Area-Wide Management of the Invasive Garden Ant *Lasius neglectus* (Hymenoptera: Formicidae) in Northeast Spain[1,2]

Sònia Rey and Xavier Espadaler[3]

Ecology Unit and C.R.E.A.F., Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

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ABSTRACT The invasive ant *Lasius neglectus* (Hymenoptera: Formicidae) has become an urban pest in northeast Spain and continental Europe and is currently expanding its range and threatens to become as serious a pest as the Argentine ant. One invading population occupying 14 ha and located in northeast Spain has been managed with a large-scale insecticide treatment, with the aim of reducing its damaging effects in houses and on human well-being. During the spring and summer periods of 2001–2002, 45 households were treated. Some non-urbanized, seminatural infested areas were also treated to avoid the pest expansion. The treatment plan was intended to attack three key aspects of the ants as well as directly killing the ants: (a) killing/destroying ant food sources, (b) limiting access to the ant's food sources, and (c) impeding access to the interior of houses. The pest control treatment involved a combination of canopy fogging, tree trunk spraying, a house perimeter injection treatment, and in-house baiting. Reduction of ant trails on trees was 73% (2001) and 68% (2002). Spots with ants at the perimeter of the house were reduced by 47% (2001) and 30% (2002). Both the objective assessment of the treatments and the positive opinion of the inhabitants of the houses confirmed the efficacy of the treatment program.

KEY WORDS Hymenoptera, Formicidae, invasive ant, *Lasius neglectus,* pest species, supercolony, tree, tramp species

Lasius neglectus Van Loon, Boomsma & Andrásfalvy (Hymenoptera: Formicidae), the invasive garden ant, is a poorly known species probably originating from Asia Minor, where it is found in natural steppe habitats (Seifert 2000). In that area, it has not been reported as a pest. However, in West Mediterranean areas and Central Europe, the pest status of this ant was evident from the first reports (Boomsma et al. 1990, Van Loon et al. 1990) and has been confirmed by recent studies (Passera 1994, Espadaler 1999, Tartally 2000, Seifert 2000). Re-

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[2]Trade names are intended only to give specific information. The CREAF and the UAB do not endorse or guarantee any product and do not recommend one product instead of another that might be similar.

[3]Corresponding author: C.R.E.A.F., Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. Tel: 34 93 5812768; Fax: 34 93 5814151; E-mail: Xavier.Espadaler@uab.es

cent publications concerning continental Europe (Seifert 2000, Dekoninck et al. 2002) and the ant's status in Spain (Espadaler 1999, Espadaler & Rey 2001) indicate that this species has the potential of becoming as serious a problem in Europe as the Argentine ant (*Linepithema humile*) is currently (see Table 1 for known localities in continental Spain). A peculiarity of this ant is its penchant for electrical equipment and circuitry (Jolivet 1986; as *L. alienus*), producing similar damage to that described for *Solenopsis invicta* (Vinson & MacKay 1990). A summary of the limited knowledge on the biology of this species and some of its effects can be found at http://www.creaf.uab.es/xeg/Lasius (last accessed 13 November 2004).

Lasius neglectus ants live in large polygynous supercolonies with no apparent within-colony boundaries and no hostility between nests. Nutrition is heavily dependent on carbohydrates (aphid and other insect-producing honeydew, floral and extrafloral nectar; unpublished observations). They display intranidal mating and adoption of the newly inseminated queens (Van Loon et al. 1990, Boomsma et al. 1990, Espadaler & Rey 2001). Throughout the colonized areas, queens are found isolated or in groups, but always with attending workers. Queen number in the supercolony treated in this work has been estimated to be in the tens of thousands (Espadaler et al. 2004). The rapid range expansion of this ant throughout Europe in the past 20 years appears to be mediated by passive, anthropogenic transport via plant pots, garden soil, or in similar ways.

Invasion of homes by *Lasius neglectus* is highly variable and seasonal; typically it corresponds with the maximum activity period of the ant. It usually begins in early spring (April) and ends by mid autumn (October) (unpublished data). Homeowners' complaints about this ant indoors are generally associated with increasing numbers of ants outdoors, usually due to increasing temperature and food availability. In early spring there is an explosion of ant numbers outdoors, and activity levels increase very quickly. *Lasius neglectus* workers remain active 24 hours a day during this activity period (unpublished data), meaning large numbers of ants foraging up and down trees throughout spring, summer, and until mid autumn. The ants, apart from nesting outdoors and infesting lawns, trees, and shrubbery, also entered the houses either through electrical conduits or holes in walls, doors, or windows. Indoor damage includes short-circuits in electrical appliances (alarms, swimming pool filters, lights, plugs; Fig. 1) and the nuisance of having ants inhabiting the house structure. The presence of large numbers of ants outside does not necessarily indicate likelihood of invasion indoors. Only when homeowners complained about indoor ant presence did we consider the house as invaded. Currently, there are no control recommendations for the invasive garden ant. The initial control strategy involved:

1. Fogging tree crowns with insecticide to kill aphids and other honeydew producing insects, the main food source of the ants.
2. Spraying tree trunks with a highly persistent insecticide to kill ants moving up and down the trees.
3. Injecting the perimeters of the houses, around the foundation, with residual insecticide to create a barrier to ant traffic.
4. Establishing in-house baits in invaded areas. House owners received commercial baits enclosed in a childproof plastic container that contained an insecti-

Table 1. Geographic coordinates and climatic characteristics of all known populations of *Lasius neglectus* in Spain (June 2004). Only the first three have been reported to be invasive.

Population	Coordinates	Elevation (m)	Mean rainfall (mm)	January mean temperature (°C)	July mean temperature (°C)	Mean annual temperature (°C)
Seva	41.80N, 2.26E	650	775	4.5	21.5	11.5
Taradell	41.88N, 2.30E	650	775	4.5	21.5	13.5
Matadepera	41.61N, 2.30E	570	775	6	22.5	13.5
Les Planes	41.46N, 2.08E	230	725	7.5	22.5	14.5
Sant Cugat	41.50N, 2.10E	130	725	6.5	22.5	13.5
Barberà	41.51N, 2.13E	130	575	7.5	23.5	15.5
Lliçà de Vall	41.59N, 2.24E	125	625	7.5	23.5	14.5
Ripollet	41.50N, 2.14E	105	575	7.5	23.6	15.5
Bellaterra	41.43N, 2.10E	90	675	6.5	23.5	14.5
Cerdanyola	41.48N, 2.14E	80	625	6.5	22.5	14.5
Barcelona	41.38N, 2.15E	20	575	9.5	24.5	16.5

Climatic data from the *Atles Climàtic de Catalunya. Termopluviometria* (1997). Institut Cartogràfic de Catalunya (http://www.icc/es).

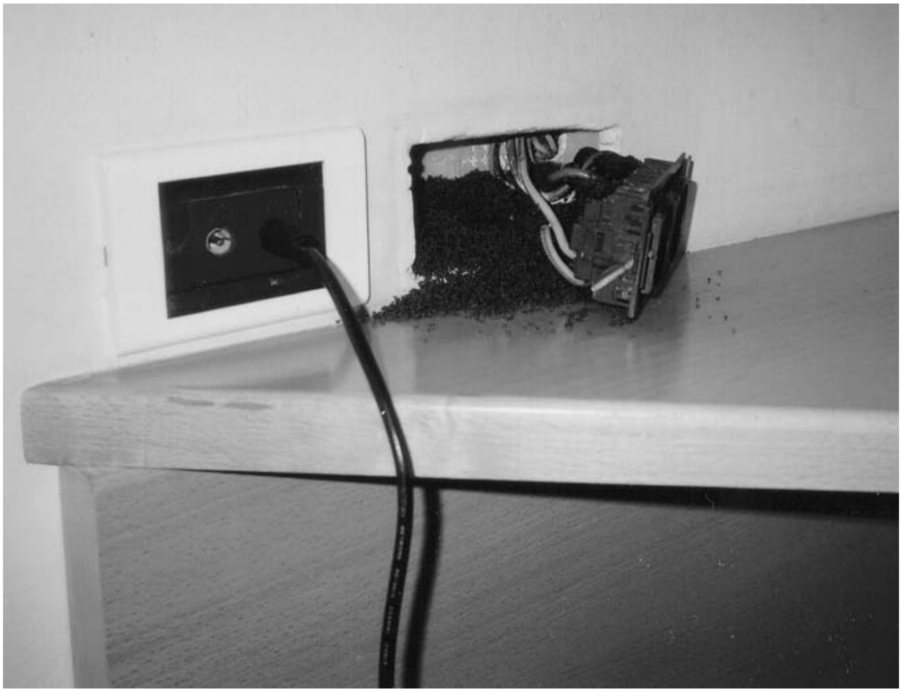

Fig. 1. Dead *Lasius neglectus* ants inside an electrical outlet. Ants were killed by applications with available commercial spray (brand unknown) a few days before. Spraying was routinely done every 2 weeks, with similar results as those shown here. (Photo courtesy of Montserrat Jorba, Matadepera, Spain).

cide and an attractant, to be applied where necessary. This was aimed at killing the ants that remained in the interior of the house after the barrier treatment.

In the first year (1999), one house was effectively treated. Therefore, in the following year (May 2000), four houses were treated using the same protocol, with similar positive results. During both years, the effectiveness and persistence of the treatments were assessed and were found satisfactory. Therefore, an informative meeting for the community was held and complete agreement was reached for a general management plan involving the entire infested area. During spring 2001 and 2002, the whole affected area was treated, using the same protocol. Results of this large-scale pest management project in a suburban area are presented here.

Materials and Methods

Study site and location. The very large, highly polygynous colony of *L. neglectus* occupies at present a continuous surface of >14 ha in a suburban area in northeast Spain, near Barcelona, at 650 m a.s.l. The climate is Mediterranean, with a wet spring and autumn and a dry winter and summer. Mean annual temperature is 11.5°C and mean annual rainfall 775 mm. The residential area affected contains widely spaced houses, and many sites have seminatural vegetation (mostly holm oaks, evergreen oaks, and pine trees). Irrigated gardens are landscaped with native trees and exotic bushes and other ornamental plants. *Lasius neglectus* has not been observed in natural habitats in Spain, but only in urban, suburban, or disturbed areas where the original vegetation has been cleared, substituted, or heavily modified (unpublished data). A peculiar finding is that only 3 out of the 11 populations in Spain have been reported to invade houses. These three populations show all the characteristics of the tramp invasive ants (Passera 1994); in particular, all other native ants are nearly absent or very rare in the invaded areas (in preparation).

Treatments. Treatments were applied during spring and summer 2001 and 2002 (May–June) after the ants finished hibernating. Treatment products are shown in Table 2.

1. Tree crowns were treated with a mixture of two pyrethrins, Efitax® (α-cypermethrin 4% p/v, emulsifiable concentrate [EC]) and Confidor® 20 LS (imidacloprid 20% p/v, soluble liquid [SL]), both diluted at 0.1% (100 cc per 100 l of water) in water and applied with a cannon. The capacity of the container where the product was diluted was of 600 l. Some applications were repeated due to periods of heavy rain (a total of 18 days between May and June) that occurred just after the application. The objective was to kill aphids in the pest-affected trees.
2. Tree trunks were treated with Fendona® 6SC (60 gr/l α-cypermethrin), a highly persistent, resin containing contact insecticide. It was diluted in water to 6% (100 cc per 15 l of water) and sprayed directly with a 15-l hand-pressurized backpack sprayer.
3. Perimeter treatment around the homes: Baythion® 50 LE (foxim 50% p/v, emulsifiable liquid [EL]) was diluted to 0.1% in water and was injected into the soil every 50 cm in the soil adjacent to and around the structure. The perimeter of nonurbanized (= without a house) lots were treated with this injectable insecticide to avoid further expansion of the pest.
4. Homeowners used a bait treatment inside a container (Blattanex®, foxim 0.08% + sugar matrix), with a delayed action toxicant, strategically placed next to ant trails in the bathroom, kitchen, and garage. The number of baits used in each house ranged from 5 to 10 and their effectiveness was assessed visually by the owners.

Nonurbanized infested areas were also treated to prevent further expansion of the pest. In these areas, all trees and bushes were fogged, the perimeter injected, and all tree trunks treated. A maximum of 45 affected houses were treated and assessed as described below. This treatment started on 21 May and finished on 1 August in the first year (2001). In the second year of treatment (2002), it started on 9 May and finished on 21 June.

Assessment. Three different treated areas (T1, T2, T3) were identified and contrasted with two nontreated infested areas (C1, C2) designated as controls. The areas were separated by approximately 100 m. Effectiveness of the treatment was checked by a survey of ants on trees (2 years), around houses (2 years) and in the ground (1 year), using the following protocol:

Table 2. Products, dosage, and methods of application[a] used for the global treatment of a supercolony of *Lasius neglectus* (Seva, Barcelona, Spain).

Treatment and formulation	Dose/ concentration	Type and site of application	Application size	Volume
Fendona 6SC (60 g/l α-cypermethrin)	100 cc/15 l (6%)	Backpack sprayer on tree trunks	25 trees/ backpack (15 l)	600 cc/tree
Baythion 50LE (foxim 50% p/v, EL)	100 cc/100 l (0.1%)	Injection in soil	200 injections/ house (60 m house perimeter)	500 cc/injection; 100 l/house
Efitax (α-cypermethrin 4% EC) + Confidor 20LS (imidacloprid 20% p/v, SL)	100 cc/100 l (0.1%)	Canon spray on tree crown	200 l/garden or lot	4 l/tree
Blattanex (foxim 0.08% + sugar matrix)		Bailts in containers	5 to 10 baits/house	

[a]The rate, units, and times are approximate data for a standard 500 m^2 lot with a 200 m^2 construction. Gardens contain a mean of 50 trees plus ornamental plants and bushes and are usually surrounded by an enclosure of bushes (*Prunus laurocerasus, Thuya* sp.).

A. Trees: Thirty trees inside each of the five different areas were numbered and marked with permanent white paint (Tippex) and their diameter breast height (DBH) was measured. The DBH is also an indirect measurement of crown volume and gives an estimation of the possible aphid population and, indirectly, the attending ants. A total of 150 trees were marked (60 trees in the control and 90 in the treated plots). The number of ant trails on tree trunks was noted before and after the treatments at 24 h, 3 days, 7 days, 15 days, and 31 days after the treatment. Control areas were also checked before and after treatments at the same intervals.

B. Perimeters: A number of spots on the ground were made with permanent white paint (Tippex). Within a 20-cm radius of each spot, we noted the presence or absence of *L. neglectus* workers (17–20 spots per house, spaced 3 m apart, around the perimeter). A total of 151 spots were made (60 in 3 houses at T1, 57 in 3 houses at T2, 34 in 2 houses at T3).

C. Ant population density in soil within the infested area was assessed in May 2002, before chemical treatments, by sampling with a manually operated soil auger that extracted a volume of approx. 300 cm^3 (10-cm diameter, 15-cm deep). At 50 random points, we took four soil cores at the extremes of a 1-m square. The extracted soil was mixed repeatedly over a white plastic box (surface 25 × 40 cm), and queens and workers were counted. This was repeated in May 2003, after the large-scale control treatment of 2002, yielding a replicate for the first soil survey and an indirect estimate of the effectiveness of the control treatment on the number of ants (workers + queens).

Analysis within the same year. *Ants on trees.* H_0: there is no significant interaction between ant counts and the time variable. This would be true if the regression lines of treated and untreated plots were parallel. A significant result means that the controls and treatments are different over time.

Data on the number of ant trails and the DBH of each marked tree was log transformed for analysis. The DBH was used in all subsequent analyses as a covariate. The number of ant trails per tree before and after treatment was analyzed with ANOVA. As measures were not equally spaced, the SAS Procedure Mixed (SAS Institute 2001) was used with time (1, 3, 7, 15, 31 days) as the repeated measures factor and area as a fixed factor.

Ants around houses. H_0: there is no significant interaction between ant counts and the time variable. This would be true if the regression lines of treated plots had a slope not different from zero. Data on the number of points with ants for each perimeter of the houses were arcsin transformed (proportion of points with ants as compared to pretreatment counts) and analyzed by ANOVA. As above, the SAS Procedure Mixed was used with time (1, 3, 7, 15, 31 days) as the repeated measures factor and area as a fixed factor. No controls are available for this variable, hence we are testing the effect of time on the number of points with ants around the perimeter of the houses.

Analysis between years. H_0: there is no significant time effect on ant counts over the course of the experiment in treated areas. A repeated measures ANOVA was applied to compare the level of ant activity (rows on trees and house perimeter points) *before* the treatments that took place during two years (2001–2002) and also to compare the activity level in the next year (2003) at the same time period. Frequency data for worker presence/absence in cores for both years were tested with a test for differences in proportions, two sided. Those analyses were run under STATISTICA 6.1 (StatSoft 2003).

Results

A simple linear regression analysis was performed to check the dependence of ant trails on tree size (DBH). Before any treatment was carried out (spring 2001), an ANOVA indicated the homogeneity of the five areas before treatments in the number of ant trails per tree ($F = 2.15$; df = 4, 144; $P = 0.077$). The level of pest infestation in the affected area was immediately reduced after the combined insecticide treatments and remained low for ~2 years. Dead ants were found in the thousands at the base of all treated trees. Similarly, an ANOVA indicated the homogeneity of the three treated areas before treatments in the number of points with ants before the treatment took place. Because houses were of different sizes and they differed in the number of marked points, this number was introduced as a covariate. No differences were found (ANOVA, $F = 0.54$; df = 2,4; $P = 0.617$), thus marked areas were considered homogenous for the level of pest infestation prior to treatment.

Treatment of tree trunks. There was a positive correlation between the numbers of ant trails on trees before the treatment and their DBH ($R^2 = 0.34$; $P < 0.001$). In 2002, 1 year after the first treatment, this relationship ceased to exist in the treated trees ($R^2 = 0.01$; $P = 0.28$) although, as expected, it was maintained in trees from control areas ($R^2 = 0.38$; $P < 0.001$; Fig. 2).

An immediate effect of the treatment was a sharp drop, in both years, in the number of ant trails on trees (Fig. 3; Year 2001: ANOVA, $F = 47.26$; df = 20, 725; $P < 0.001$. Year 2002: ANOVA, $F = 6.86$; df = 20, 725; $P < 0.001$) and this was maintained for a significant period of time. After the first treatment in 2001, the population did not reach (data at May 2003) the high level of activity previously detected (Table 3; Fig. 4A). The controls and treatments were different over time. This was maintained throughout the two next years in the different treated areas; therefore, an important effect on the population was achieved with this first treatment ($F = 4.32$; df = 4, 174; $P = 0.002$; without the control areas as no differences were found).

Treatment of house perimeters. There was a decrease in the number of workers detected at perimeter points after the first (2001) and second (2002) treatments with soil injections (Fig. 5; Year 2001: ANOVA, $F = 48.37$; df = 5, 25;

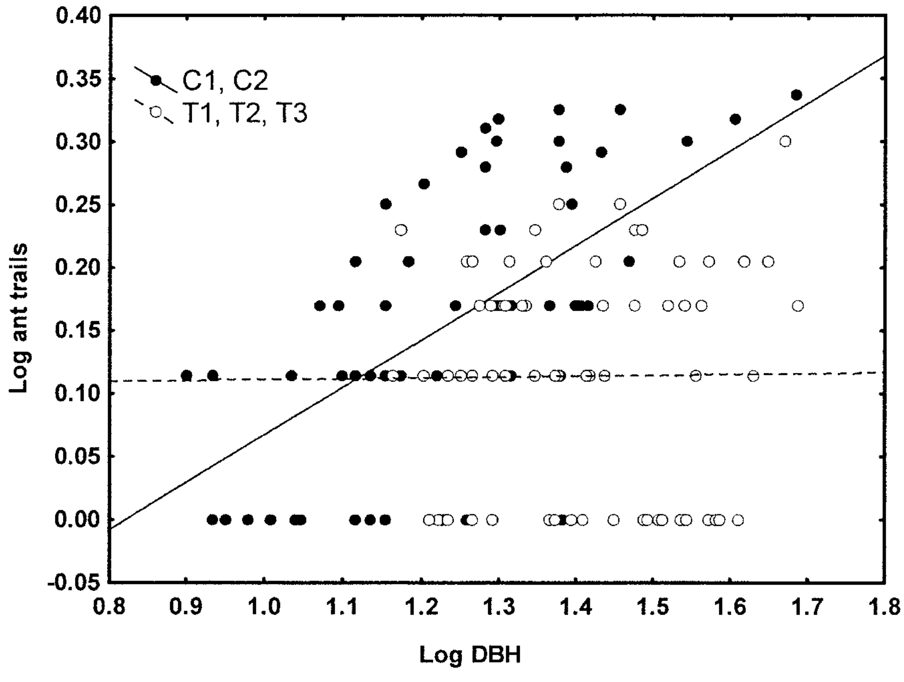

Fig. 2. Relationship between the number of *Lasius neglectus* ant trails found in each tree trunk and the diameter of the tree trunk (*Quercus ilex*) in the control areas (C1, C2; $R^2 = 0.38$; $P < 0.001$) and the treated areas (T1, T2, T3; $R^2 = 0.01$; $P = 0.28$) in 2002. In the treated area, the relationship is broken. Population of Seva, northeast Spain. Fitted simple regression line.

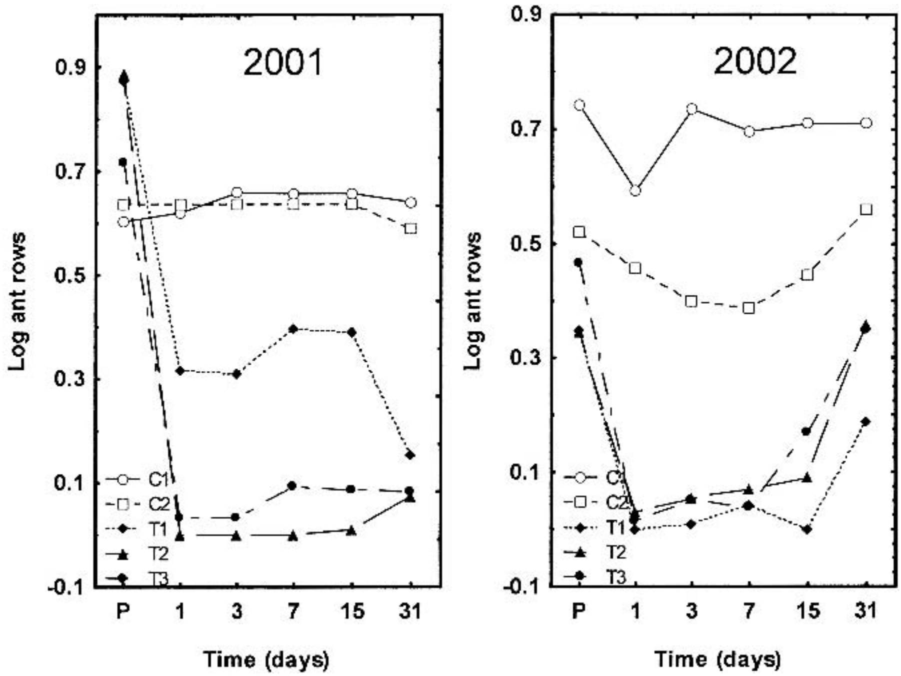

Fig. 3. Mean number of ant trails (log10 transformed) on tree trunks before the treatment (P) and after 1, 3, 7, 15, and 31 days. C1 and C2: control areas; T1, T2, T3: treated areas. Data from 2001 and 2002. Heavy rains in 2002 diminished the effect of trunk sprays.

$P < 0.001$. Year 2002: ANOVA, $F = 7.92$; df = 5, 25; $P < 0.001$). In the second year there was not such a drop and population recovery appeared to be faster. The heavy rains after some of the treatments could be the cause of this decreased effectiveness. The number of points with ants before the treatments (pretreatment level, Table 3) over the 3 years decreased significantly every following year (Fig. 4B; ANOVA, $F = 19.07$; df 2, 10; $P < 0.000$; also without control areas).

Table 3. Mean (± SE) number of rows per tree or points with ants around a house before any treatment (2001), immediately before treatment (2002), and at the comparable time in 2003, when no further treatment was done. Data pertain only to treated areas.

	2001	2002	2003	*n*
Ant trails/tree	7.2 ± 0.6	1.9 ± 0.2	0.6 ± 0.1	8 premises, 90 trees
Points with ants/house	16.2 ± 1.0	8.6 ± 1.1	6.0 ± 1.6	8 houses; 17–20 points/house

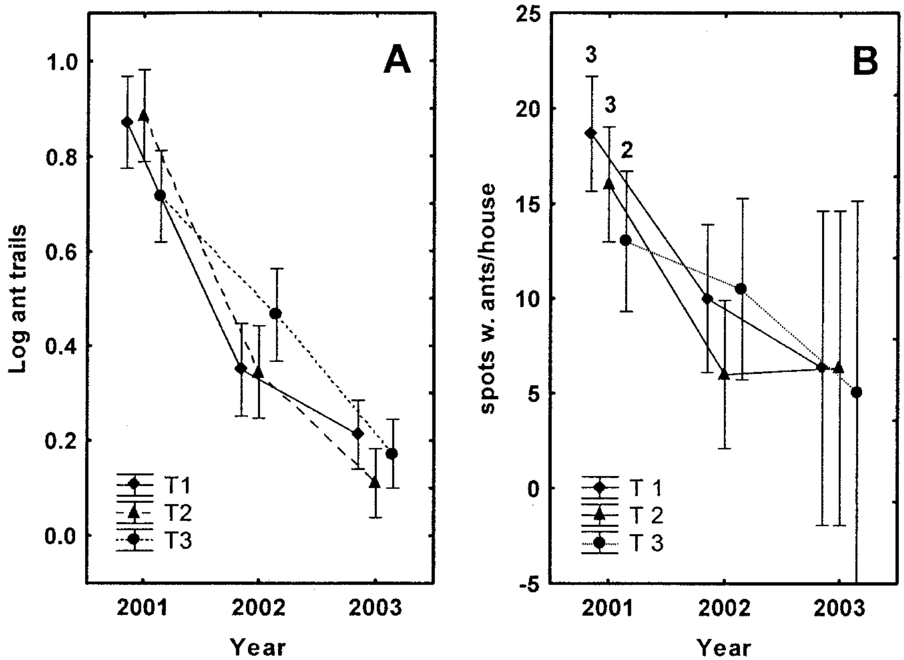

Fig. 4. Mean (±95% c.i.) number of ant trails (log10 transformed) on tree trunks (A) and number of perimeter spots with ants per house (B; digits over figures indicate number of houses) in the 3 treated zones (T1, T2, T3; n = 30 trees per zone) before any treatment (2001), immediately before the treatment of 2002 (2002), and at the equivalent time (May–June) in 2003, when no further chemical treatment was applied.

Queens and workers in soil. Queens were present in only three (year 2002) and two (year 2003) of the 200 soil cores taken in each of these years. In 2002, two cores had a single queen, and in the third we recovered two queens (one alive, one killed). In 2003, both of the cores had only one queen. In 2002, workers were found in 35 of the 50 random points and in 84 of the 200 (four per point) cores, suggesting a uniform presence within the area. This last sample unit had a mean ± SD of 6.28 ± 20.0 workers/core (range: 0–173). Given the area of the sample device (78.5 cm^2), an extrapolation to the entire occupied surface gives a rough estimate of 1.12×10^8 workers in the soil, for the entire colony (workers foraging on trees are not included). In 2003, workers were found in 30 of the 50 random points and in 60 of the 200 random cores, with a mean ± SD of 1.66 ± 5.9 workers/core (range: 0–71). The worker population in soil, after the chemical control of 2002, was estimated to be 2.9×10^7. Frequency data for worker presence/absence in cores for both years are different ($P = 0.012$; test for differences in proportions, two-sided). The number of workers per core is also different for years (Mann-Whitney U-test; $U = 17159$; $Z = -2.89$; $P = 0.0038$; corrected for ties, two sided).

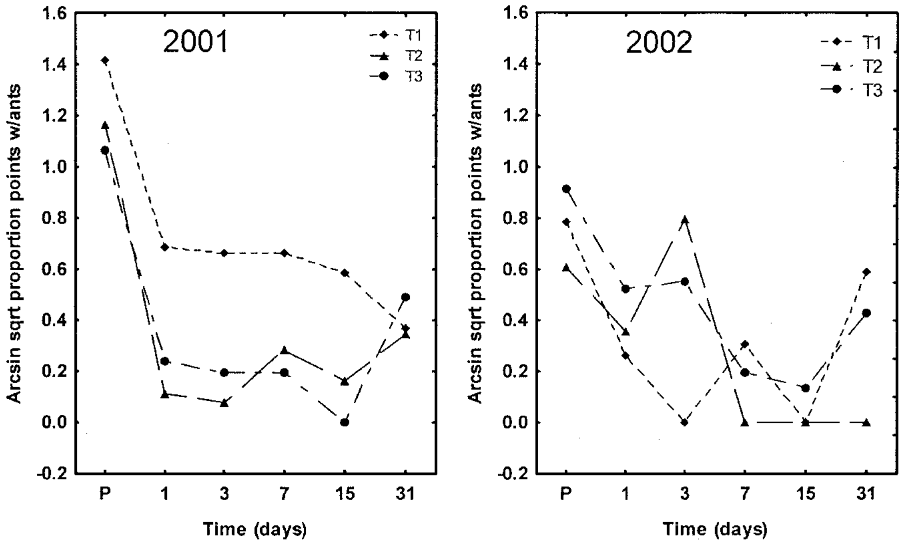

Fig. 5. Mean number of perimeter spots (arcsin transformed) with ants per house before the treatment (P) and after 1, 3, 7, 15, and 31 days. T1, T2, T3: treated areas. Data from 2001 and 2002.

Discussion

Only rarely have *Lasius* ants been reported as domestic (Green & Kane 1958, Kane & Tyler 1958, Jolivet 1986) or agricultural pests (Thompson 1990). The generic biological profile (food habits based on honeydew; absence of a sting; usually inconspicuous and timid behavior) is only exaggerated in *L. neglectus*, due to the polygyny and unicolonial structure of the populations. Therefore, potential damage may be enormous, as is the case in the colony studied in this work. To our knowledge, this is the first large-scale control trial to be applied to a *Lasius neglectus* supercolony. The combined treatments carried out on the invasive garden ant have been effective in controlling the large polygynous colonies of these ants. Similar problems have been detected with other invasive ants such as the Argentine ant (*Linepithema humile*, Hymenoptera: Formicidae*)*, the pharaoh ant (*Monomorium pharaonis*, Hymenoptera: Formicidae) in North America. Controlling these ant tramp species has always been problematic and their complete eradication has been deemed very difficult (Myers et al. 2000). Different control strategies have been tested, most of them based on barrier sprays (Moreno et al. 1987, Blachly & Forschler 1996, Rust et al. 1996, Klotz et al. 2003, Pereira 2003, Pranschke et al. 2003). Other safer combinations to decrease exposure to insecticides (Klotz et al. 1997) have been tested with good results.

Our approach is a combination of several treatments: three broad-spectrum insecticides with a high persistence, contact effect, and different application methods helped with a bait application inside the houses. Concerning the control of this pest ant, we detected a sharp decrease in the ant population. The decline of the ant population in soil (queens + workers) is based on a single comparison before and after chemical treatment. The decrease in the proportion of soil cores with workers and in the number of workers per soil core 1 year after the control treatment shows the efficacy of the chemical treatment and provides hope for eventually limiting of the colony. The number of ants in trees and house perimeters decreased significantly in all treated houses. Homeowners' opinions about treatment results were generally encouraging; there were only a few complaints when rains occurred just after some of the applications and the insecticide was washed away. In these cases, the treatment had to be repeated. This was a problem in 2002, when rains persisted for a total of 18 days during the 2 months of the treatment followed by an extremely hot summer. This increased economic cost and reduced the effectiveness of the treatment, as shown in the results section. Levels of pest infestation were clearly lower during the second year (2002) after one treatment and they never reached the high levels found at the beginning of the study. The decrease in ant trails on trees (73% reduction in 2001; 68% reduction in 2002) and areas with ants per house perimeter (47% reduction in 2001; 30% in 2002; Table 3) were lower in the second year, although it was still significant. Our experimental results were corroborated by the opinions of some of the homeowners and confirmed by visual inspection at the base of trees and was not merely repellency, with the ants moving from the treated area (Costa & Rust 1999).

We believe that an insecticide application earlier in the year would have increased the efficiency of the treatment. When the ant colony finishes hibernating and restarts the foraging behavior and normal activity, hibernating larvae of males and queens are the first stages to mature. A global application at this key period would eliminate by direct contact (soil injection) the number of sexuals produced. Although there have been tests with alternative substances (Vogt et al. 2002), conventional chemical treatment is still the best tool to control ant infestations (Williams 1994) such as in the case described here. At present, no parasites or pathogens of *L. neglectus* are known in native or introduced populations. A survey, posited at finding local parasitoids (Diptera: Phoridae), has been planned for 2005.

Attempts should be made to stop new introductions, especially in those localities where the climate fits the conditions under which this species seems to thrive in the Iberian Peninsula, that is, at more than 500 m altitude. It is perhaps also important that these areas are in the higher elevations, with relatively more rainfall and milder July temperature. Climate seems to affect the developing potential of this ant, although this may be different at other latitudes. To prevent further introductions, continuous surveillance when moving soil and turf and transplanting pot plants is the most reasonable strategy. This requires sustained awareness and community and public support.

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