

Five transects (T1 to T5) were established in the area, each with 5 stations spaced 10 m apart (S1 to S5). The transects were positioned to avoid streets or buildings, with all the stations being placed on soil (Fig S1 in Supp. Mat.). Each station consisted of a circular (*ca.* 8 cm in diameter) white plastic plate with half a hydrogel sphere (*ca*. 1 cm in diameter) placed in the center, flat surface down. This procedure allowed us to see all the ants feeding from the hydrogel, as a preliminary assay showed that using an entire hydrogel sphere made assessment difficult. The hydrogel had been hydrated with 20%w/w sucrose solutions as detailed above. Hydrogel half-spheres were kept in the solution until the moment of putting them on the station in the field.

The transects were sampled individually and sequentially. First, we established the five stations. Then, we took a photo at each, with this time being considered as time 0. Then every 30 minutes for two hours we recorded 1 min videos (4 videos in total: at 30, 60, 90, and 120 min). After the recording we carefully took a sample of ant species present for identification, waiting for individuals to be at least 10 cm away from the station to avoid disturbance. Ants other than *L. humile* were identified to genus in the laboratory.

To study ant activity, from each video at each station taken at the four assessment times (30, 60, 90, and 120 min), we took 5 frames, one frame every 15 seconds, and counted the number of *L. humile* individuals and of other ant species present at the station. We counted separately the ants in contact, and not in contact, with the hydrogel. For each station the number of ants present was calculated using the average of these 5 photos (i.e. when one

ant was present in each of the 5 photos, the number used was 1, but if an ant occurred in only 1 photo, the number used was 0.2). We then calculated the percentage of *L. humile* and other species from the total number of ants counted at each station. We also classified for each photo the sun/shade status of the station (0= total sun; 0.5= partially shaded; 1= total shade). Thus, the average of these values for the period of the experiment was taken as an index that characterizes the sun/shade status of each station with values from 0 to 1, being the extremes 1 = total shadow and 0 = total sun. We then condensed this index into three categories: from 0 to 0.3 = sunny, from 0.4 to 0.6 = partial shade and from 0.7 to 1 = full shade.

T1, T3 and T5 were assessed during late morning, and T2 and T4 were assessed over the early afternoon. Additionally, after the 2h-experiment, we visited all the stations at least two more times over the next two days to assess sun/shade status at other times of the day. This determined whether each station had a different sun/shade category than during the two-hour experiment. Simultaneously we also casually noted ant activity on the stations.

2.2.3. Statistical analysis

Statistical analyses were performed using R version 3.6.2 (R Core Team 2019). For the laboratory experiments, most variables were analyzed using the non-parametric Kruskal-Wallis test with significance among treatments determined post hoc using Dunn's Test. For variables where homoscedasticity assumptions were not met, we ran a GLS model applying a constant variance function and used package 'nlme' to conduct analyses, with significance among treatments determined post hoc using Tukey’s test.

For the field experiments, we recorded different variables from the photos of the videos. For all the measurements we only considered two classifications, *L. humile* and *others* (the rest of other ant species taken together). Firstly, we analyzed the frequency of stations visited by at least one *L. humile* individual at any time in relation to the sun/shade categories by a Fisher Exact Probability test.

To determine if the presence of at least one *L. humile* worker during the first hour affected the probability of total monopolization (100% of the ants) by this species in the last recording of each station, we categorized the stations in two ways; 1) with or without *L. humile* in the first hour and 2) if at least one ant of another species touched the hydrogel or not at the last recording of each station. We excluded one station from this analysis as there were no ants in its last recording. The independence of these two variables was assessed by a Fisher Exact Probability test.

We further analyzed the percentage of *L. humile* at the last recording of each station (as response variable) using a linear model accounting for interactions with the two explanatory variables: sun/shade status (sunny, partial shade, full shade) and 'first arrival recorded' (*L. humile* or others). We excluded one stations from this analysis as there were no ants in its last recording. As the normality assumption was not met, we used the non-parametric Kruskal-Wallis test, and the interaction was further analyzed using Dunn's Test.

3 RESULTS

3.1. Laboratory Experiments

1) *Sucrose concentrations*

Estimated ingested volume varied significantly and inversely with sucrose concentration (H = 99.7; df = 3; $P < 0.001$). Ants that fed from the two most dilute solutions (20 and 30%) ingested approximately triple the volume of those that fed from the highest concentration (50%) (Fig. 2A). Feeding time decreased significantly with increasing sucrose concentration (F = 7.33; df = 3; $P < 0.0001$. Fig.2B), with ants feeding longer from the two lowest concentrations (20 and 30%). Pause time and number of pauses did not differ among the concentrations (pause time: H= 1.24; df = 3; $P = 0.74$; number of pauses: H = 5.8; df = 3; $P = 0.12$). The intake rate of the highest concentration was four times slower compared to the other three concentrations which did not differ statistically from each other (F = 36.69; df = 3; $P < 0.0001$. Fig. 2C).

2) *Hydrogel vs liquid droplet*

Almost five times more liquid was consumed from a liquid droplet than from a hydrogel (F = 13852.30; df = 1; $P < 0.0001$. Fig. 3A). Feeding time was almost twice as long at a hydrogel than at a liquid droplet (F = 193.61; df = 1; $P < 0.0001$. Fig. 3B). Ants feeding on a liquid droplet rarely paused, whereas ants feeding on a hydrogel had many long pauses between feeding events. As a result, the intake rate of ants feeding on a liquid droplet was almost three times greater than for ants feeding from a hydrogel (F = 463.94; df = 1; $P < 0.0001$. Fig 3C).

3) *Hydrogel lifespan*

The estimated ingested volume reduced significantly with increasing air exposure time (F = 1620.97; df = 4; $P < 0.0001$. Fig. 4A), with almost no liquid consumed from hydrogels exposed to air for 120 minutes. Feeding time increased significantly with exposure time (H =

55.53; df = 4; $P < 0.0001$. Fig. 4B), doubling after 30 minutes of exposure, and increasing again after 120 minutes of exposure. Pause time did not differ with exposure time (H = 3.97; df = 4; $P = 0.41$). Intake rate decreased significantly among each increasing exposure time, reaching almost zero by 120 minutes (F = 504.73; df = 4; $P < 0.0001$. Fig. 4C).

4) Field Experiment

All 25 stations were found by ants, with *L. humile* finding 14 stations. Other ants that fed from hydrogels were from the following genera: *Pheidole, Nylanderia, Brachymyrmex, Crematogaster, Solenopsis* and *Paratrechina*. The most individuals observed simultaneously at a station was 69 ants (Table 1).

Firstly, we described a non-target species observation and then different aspects related to the dynamics of the foraging visits for both *L. humile* and other ant species.

At three stations on T5 (Gray areas in Fig.5), we saw a chalk-browed mockingbird (*Mimus saturninus*) picking up the hydrogels (Video S1 in Supp. Mat). At another station from T1, the hydrogel disappeared between 30 and 60 mins (just remaining a tiny piece of hydrogel), presumably taken by a bird. Apart from these cases, no other animals were observed feeding on the hydrogels.

The occurrence of *L. humile* at stations had great variability. For all timeframes combined, there was no record of *L. humile* at 44% of the stations, whereas it was the only species present at 8% of the stations (Table 2). In the remaining 48% of the stations both *L. humile* and other species were recorded. But if we only take into account ants in contact with the hydrogel, then *L. humile* was the only species recorded at 24% of all stations.

Regarding the first ant discovering each station, *L. humile* was the first in 6 of the 25 stations. Other species were found first at 17 stations, and at 2 stations there was a simultaneous record of both *L. humile* and another species. Most (60%) of these first recordings were at time 0 (i.e. after just two minutes of placing the hydrogel), comprising 16% *L. humile,* 40% other species, 4% both *L humile* and another species.

To analyze some factors that could affect the final result of the station, we first evaluated if the presence of other species in contact with the hydrogel in a station at the end of the experiment is independent or not of the fact that *L. humile* discovered the station in the first hour. Effectively, the presence of *L. humile* in the first hour strongly affected the final result of the access to the hydrogel for other species (Fisher exact test: p-value =0.00002; N= 25). When a station was found by *L. humile* within the first 60 minutes, the probability that no other ant species would be in contact with the hydrogel at the end of the experiment was high (0.83). The mechanism of this usurpation of the hydrogel was readily observed, with *L. humile* aggressively displacing other ant species (Video S2 illustrates this behavior with images of the preliminary assay; Supp. Mat.). Besides, the other ants quickly abandoned the station when they detected the presence of *L. humile*.

We also analyzed the impact of sun exposure on the presence of *L. humile* at stations at any time during the evaluation. Shading greatly influenced *L. humile* presence at stations (Fisher exact test: P = 0.0175, Fig.5), with *L. humile* present at 90% of fully shaded stations, 33% of partially shaded stations and 33% of sunny stations. Taking into account the sun/shade

status of the stations considering different times of the day, there was no fully shaded station without *L. humile*, but only 20% of the sunny stations had *L. humile* present.

Finally, we evaluated how the percentage of *L. humile* in the last recording of each station was affected by two factors, the sun/shade status of the station and who was the first ant to discover the hydrogel, analyzing their interaction. There was a significant interaction between the sun/shade status and who was the first to discover the hydrogel, which affected the percentage of *L. humile* in the last recording ($H = 18.099$, $df = 4$, $P = 0.001$). On the one hand, of the ten stations in shade, *L. humile* was the first ant recorded at three (T1S1, T1S4, T4S3), but by the end of the experiment it dominated (≥90% of individuals at a station) eight stations. On the other hand, of the twelve stations that were in the sun, *L. humile* was the first ant recorded at three (T3S1, T4S1, T4S4), and it dominated only two at the final assessment time. It is worth noting that those two stations in the sun dominated by *L. humile* were two of the ones where it arrived first (T4S1, T4S4). In other words, *L. humile* visited few stations located in the sun, and had a very low probability of dominating those stations, and this could have only happened if it arrived first. On the contrary, in the shade, regardless of whether it was the first one to arrive or not, it had a high probability of finding the station and dominate it in two hours.

4. DISCUSSION

Our experiments have provided new insights into the functionality of hydrogels for use in ant management. In laboratory experiments, we quantified for the first time *L. humile* workers imbibing fluid from hydrogels into their crops. We showed that increasing the sugar concentration increases feeding time but decreases the amount of liquid ingested. We confirmed that feeding from a hydrogel is more constrained than from a liquid droplet. Ants

ingested less solution from the hydrogel, and they spent less time feeding. Finally, we showed that ingestion volume and intake rate decrease as hydrogels dehydrate, but feeding time increases under laboratory conditions.

Feeding behavior on hydrogels containing different sucrose concentrations showed some similar and some contrasting results compared to when ants feed directly from liquid droplets. Ingestion volume from hydrogels decreased as sucrose concentration increased, consistent with results of *L. humile* and other ant species feeding from liquid droplets. [18, 23, 24] However, ants spent less time feeding from hydrogels with increasing concentrations. This was unexpected because when feeding from liquid droplets, *L. humile* increases feeding time with increasing sucrose concentration [18], as do other ant species. [23-25] The intake rate (volume ingested in a given time) remained constant until the sucrose concentration was 40%, after which the intake rate reduced. This is consistent with published studies on ants feeding on *ad libitum* sources showing intake rates start to decrease between 30-50 % sucrose.[23, 26, 27] Therefore, hydrogels containing 20-30% sucrose seems appropriate for use with *L. humile*.

To date, broadscale field applications of hydrogels have used approximately 30% sucrose solutions [9], and this protocol is in line with our finding that 20-30% sucrose solutions maximize uptake. Exactly which concentration to use will likely be situation-dependent, with areas having greater natural carbohydrate supply possibly needing higher sucrose concentrations to attract foraging ants to hydrogels over other carbohydrate sources.

Hydrogels rapidly dehydrate [10], slower as relative humidity or substrate moisture increases, and faster with high sucrose concentration.[12, 13] Hydrogel age influenced liquid uptake under our laboratory conditions. By two hours of air exposure at T=26±1°C and RH=56±6%, ants

attempted to feed for a long time but almost no solution was imbibed. Other studies, when offering hydrogels of different ages to laboratory colonies, reported a decrease in ant attendance as hydrogels aged.[10, 12] This is reasonable, considering the extremely low individual intake rate that we recorded after the hydrogels had been exposed to air for more than an hour. The useful lifetime of the hydrogel to deliver solution was shorter than hydrogel dehydration in other studies, both under laboratory conditions and under the sun [10, 12], or using alginate hydrogels. [28] However, we did not measure hydrogel liquid loss, just individual ant behavior, and so we cannot make a direct comparison of dehydration with other works. Potentially our hydrogels were less hydrated from the beginning, or an uncontrolled factor (e.g. room airflow) could have dehydrated hydrogels in addition to room temperature and relative humidity.

The effective lifespan of hydrogels has differed greatly among published research for both laboratory and field experiments, and the same has been found with our results. For example, Krushelnycky (2019) found that ant attraction to hydrogels in the field generally declined after 30 minutes, probably as a consequence of hydrogel dehydration because the hydrogels lost >50% of their water within an hour of full sun exposure. Conversely, we found recruitment continued increasing after this time and often ant attendance remained high until the end of recordings at two hours. Likewise, in the laboratory, even though at 120 min ants were not successful imbibing much liquid they persisted attempting to feed. The persistence of ants attempting to feed is consistent with the response of the ants when they detect sugar.[29, 30] Ultimately, variations in temperature, humidity, shading and even hydrogel size and shape will influence this variability.

The overarching implication of liquid uptake relative to hydrogel lifespan is that application timing is critical as ants need to be given adequate time to feed from the hydrogels. But

appropriate timing of hydrogel dispersal will be dependent upon local environmental conditions and ant activity. For example, in hot environments such as northern Australia where treatments are being conducted against yellow crazy ant, *Anoplolepis gracilipes* [31], hydrogel baits are predominantly dispersed in the late afternoon so that the hydrogels are fresh when *A. gracilipes* recommences foraging as temperatures reduce, and so that workers can feed from the hydrogels at night when its activity is greatest. Conversely, hydrogel treatments for *L. humile* [9] have been conducted throughout the day in cooler temperate environments where the ants are active during the day and hydrogels don't dehydrate rapidly.

Our field experiment showed the importance of hydrogel location in relation to sun exposure, not only for hydrogel lifespan but for species visiting and dominating the hydrogel, with the presence and dominance of *L. humile* predominantly in shaded locations. Additionally, our experiment showed that, mostly, *L. humile* did not find hydrogels quicker than other ant species, but it behaviorally dominated them more consistently, particularly in shade. This conforms with its behavior in the invasive range, where it also dominates numerically.[32] This efficient interference competition of the Argentine ant may have positive consequences for native ants in areas where toxic baits are used in management programs for *L. humile*, as they expel the native ants from the baits. [33]

Presumably, it is not the shade that increases the probability of finding *L. humile*, but the coverage level, microclimatic aspects that maintain higher humidity, heterogeneity of the landscape offering nesting sites, etc. [34] In our study, performed in its native range, *L. humile* presence varies significantly depending on the characteristics of the area, being only found in forests, isolated trees, shrubs, along concrete slabs surrounded by dense vegetation, in

concrete walls, or buildings in general. This also coincides with other studies in invaded ranges [35], as highly disturbed areas commonly offer a fragmented setting.[36] Considering this, along with our results, it would be convenient for control strategies in small areas where the ant target species is patchily distributed, for hydrogels to be dispersed locally rather than over entire areas as it will lead to more efficient treatment.[14] Our results also suggest that hydrogels placed in shaded locations may provide greater treatment efficacy and lower non-target impacts.

Apart from *L. humile*, we found species of the genera *Pheidole, Nylanderia, Brachymyrmex, Crematogaster, Solenopsis,* and *Paratrechina* at the stations. This shows that potentially the hydrogels will have broad applicability for use in ant control for different species, potentially more so than standard granular ant baits that are mostly specific to seed consumer and leaf-cutting ants. We also found that non-target insects were not interested in the hydrogels, consistent with findings of other studies.[37] The highest engagement with non-target organisms was 3 of 25 hydrogels being eaten by birds (probably just one bird). No other publications have reported bird attractancy to hydrogels, even when specifically investigated.[16] But such work to date has been conducted in "natural" areas, whereas our study was at a University where birds are very used to human presence and possibly more opportunistic. Indeed, in northern Australia, Torresian crows (*Corvus orru*) which are very opportunistic scavengers will commonly feed on hydrogels being prepared for use in an ant eradication program (Hoffmann unpublished observations).

A notable incidental observation was hydrogels that had been placed in the field during the afternoon on a sunny day that were subsequently subjected to rain over night had ants attending the next day. Because ants remained attracted to the hydrogels suggests that the

water absorbed from the rain mixes within the hydrogel with the original hydrogel liquid contents and spreads throughout the matrix, it doesn't just form a new external layer. Therefore, the efficiency of hydrogels could remain high even if rain or water from irrigation falls shortly after hydrogel dispersal or even following partial dehydration. This supports other unpublished observations of the efficacy of hydrogels containing toxicant in use for invasive ant eradication (Hoffmann unpublished data) which is unprecedented for an ant bait as baits are normally ruined by rain.

Our results provide new information about the behavior of *L. humile* workers feeding from hydrogels in laboratory and field conditions. These results can be used to help actively adapt treatments to local conditions to improve ant-control or eradication programs. Clearly, hydrogels appear to have a great utility as a tool for pest ant management.

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Availability of data: Data are available from Figshare Digital Repository.

Author contribution: RJ and BDH conceived and designed research. MEC conducted the lab experiments and MEC and IRF conducted the field experiment. MEC analyzed data. MEC and RJ wrote the original draft of the manuscript. BDH and IRF reviewed and edited the manuscript. All authors read and approved the manuscript.

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Figure captions

Figure 1 Experimental setup (A). The bridge and the arena viewed from above (B) and from the side (C). Position of an ant contacting a hydrogel cube (D).

Figure 2 Liquid uptake metrics for ants feeding on four sucrose concentrations (20%, 30%, 40% and 50% w/w) absorbed within a hydrogel. A: Estimated ingested volume (µl); B: Feeding time (s) and C: Intake rate (nl/min). Letters indicate statistical separation (Dunn Test, $P < 0.05$). $N_{total} = 120$

Figure 3 Liquid uptake metrics for ants feeding on 20%w/w sucrose solution from a liquid droplet or from a hydrogel cube. A: Estimated ingested volume (µl); B: Feeding time (s); C: Intake rate (nl/min). Letters indicate statistical separation (Dunn Test, $P < 0.05$). $N_{total} = 60$

Figure 4 Liquid uptake metrics for ants feeding on hydrogel cubes containing 20%w/w sucrose solution at different are exposure times (0, 30, 60, 90 and 120 min). A: Estimated ingested volume (µl); B: Feeding time (s); C: Intake rate (nl/min). Letters indicate statistical separation (A and C: Tukey Test; B: Dunn Test, $P < 0.05$). $N_{total} = 90$

Figure 5 Relative frequencies of ants visiting the stations over time (at 0, 30, 60, 90, and 120 min). Stations were located along the 5 transects (from T1 to T5) with 5 stations each (from S1 to S5); i.e. 25 stations in total; from A to Y). *L. humile* (black bars) and other species (white bars) were counted from individual digital photos. Five photos were averaged for each time to give the number of ants per station and per time (except for time 0 which had

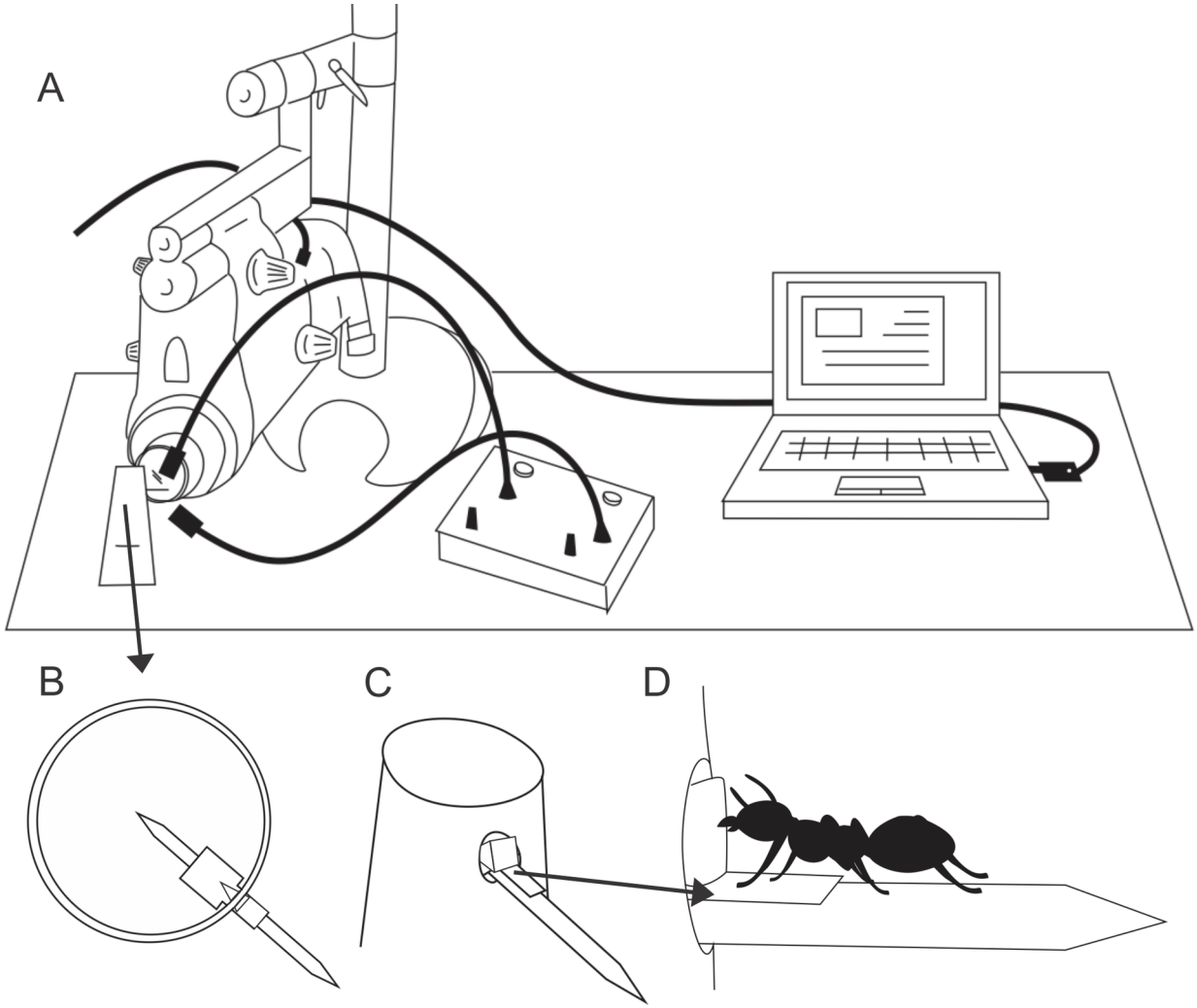
only one photo). Open circle, black circle and the combination of both represent the sun/shade index categories of sunny, full shade and partial shade respectively.

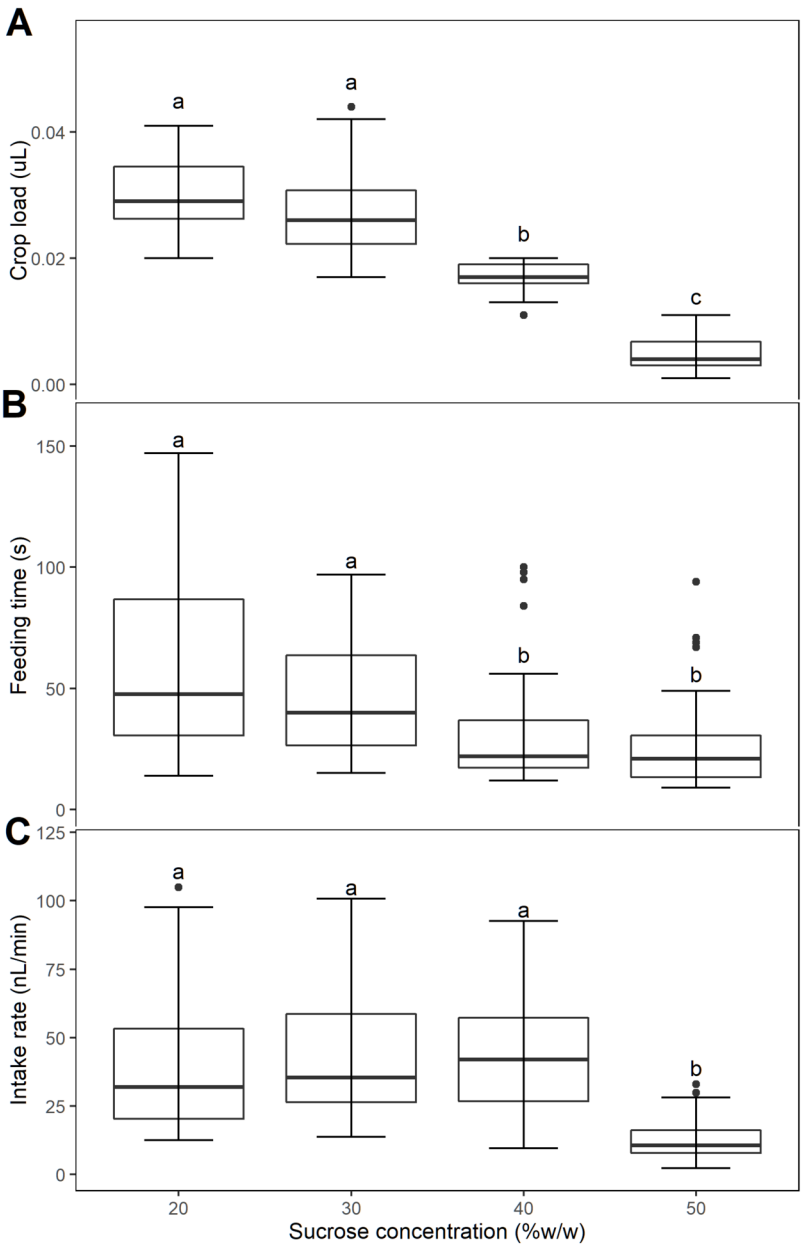
Table 1 Maximum number of ants recorded in a single photo of a station in each time period. Information in parentheses are transect and station numbers.

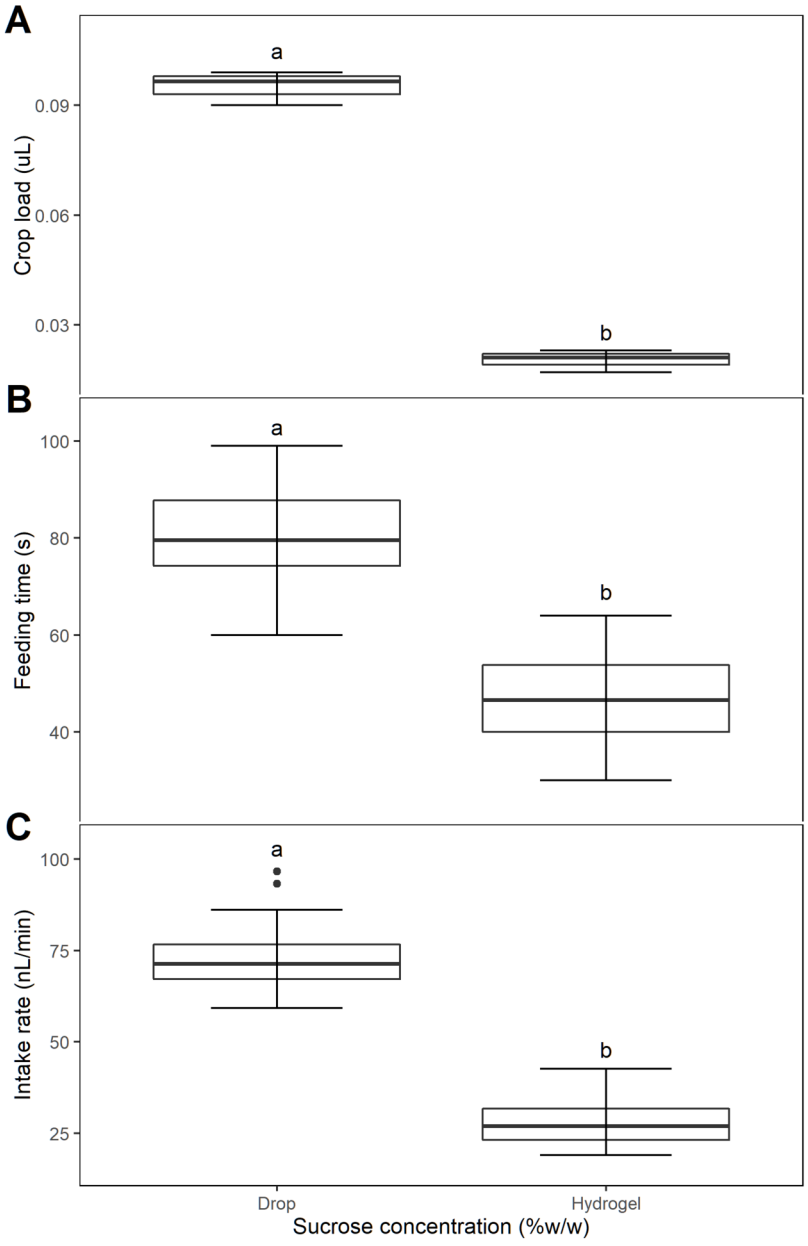
TIME (min)	*L. humile*	*Other* species
0	16 (T4S4)	13 (T3S5)
30	24 (T4S3)	54 (T5S5)
60	48 (T1S4)	69 (T5S4)
90	36 (T4S3)	35 (T2S5)
120	34 (T4S3)	36 (T2S5)

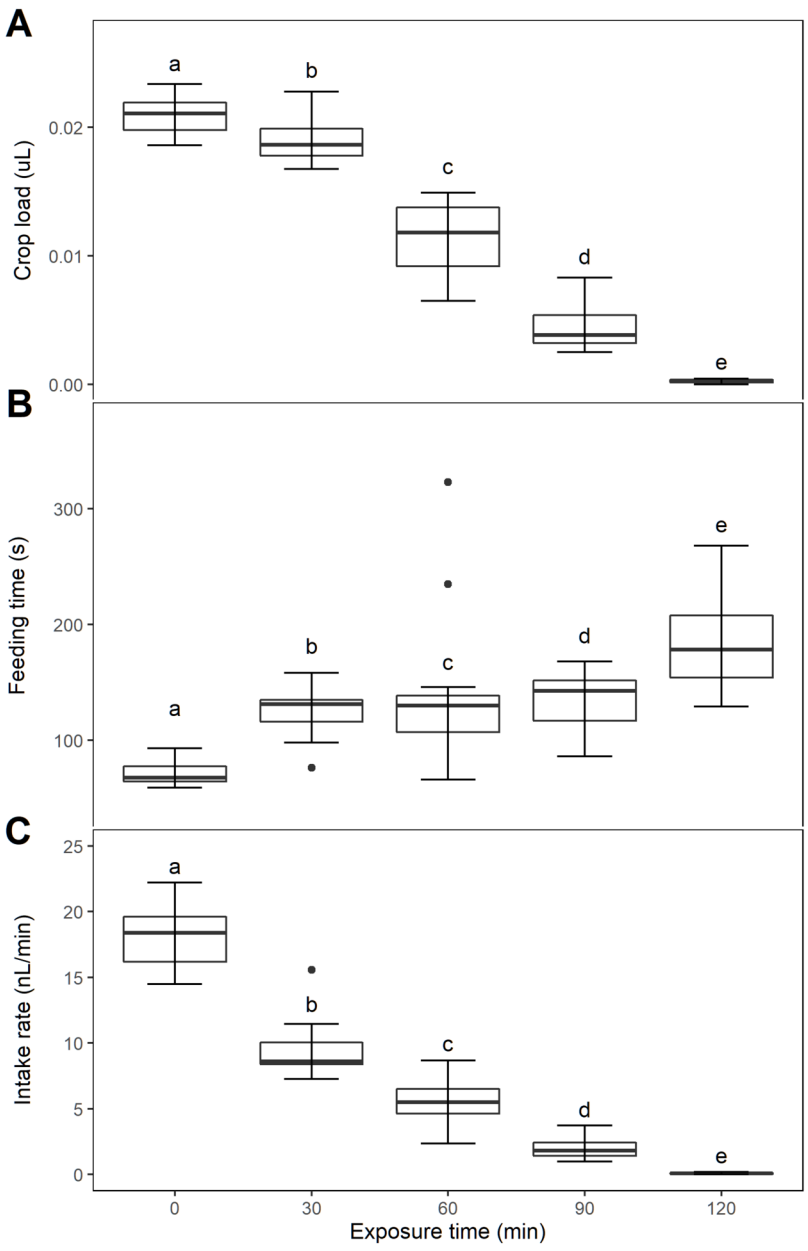
Table 2 Occurrences of ant activities at the 25 stations. Data in parentheses are % stations.

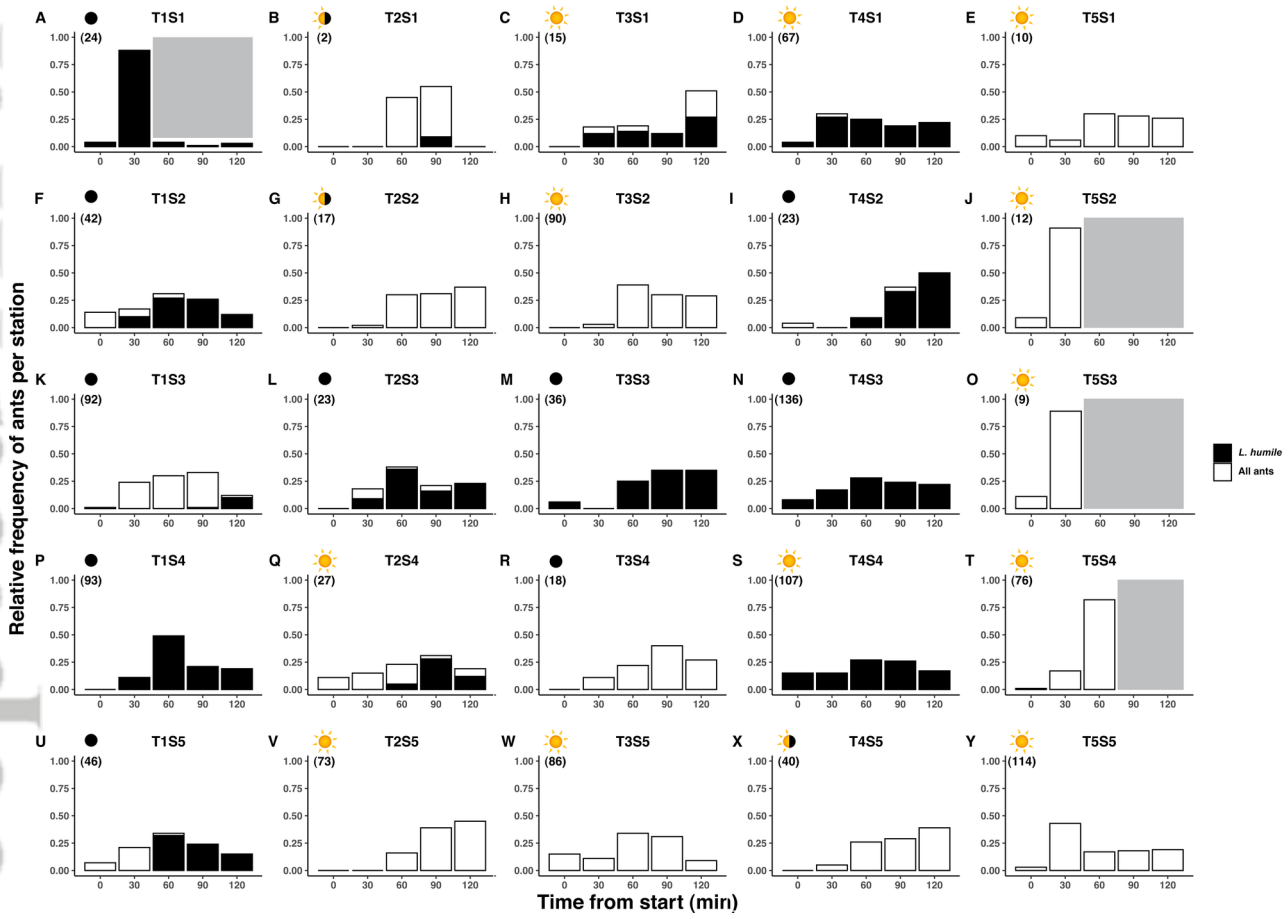
Observation	Only *L humile*	Only others	Both[a] or None[b]
Present at the station at all times	**2** (8)	**11** (44)	**12**[a] (48)
Contacting the hydrogel at all times	**6** (24)	**11** (44)	**8**[a] (32)
Present at the first recording time	**6** (24)	**17** (68)	**2**[a] (8)
At least one ant present during the first hour	**2** (8)	**13** (52)	**10**[a] (40)
>90% ants in last recording	**10** (40)	**11** (44)	**4**[b] (16)
Contacting hydrogel in last recording	**10** (40)	**11** (44)	**3**[a] (12) **1**[b] (4)











↓ Volume ingested FOR { Concentrations > 30% w/w
Increasing time of air exposure

↑ Foraging activity FOR { Shadowy
or partially shadowy places