International Journal of Pest Management

ISSN: 0967-0874 (Print) 1366-5863 (Online) Journal homepage: http://www.tandfonline.com/loi/ttpm20

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To cite this article: Roxana Josens, Francisco Sola, Jimena Lois-Milevicich & William Mackay (2016): Urban ants of the city of Buenos Aires, Argentina: species survey and practical control, International Journal of Pest Management, DOI: 10.1080/09670874.2016.1239035

To link to this article: http://dx.doi.org/10.1080/09670874.2016.1239035

INTERNATIONAL JOURNAL OF PEST MANAGEMENT, 2016
http://dx.doi.org/10.1080/09670874.2016.1239035

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Urban ants of the city of Buenos Aires, Argentina: species survey and practical control

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ABSTRACT
Ants are among the most diverse, abundant and ecologically significant organisms on earth. They have colonized almost all existing habitats, including urban areas, where they may pose serious problems for human activities. Here, we present different aspects of our studies on urban ants in the city of Buenos Aires aimed at collecting information on the species present in the city and at improving bait control strategies via laboratory assays. The use of these baits represents a control strategy that is environment-friendly as it avoids indiscriminate pesticide release. Moreover, we show that our baits exhibit higher efficiency when compared to a commercial bait, as it is optimized in terms of the ants' feeding behavior even when both have the same active compound and at the same concentration. This work represents the first integrative study on urban ants in the city of Buenos Aires and indicates that the control of invasive species in urban settings may be improved by increasing the scientific knowledge of the biology of the target species.

ARTICLE HISTORY
Received 22 April 2016
Accepted 26 August 2016

KEYWORDS
Social insect; urban pest; hospital ants; bait; *Camponotus mus*

1. Introduction

Eusinanthropic species are adapted to live in very disturbed areas, with a high density of human buildings. Insects of urban areas, such as many ant species, are examples of this adaptation. Some species may go unnoticed, while others constitute noxious pests that need to be removed or controlled continuously due to their negative impact on human activities. In a recent review on urban ants, Santos (2016) classified most studies into four categories: (1) diversity and ecology, (2) ant pest control including both chemical and biological control, (3) invasive ants and (4) public health.

In that review, as well as in a previous one (Chacón de Ulloa 2003), it is clear that there is no information on urban ants in Argentina (Santos 2016).

Buenos Aires is similar to most cities with mild climates in having many undesirable ant species which bite or sting, with dramatic consequences for allergic people, infants or the elderly. Some species are considered "structural" pests in the sense that they can damage wood structures or poles, house foundations, insulation materials, etc. during the construction of their nests and galleries. Cracks in houses favor the foundation of nests. Metal plates, moldings, hollow bricks and doors, and even motors and electric circuits offer nesting sites for diverse species. When nests are built inside electric equipment or installations (Vinson & Mackay 1990; Bueno 1997; for Buenos Aires: R. Josens and F. Sola pers. obs.), they can be the source of short circuits and serious problems. For example, in October 2006, the whole campus of the University of Buenos Aires was deprived of electricity during three days due to a short circuit caused by the presence of an ant nest within an electric server (*technical report* from 6 November 2006). Other ants may constitute a significant nuisance in gardens, directly by leaf-cutting activity or indirectly by tending homopterans on ornamental plants (Smith 1965; Rust & Su 2012).

Finally, ants can cause significant public health problems by transporting pathogenic organisms (Beatson 1972; Bueno & Campos-Farinha 1999; Santos et al. 2009), acting as mechanical vectors for diseases and nosocomial infections (Beatson 1972; Fowler et al. 1993; Olaya & Chacon 2001; Moreira et al. 2005; Olaya-Masmela et al. 2005). They transport pathogens on their body surface as well as in their digestive tract (Beatson 1972; Fotedar et al. 1989; Moreira et al. 2005; Xue et al. 2009; Garcia & Lise 2013). Pathogens can be released via regurgitation or excretion of feces. Ants are a more serious problem than other insects due to their minute size, as they can enter places that are inaccessible for larger insects (such as cockroaches, flies, mosquitoes, etc.).

The indoor environment has been shown to be an important source for human exposure to several pesticides through inhalation of volatile compounds, and

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Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/09670874.2016.1239035.

other non-volatile compounds through the indoor dust (Harrad et al. 2006; Vorkamp et al. 2011; Dirtu et al. 2012). Liquids, powders, emulsions, sprays or concentrated suspensions of insecticides spread active toxic compounds from one site to another in air and dust streams. They can remain in house dust and airborne particles following application (Leng et al. 2003, 2005; Obendorf et al. 2006; Julien et al. 2008). In recent years, these pathways have been recognized to have a significant influence on human health (Abdallah et al. 2008; Ali et al. 2011; Van den Eede et al. 2011).

Habitat sensitivity refers to the resistance and resilience of the “natural environments” against disturbance (Bax & Williams 2001). As well, the term “sensitive” can be used with pest control in that the use of pesticides in protected environments or some specific spaces in urban settings is not advised (Gore et al. 2004; Choe et al. 2010; Suckling et al. 2010). People who frequent these particular urban sites may be more susceptible or vulnerable to pesticide exposure than the general population. Examples of such sensitive sites are schools, childcare centers, community health centers, nursing homes and hospitals (Fu et al. 2009; Choe et al. 2010; EPA 2016). Similarly, places where asepsis is required as in surgical operating rooms and where food, serums or vaccines are manufactured or processed can be considered sensitive places (Fu et al. 2009).

In recent years, awareness concerning the risks of indiscriminate use of insecticides has grown. Integrated pest management (IPM) promotes pest monitoring and the prevention and reduction of insecticide use (Lewis et al. 1997; Juneau et al. 2011). For agro-ecosystems, IPM encourages natural pest control mechanisms such as the use of biological control agents (Benjamin & Wesseler 2016). IPM favors the use of physical barriers, traps and baits as control methods that are less harmful for humans and environmental health in sensitive urban areas. Toxic baits are recommended for controlling ants and cockroaches in such areas (Owens 2003; Pampiglione & Velo 2010). The main advantage of toxic baits is that they include a small amount of toxicant, which is dissolved in the food bait formulation, thereby reducing its dispersion in the environment and minimizing human exposure. In addition, baits act specifically on the target insect.

Ants are eusocial insects that live in a cooperative society, with only a few reproductive individuals and a vast majority of sterile workers (Hölldobler & Wilson 1990). The latter performs all tasks related to the maintenance, foraging and growth of the colony. Workers change tasks during their life; older individuals deal with work occurring outside the nest such as nest defense and foraging. Thus, the number of ants leaving the nest represents only a small proportion of the members of the colony; the rest remains in the nest.

Toxic baits for ants are food resources to which a lethal active component is added. Foragers collect the bait due to a phagostimulant, bring the bait back to the nest and distribute it among colony members including the queen and the sexual reproductives. This is an important advantage as localization or access to the nest is not necessary (Klotz et al. 1997; Bonnefoy et al. 2008). The toxicant must have a delayed action in order to give the ants enough time for foraging, recruitment and distribution among the colony members, especially the queen (Stringer et al. 1964).

In Argentina, there are two general types of ant baits available on the market: dry granulated pellets aimed at leaf-cutting ants, and gels or viscous liquids contained in syringes, which are aimed primarily at urban nectivorous ants. These ants rely on sugary solutions as an important part of their diet, which they normally obtain from extrafloral nectaries or from attended homopterans. However, even nectivorous ants reject commercial sugary baits in many situations, limiting their effectiveness (O’Brien & Hooper-Bui 2005; McDonald 2012). Sugary gel baits are generally ineffective for omnivorous ants that rarely ingest sugary solutions. Therefore, lipid attractants or protein-based matrices should be used to make baits more attractive for such species (Stanley 2004; Rust & Su 2012).

Bait acceptance is crucial for the success of toxic baits, and it varies with the species and the current conditions. It is necessary to identify the target species and have rigorous research for testing toxic compounds and adequate baits against each pest ant species (Stanley 2004).

In order to develop effective strategies for ant control, we include different topics we have been working on during the past years: (1) a list of species of Buenos Aires; (2) a focal survey in the green areas of a public hospital and (3) an experiment on toxic bait ingestion on a particular urban pest species (*Camponotus mus*).

2. Results, observations and discussion

2.1. Ants of the city of Buenos Aires

The city of Buenos Aires is located in the central-eastern region of Argentina, on the western shore of the Rio de la Plata (34° 35′ S and 58° 26′ W), within the basin of the Paraná River. It is in the native range of some of the most widely distributed and destructive invasive ant species (*Linepithema humile*, *Solenopsis invicta*, *Solenopsis richteri*, *Wasmannia auropunctata*) (Lowe et al. 2000).

The weather is temperate with monthly precipitation that varies between 50 and 154 mm and average temperatures between 25.1 °C in summer (January) and 10.9 °C in winter (July). There are approximately 3 million inhabitants, more than 12 million taking into account the vast suburbs surrounding the city.

Ants enter buildings searching for food, nesting sites, humidity and warmth, especially in the temperate climate of Buenos Aires with cool winters. In cold weather or seasons with high rainfall, ants migrate into warm or dry places (as in other cities, see Gordon et al. 2001). The latter became evident when we performed ant control in a children's hospital. *Nylanderia fulva* was particularly abundant outside buildings close to the heating-pipe outlets, a fact that was accentuated when the weather turned colder (Josens et al. 2014). Another example was seen in early fall 2016: several cars in a parking area of the campus of the University of Buenos Aires were invaded by ant colonies of *L. humile* (R. Josens pers. obs). Nesting in a car was also observed for *C. mus* (Calcaterra L. personal communication 2016.). This behavior clearly favors dispersion of these species.

Here we present an updated list of ant species of the city of Buenos Aires (Table 1). To our knowledge, this is the most complete and recent list of ant species specific to an urban city in this latitude of South America. We collected a total of 60 species belonging to 27 genera, which correspond to 10% and 36%, respectively, of the total number of ant species and genera cited for Argentina (AntWiki 2016). Species were distributed as follows (tribe:genus:species): Amblyoponinae (1:1:1); Dolichoderinae (2:3:5); Ectatomminae (1:1:1); Formicinae (4:5:13); Myrmicinae (4:14:34); Ponerinae (1:1:2); Proceratiinae (1:1:1) and Pseudomyrmicinae (1:1:3).

The largest and most conspicuous species in Buenos Aires are two different black ants: the leaf-cutting ant *Acromyrmex lundi* and the carpenter ant *C. mus*. Whereas, *A. lundi* is an important pest affecting garden plants and can nest in the ground in green areas or building foundations, *C. mus* can nest in wood (both standing trees or structural timbers) and in foundations and cracks in buildings, but the most common nesting site is under roofs.

We found two exotic species in our samples: the pharaoh ant *Monomorium pharaonis*, and the pavement ant *Tetramorium caespitum*. Both were captured outside our main study area on the University campus. Pharaoh ants were found inside a building in the middle of the city, while pavement ants were collected from residential buildings. Pavement ants responded better to lipid and protein food than to carbohydrates (R. Josens pers. obs.). Both species were previously reported in the city of Buenos Aires (pharaoh ant: Josens et al. 2014; pavement ant was mentioned in Steiner et al. 2008) and were the only introduced ants and both were associated exclusively with buildings.

Some of our native species, such as *L. humile* and *S. richteri*, are known to be numerically and behaviorally dominant (i.e. one species monopolizes resources displacing others) when they act as invasive species (Tsutsui & Suarez 2003; Silverman & Brightwell 2008). However, they behaved differently in our study zone and only appeared as dominant species in patches or specific small areas. Opportunistic or inconspicuous ants (*Brachymyrmex* spp., *Pheidole* spp.) tended to be more widespread and numerous in almost all areas sampled.

In an ongoing study based on visual evaluation of a municipal building line (boundary between private property and the road or public space), we found in two sites of the south of the city (neighborhoods of La Boca and Barracas) that *L. humile* is neither abundant nor dominant in the blocks analyzed. In those locations, it shared trails with *Pheidole* spp. without reciprocal aggression. These species do not overlap in the blocks analyzed in the neighborhood of Palermo. In this latter case, *L. humile* is highly abundant and exhibits trails longer than 160 m, with several bifurcations towards buildings and street trees. No other ant species was observed along these trails. *L. humile* is also dominant in many areas on the campus of the University of Buenos Aires. These ants nested in a four-story building while it was still under construction. In November 2015, half of the third floor was opened for use. Ants were found in laboratory equipment (vibratome, thermal bath and weighing scales) by early February 2016. We were asked to resolve the situation. After few days of baiting, we no longer observed any ant activity within or around the laboratory equipment.

We found that the city of Buenos Aires contains a relatively high diversity of ants, especially in its green areas. The number of species is similar to those of other cities (Klotz et al. 1995; Yamaguchi 2004; Chacón de Ulloa et al. 2006; Pecarevic et al. 2010). In addition, the number of species reported in our survey was a little higher than that found in non-urban sites of the Buenos Aires Province (Heller 2004: 27 species; LeBrun et al. 2007: >40 species; Calcaterra et al. 2016: 49 species); yet, for the whole Province of Buenos Aires, the number of species reported reached more than 145 in a review of Argentina's ants (Cuezzo 1998).

An important result of our sampling studies was the discovery of a new ant species *Pheidole acutilobata* (Mackay et al. 2011). This species was also found nesting inside buildings and it did not respond well to sugary baits (Josens et al. 2014).

We hope that this list will constitute a valuable tool for further research on ants of Buenos Aires, in Argentina, and more generally for urban ants.

2.2. Survey in a pediatric hospital

Our group has been consulted on several occasions where commercial baits were not effective for controlling household ants. Our most relevant and best documented work carried out was in the Children's Hospital Ricardo Gutiérrez (Josens et al. 2014).

We discovered a large number of ant species in its green spaces during an initial evaluation, collecting a

Table 1. Ant species of the city of Buenos Aires (tribes are based on Ward et al. 2015).

Subfamily	Tribe	Species	Determined by
Amblyoponinae		*Stigmatomma armigerum* Mayr, 1887	J. Lattke
Dolichoderinae	Leptomyrmecini	*Dorymyrmex pyramicus* Roger, 1863	F. Cuezzo
	Leptomyrmecini	*Dorymyrmex brunneus* Forel, 1908	F. Cuezzo
	Leptomyrmecini	*Linepithema micans* (Forel, 1908)	F. J. Sola
	Leptomyrmecini	*Linepithema humile* Mayr, 1868	F. J. Sola
	Tapinomini	*Tapinoma melanocephalum* (Fabricius, 1793)	F. J. Sola
Ectatomminae	Ectatommini	*Gnamptogenys triangularis* Mayr, 1887	W. Mackay
Formicinae	Camponotini	*Camponotus mus* Roger, 1863	R. Josens
	Camponotini	*Camponotus bonariensis* Mayr, 1868	W. Mackay
	Camponotini	*Camponotus punctulatus* Mayr, 1868	F. Sola
	Lasiini	*Nylanderia steinheili* (Forel, 1893)	W. Mackay
	Lasiini	*Nylanderia silvestrii* Emery, 1906	W. Mackay
	Lasiini	*Nylanderia fulva* Mayr, 1862	F. J. Sola
	Myrmelachistini	*Brachymyrmex brevicornis* Emery, 1906	E. Quirán
	Myrmelachistini	*Brachymyrmex patagonicus* Mayr, 1868	E. Quirán
	Myrmelachistini	*Brachymyrmex australis* Santschi, 1922	E. Quirán
	Myrmelachistini	*Brachymyrmex fiebrigi* Forel, 1908	E. Quirán
	Myrmelachistini	*Myrmelachista nodigera* Mayr, 1887	F. J. Sola
	Myrmelachistini	*Myrmelachista gallicola* Mayr, 1887	F. J. Sola
	Plagiolepidini	*Acropyga exsanguis* Wheeler, 1909	F. J. Sola
Myrmicinae	Attini	*Acromyrmex lundi* Guérin-Méneville, 1838	F. J. Sola
	Attini	*Acromyrmex heyeri* Forel, 1899	W. Mackay
	Attini	*Apterostigma steigeri* Santschi, 1911	F. J. Sola
	Attini	*Apterostigma pilosum* Mayr, 1865	F. J. Sola
	Attini	*Cephalotes jheringi* Emery, 1894	W. Mackay
	Attini	*Cyphomyrmex rimosus* (Spinola, 1851)	F. J. Sola
	Attini	*Cyphomyrmex daguerrei* Santschi, 1933	F. J. Sola
	Attini	*Pheidole rosula* Wilson, 2003	W. Mackay
	Attini	*Pheidole acutilobata* Mackay, 2011	W. Mackay
	Attini	*Pheidole radoszkowskii* Mayr, 1884	F. J. Sola
	Attini	*Pheidole rosae* Forel, 1901	F. J. Sola
	Attini	*Pheidole bergi* Mayr, 1887	F. J. Sola
	Attini	*Pheidole breviseta* Santschi, 1919	F. J. Sola
	Attini	*Pheidole triconstricta* Forel, 1886	F. J. Sola
	Attini	*Pheidole cordiceps* Mayr, 1868	F. J. Sola
	Attini	*Pheidole nitidula* Emery, 1888	F. J. Sola
	Attini	*Pheidole humeridens* Wilson, 2003	F. J. Sola
	Attini	*Strumigenys infidelis* Santschi, 1919	W. Mackay
	Attini	*Strumigenys louisianae* Roger, 1863	F. J. Sola
	Attini	*Trachymyrmex pruinosus* Emery, 1906	F. J. Sola
	Attini	*Trachymyrmex tucumanus* Forel, 1914	F. J. Sola
	Attini	*Wasmannia auropunctata* (Roger, 1863)	F. J. Sola
	Crematogastrini	*Crematogaster torosa* Mayr, 1870	W. Mackay
	Crematogastrini	*Crematogaster evallans* Forel, 1907	W. Mackay
	Crematogastrini	*Crematogaster quadriformis* Roger, 1863	F. J. Sola
	Crematogastrini	*Nesomyrmex spininodis* Mayr, 1887	F. J. Sola
	Crematogastrini	*Tetramorium caespitum* Linnaeus, 1758	W. Mackay
	Pogonomyrmecini	*Pogonomyrmex naegelli* Emery, 1878	W. Mackay
	Solenopsidini	*Monomorium pharaonis* Linnaeus, 1758	F. J. Sola
	Solenopsidini	*Solenopsis richteri* Forel, 1909	W. Mackay
	Solenopsidini	*Solenopsis sulfurea* (Roger, 1862)	F. J. Sola
	Solenopsidini	*Solenopsis picea* Emery, 1896	F. J. Sola
	Solenopsidini	*Solenopsis metanotalis* Emery, 1896	F. J. Sola
	Solenopsidini	*Solenopsis clytemnestra* Emery, 1896	F. J. Sola
Ponerinae	Ponerini	*Hypoponera argentina* Santschi, 1922	F. J. Sola
	Ponerini	*Hypoponera opaciceps* Mayr, 1887	F. J. Sola
Proceratiinae	Proceratiini	*Discothyrea neotropica* Bruch, 1919	F. J. Sola
Pseudomirmicinae		*Pseudomyrmex holmgreni* (Wheeler, 1925)	F. J. Sola
		Pseudomyrmex gracilis Fabricius, 1804	F. J. Sola
		Pseudomyrmex phyllophilus Smith, 1858	F. J. Sola

total of 4767 individuals in 24 pitfalls. The relative frequencies of the genera identified in our hospital samples are shown in Figure 1. The most abundant genus was *Pheidole* (three species) representing 52% of the total abundance, followed by *Nylanderia* (only *N. fulva*) representing 34%. These two genera were found in 100% of the traps, although in different proportions. Other genera were found in several traps but in much lower abundance: *Solenopsis* (only *S. albidula*) was found in 91.7% of the traps, *Brachymyrmex* (two species) in 70.8% and *Acromyrmex* (only *A. lundi*) in 12.5%. Finally, *Strumigenys* and *Gnamptogenys* were each found in only two pitfalls (8.3% of the traps available), and in each case, only a few individuals were collected (Josens et al. 2014). We have found 15 different species belonging to 12 genera in outdoor and indoor

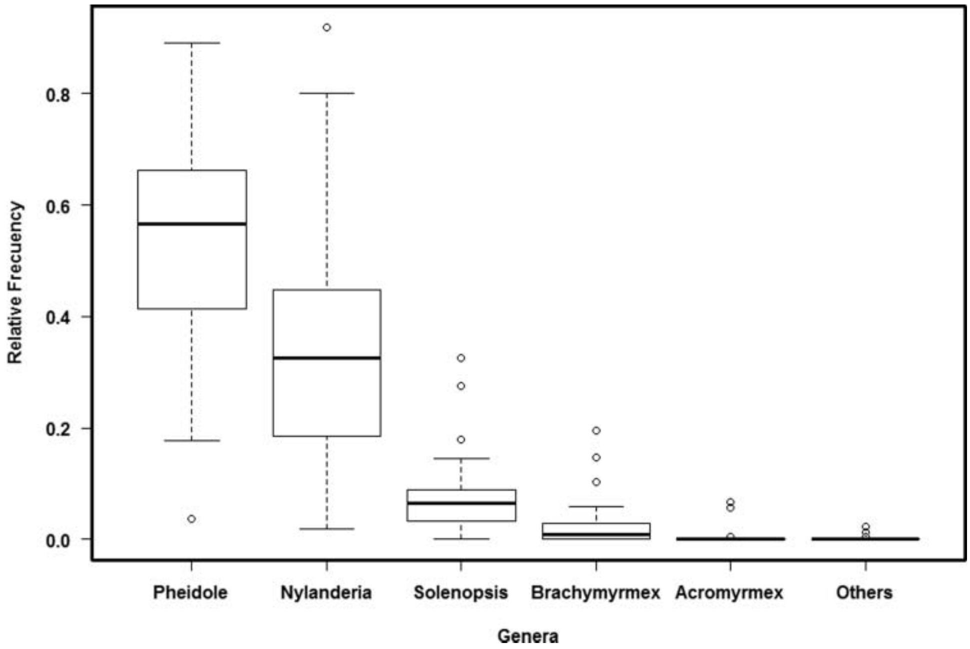

Figure 1. Relative frequency per pitfall for each ant genus found in green areas of a children hospital of the city of Buenos Aires (adapted from data of Josens et al. 2014). The boundaries of the box indicate the 25th and 75th percentiles. A line within the box marks the median. The ends of the whiskers represent the non-outlier range. Circles show outliers.

areas of the hospital (Josens et al. 2014). This high number of species in a hospital is similar to the instance reported for the southernmost states of Brazil, which in turn are the states with the highest records of ant species per hospital from Brazil (Monteiro de Castro et al. 2015).

While working in the hospital, we confirmed on several occasions that a commercial bait was not effective, contrary to our sugar baits which had exactly the same toxicant and in the same concentration (Josens et al. 2014). This difference would be due to different factors, either intrinsic or extrinsic, rather than to the active compound and its concentration.

The reasons for the low efficacy of a bait may be variable over time. On the one hand, the bait attractant is food and therefore competes with other simultaneously available food sources. On the other hand, colonies exhibit seasonal variations in their requirements of carbohydrates and proteins, which affect their tendency to accept or reject toxic baits (Sudd & Sudd 1985; Stein et al. 1990; Falibene & Josens 2014).

The poison may be repellent due to its taste and therefore ultimately rejected (Klotz & Williams 1996; Hooper-Bui & Rust 2000). Rejection of toxic compounds varies between ant species, as certain species reject specific toxicants while others accept these compounds and reject others (Sola et al. 2013). Therefore, the advantage of scientific intervention in ant control relies on the ability to recognize species and a knowledge of their food preferences, behavior and biology. This is a crucial point which may also explain the difference in the success of urban ant control when using baits exclusively, as required in sensitive sites. The vast majority of people does not know how to identify species, and often place pellet baits (made for leaf-cutting ants) for nectivorous ants or vice versa.

2.3. Testing individual bait consumption and bait efficacy in the laboratory

We conducted laboratory assays in which we compared two groups of ants (*C. mus*) of similar sizes to determine whether individual feeding behavior could give us some indication of the efficacy of baits. Ants of one group were individually offered a drop of a commercial bait whose active compound is boric acid (2% w/w) while ants in the other group received a bait based on a 30% (w/w) sucrose solution including exactly the same active compound in the same concentration as the commercial bait. Different behavioral variables were recorded and post-ingestion mortality was determined (for methodological details see: Josens et al. 1998; Sola et al. 2013).

Our results showed that consumption of the commercial bait was 35% lower than consumption of our bait, similar to what happened at the hospital (Figure 2(a,b), respectively). The difference in time required for the individual ingestion of the baits was even more remarkable. The feeding time took one-fourth as long for our bait. Moreover, our bait induced less feeding interruptions so that the difference in total feeding time – from the beginning until the end of ingestion – between both baits was even more accentuated (Figure 2(c)). In a normal control situation, ants consume baits and return to the nest to empty their crops, transferring their contents to nestmates. They then return to the baits and fill their crops again, which would make our bait even more effective over time. The difference in toxicant transported to

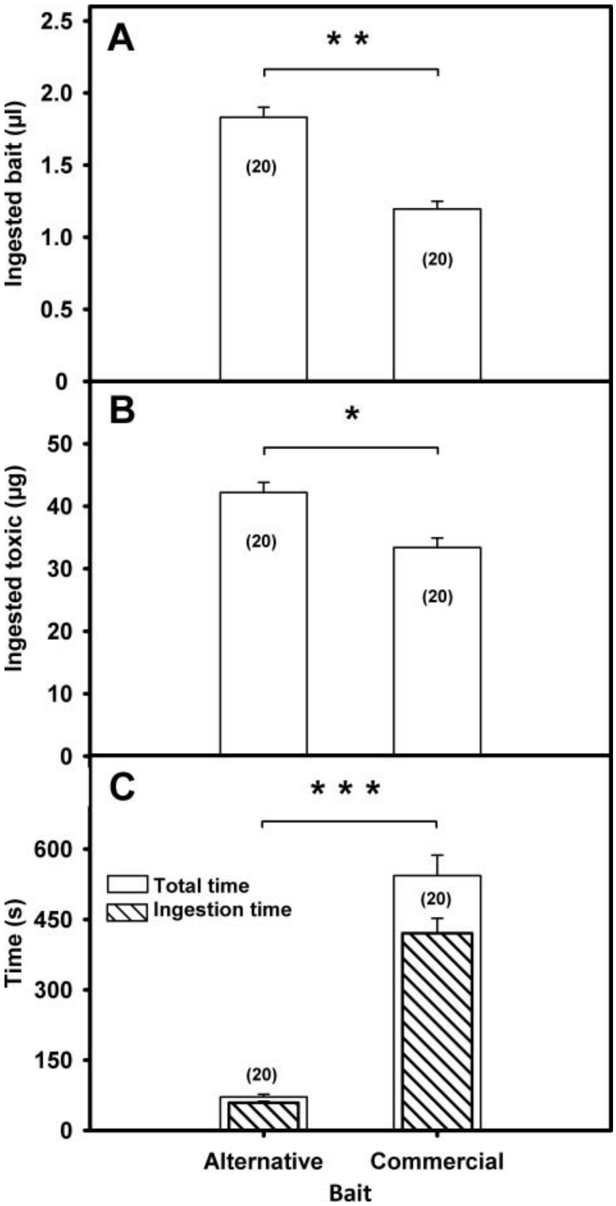

Figure 2. Bait feeding assays: (A) ingested bait (μl), (B) ingested toxic compound (μg) and (C) total time (white bars) and ingestion time (stripe pattern bars) (both in seconds) measured during a single ingestion of a bait: commercial or alternative (both contained the same active compound at the same concentration). For all variables, both baits differed significantly (generalized linear mixed model (GLMM); (A): normal family with identity link function; $F_{1,4} = 51.4$, $p = 0.002$. (B) Normal family with identity link function; $F_{1,4} = 13.18$, $p = 0.0221$. (C) Gamma family with inverse link function; total time: $X^2_1 = 26.26$, $p < 0.0001$; ingestion time: $X^2_1 = 22.15$, $p < 0.0001$).

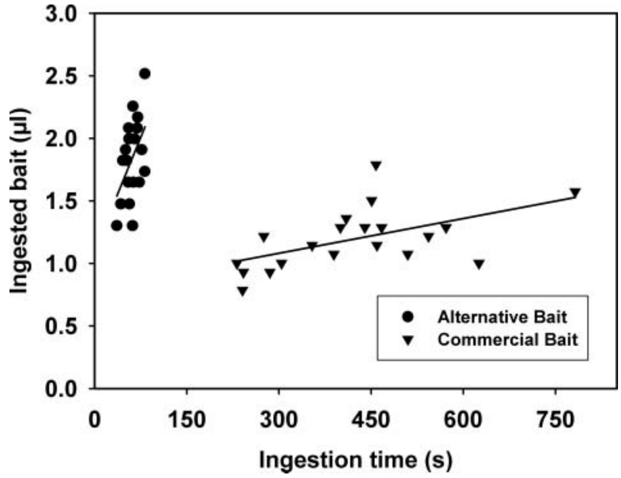

Figure 3. Bait feeding assays: ingested bait (μl) as a function of time (s) during a single ingestion of a bait: commercial or alternative (both containing the same active compound and in the same concentration). The slope of the linear regression for each group represents its intake rate (least square method for multiple regression; $R^2 = 0.68$, $p < 0.05$).

the nest would be amplified between baits in successive foraging cycles.

The value of the slope relating the volume consumed with the time of ingestion represents the intake rate and indicates the speed of ingestion for each bait. Figure 3 shows that the commercial bait is consumed 11 times more slowly than our bait, probably due to their different viscosity. This physical property affects significantly the speed of fluid ingestion in sucking feeding insects; the more viscous a liquid, the more slowly it is ingested (Josens et al. 1998; Medan & Josens 2005; Sola & Josens 2016).

We also assessed the mortality in ants induced by a single ingestion of one of the two types of baits or a control sucrose solution (30% w/w) without the active compound. In order to record the mortality, we separated workers from the nest and we checked mortality daily.

As expected, control ants died significantly later than the two other groups of ants that consumed a toxic bait (Figure 4). There was a significant difference in mortality between both baits as well. The ants that ingested the commercial bait took 2.2 more days to die which means 68% extra time. In other words, our alternative bait induced a mortality that was faster than the

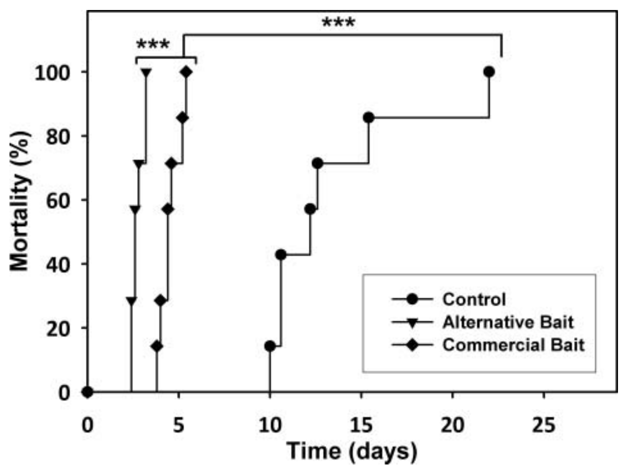

Figure 4. Bait feeding assays: mortality (%) during the days after a single ingestion of one bait: commercial or alternative (both containing the same active compound at the same concentration) or a control solution of 30% (w/w) sucrose without an active compound. (Kaplan–Maier analysis: $p < 0.0001$ for each comparison).

commercial bait. These results confirmed our experience on ant control in different buildings in which our bait proved to be efficient where available commercial baits had failed.

2.4. Final conclusions

The list of species of Buenos Aires City is long. Urban habitats are likely to provide a more heterogeneous environment, promoting species coexistence (Palmer 2003). This allows highly dominant species to coexist with other likely competitors. Even in the hospital, the number of species was high and comparable to other records from warmer climates. However, this high number of species makes it difficult for ordinary people to identify the species. The best way to control harmful ants is by identifying the target species and knowing its biology and response to baits and the active compounds and formulations, which varies from species to species.

All in all, the comparison between commercial baits and our alternative baits shows that the latter represents a more suitable and attractive control strategy due to its faster and higher consumption and the rapid induced mortality, despite the fact that both included the same active compound and in the same concentration. Urban ants require effective control strategies and thus the development of products that do not sacrifice efficiency in favor of commercial aspects but rather optimize it in terms of the biology of the target species. Moreover, the development of these products should be accompanied by an increase of social awareness on the undesirable consequences of indiscriminate pesticide release and by the promotion of public health policies regulating control strategies particularly in sensitive places of urban settings.

3. Materials and methods

3.1. Ants of the city of Buenos Aires

We have advocated using a diversity of methods to gain the greatest collection of species. We did not try to collect samples to be analyzed *quantitatively*. Therefore, no species richness quantification or standardization of the sampling effort or the number of samples was made.

Different sampling methods were used such as direct sampling by active searching, baits, leaf litter sampling, soil sampling and pitfall traps. Some city locations were more represented than others and were the object of more detailed studies (e.g. the hospital mentioned earlier, or the Campus of the University of Buenos Aires-UBA; Figure 5). In order to attract different ant species on the Campus of the UBA, four different types of food were offered as baits: commercial canned tuna in oil, commercial canned corned meat, honey-water and raw lean beef. In addition, the Laboratory of Social Insects of the University of Buenos Aires has been receiving ant samples from different parts of the city of Buenos Aires, provided both by pest control companies and affected city dwellers. Ants were identified to species, using the keys of Kusnezov (1978) and Fernández (2003), complemented with genus-specific keys (Annex 1 (supplementary material)). Most identifications were done in our laboratories by Dr Francisco J. Sola and by Dr William Mackay; some samples were processed by specialists of specific genera, such as Dr Fabiana Cuezzo of the Institute-Museum Miguel Lillo (IMML), San Miguel de Tucumán, Dr Estela Quirán of the Faculty of Sciences, National University of La Pampa, Santa Rosa and Dr John Lattke of the Universidade Federal do Paraná, Brazil. Voucher specimens were deposited in the Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina, and the ant collection of the Laboratory of Social Insects (IFIBYNE; FCEN), University of Buenos Aires.

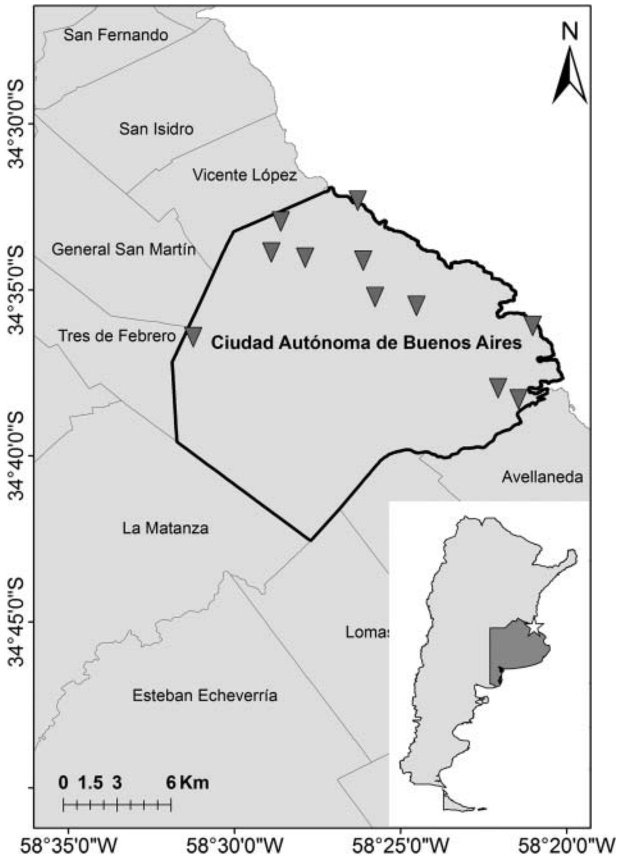

Figure 5. Map of the city of Buenos Aires. Triangles indicate the city locations that were object of a more detailed sampling.

3.2. Survey in a pediatric hospital

Sampling was performed from December 2011 to January 2012, which corresponds to late spring and summer in our latitude (for more details of the hospital, see Josens et al. 2014). The genus abundance was quantified from pitfall traps. We located 24 non-baited pitfall traps in different green spaces of the hospital grounds. This procedure was repeated twice (two weeks apart). Each pitfall trap consisted of a 200 ml plastic cup buried in the ground to the rim and half-filled with alcohol (70%). Pitfalls were left for 48 h, which allowed for sampling ants with low activity and at the times when we did not have access to the hospital. Trapped ants were identified to species or at least genera with available keys (Kusnezov 1978; MacKay & MacKay 2002; Fernández 2003; Wilson 2003). Relative abundance per genus was measured as the number of ants per genus over the total number of ants collected during the study.

3.3. Testing individual bait consumption and bait efficacy in the laboratory

Two colonies of *C. mus* (Roger) composed of around 2000 workers and one or more queens were used in the experiments. Each colony was reared in an artificial nest or container consisting of a plastic box (30 × 50 × 30 cm) with its base coated with plaster and its walls painted with fluon to prevent animals from escaping. Colonies were housed in piled acrylic plates and workers had access to fresh water, honey-water and chopped insects within the container. Nests were maintained in the laboratory for one year under natural light/dark cycles and nearly constant temperature (23 ± 3 °C). Prior to the behavioral experiments, colonies were submitted to a reduction in the carbohydrate supply.

We offered each ant one of two baits: a commercial one, PLATINUM® AB, which contains 2% boric acid, or a simple sucrose solution 30% w/w with also 2% boric acid (99.5% purity; Anedra, Research AG, Buenos Aires, Argentina).

Before each assay, a group of about five ants was allowed to feed at the experimental device to establish a pheromone trail to the sucrose solution. Afterwards, recruited ants were placed individually in an acrylic container with a cover and weighed to record the initial ant mass (Mettler Toledo AG285, resolution 0.01 mg). Ants used in the experiments were of similar sizes (ranged between 3.5 and 5.5 mg). Ants were placed one at a time at the edge of a wooden bridge that led to the recording arena (acrylic rectangle) with a drop of sugar solution in its center. Once on the arena, the ant was not disturbed to allow it to find and drink the bait by itself. The recording began when the ant introduced its mouthparts in the drop. The volume of the offered solution was always larger than the volume a single ant could ingest in one intake. After the ant finished feeding and returned to the wooden bridge, its mass was recorded again (final weight: ant plus crop load mass). Each individual ant was provided with only one bait and tested only once.

Crop load (mg) was obtained from the difference between final and initial weight. Volume of solution ingested (μl) was calculated by dividing the crop load by the density of the solution (obtained by weighing 1 ml of each solution and averaging 10 measurements).

To evaluate mortality after bait ingestion, 35 ants per treatment were maintained in seven groups of 5, according to the treatment received, until all were dead. 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, all ants had access to water and sucrose solution (30%) *ad libitum* and no additional toxicant was offered. Mortality was recorded as the cumulative number of dead ants per day.

3.3.1. Statistical analysis

We performed a GLMM for each variable. We checked normality by performing a Shapiro–Wilks test. Since ingestion of bait was normally distributed, we used the Gaussian family with an identity link function. In the case of time (total and at the source), we used a gamma family with an inverse link function. In order to compare mortality, we performed a Kaplan–Meier analysis.

Acknowledgments

We would like to thank Nahuel Marchisio, whose help was essential for our field work and Maria Agostina di Renzo for her help with the organization of tables and files of the hospital data. We would also like to thank the collaboration of Drs Fabiana Cuezzo (Universidad de Tucuman), Estela Quirán (Universidad Nacional de La Pampa) and John Lattke (Universidad Central de Venezuela, now at the Universidade Federal do Paraná, Brazil) for their help with the taxonomic identification of ant species. We thank two anonymous referees for critical reading of the manuscript. This study was supported by funds from the National Council for Scientific and Technological Research – CONICET – from Argentina (PIP 112 201101 00472), and the estate of Maxie Groce Templeton in El Paso, TX, USA.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

National Council for Scientific and Technological Research – CONICET [grant number PIP 112 201101 00472]; Maxie Groce Templeton.

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