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Biologia, ecologia i efectes de la formiga invasora *Lasius neglectus* (Hymenoptera, Formicidae) en comunitats d’artròpodes

Memòria presentada per Sònia Rey i Planellas per obtenir el grau de
Doctor per la Universitat Autònoma de Barcelona.

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Bellaterra, 2 de maig de 2007

A la meva família:
a la mare per l’origen
al Simon pel present i
als fills: Jofre, Arran i Tommy,
el nostre futur.

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Resum

Aquest treball de tesis doctoral tracta sobre la formiga invasora *Lasius neglectus* i els seus efectes sobre altres artròpodes en comunitats envaïdes al Nord-est de la Península Ibèrica. S’han estudiat: a) trets de la seva biologia reproductiva i ecologia b) els efectes que la invasió provoca sobre altres espècies de formigues natives i sobre els altres artròpodes que coexisteixen amb la colònia de *L. neglectus*; aquest darrer aspecte s’ha estudiat en tres poblacions. També es va fer un assaig de control de la plaga amb un tractament clàssic amb insecticides de contacte i una elevada persistència.

Pels estudis sobre la seva biologia reproductiva es va constatar el seu caràcter poligínic en tota la supercòlonia, amb una gran densitat de reines per supercolònia que es trobaven isolades o bé en grups de 2 o més reines dins de cada niu i sempre amb obreres. Per les dades en increment en pes sec i contingut de greixos des del naixement fins al moment de l’aparellament es va demostrar que les reines de *L. neglectus* es poden reproduir mitjançant fundació dependent tot i que el valor obtingut de la proporció del mesosoma de reines i obreres (relació entre el volum del tòrax de reines i obreres=23.83±0.15) coincideix amb el de les espècies que fan fundacions independents. Al laboratori també es van obtenir obreres a partir de reines joves acabades de fecundar (fundacions independents, haplo i pleometrótiques). Per les dades en contingut de carbohidrats es demostra que les reines tenen valors entremitjos entre les reines d’espècies que fan vols nupcials i les que no són capaces de volar, tot i que per l’àrea relativa de les ales les reines de *L. neglectus* han de ser capaces de volar. Tanmateix mai s’ha documentat cap vol nupcial en *L. neglectus* fins ara. Les reines joves alades fan fecundació intranidal i aquestes reines joves fecundades són adoptades per les reines velles i les obreres dins del mateix niu. Aquesta estratègia evita la depredació de reines joves fecundades després del vol nupcial, moment crític per a l’expansió de la colònia o la dispersió de l’espècie. Per tant aquesta expansió és per gemmació o bé a través de transport passiu, normalment afavorit per l’home. Les taxes de posta de reines velles són el doble que les de reines joves, amb un temps mitjà de desenvolupament de les obreres, però, el doble de llarg.

L’efecte més significatiu de la invasió de *L. neglectus*, tant a nivell d’abundància com de riquesa d’espècies, es va trobar sobre la resta de formigues natives. En totes les poblacions estudiades la presència de *L. neglectus* reduïa significativament la probabilitat de trobar espècies de formigues natives tant al sòl com als arbres. En el cas de l’efecte sobre altres artròpodes els resultats eren més variables: alguns artròpodes com ara els àfids es veien afavorits per la seva presència i en canvi altres ordres com ara Aràcnids, Himenòpters i Dípters es veien afectats negativament per la presència de la formiga invasora. Tanmateix aquests efectes, tant en formigues natives com en altres artròpodes, variaven en funció de la població estudiada i depenent de l’hàbitat mostrejat: capçades dels arbres o bé sòl.

En quan a les mesures per controlar aquesta invasió, es va fer un assaig amb insecticides d’una colònia de *L. neglectus*. Es van atacar tots els fronts possibles d’expansió de la invasió: a) l’interior de cases ocupades amb esquers, b) el perímetre de les cases ocupades amb injeccions amb un insecticida de contacte amb gran persistència al igual que c) els troncs dels arbres i finalment, d) es van tractar totes les capçades dels arbres de la zona ocupada per tal de matar els àfids, que són el principal aliment de *L. neglectus*. Gràcies al coneixement de les dades biològiques i ecològiques de la supercolònia de *L. neglectus* el resultat d’aquests tractaments integrats va ser satisfactori ja que disminuïa dràsticament la probabilitat de trobar formigues després del tractament i, a l’any següent al tractament, els nivells inicials de formigues eren molt menors als de l’any abans de tractar. Els tractaments es van fer dos anys seguits amb resultats prometedors i gran satisfacció per part dels habitants de les cases ocupades.

Paraules clau: biodiversitat, Formicidae, formigues invasores, Himenòpters, invasions biològiques, *Lasius*, *Lasius neglectus*, tractaments de control de plagues.

Biology, ecology and effects of the invasive garden ant *Lasius neglectus* (Hymenoptera, Formicidae) in arthropod communities

Abstract

The aim of this work is to study: a) the reproductive biology and ecology of the invasive garden ant *Lasius neglectus* and b) its effects over other native arthropod communities in invaded communities from Northeast Spain; this last aspect has been studied on three populations. A large scale insecticide treatment of the plague was also made with contact and high persistence insecticides.

Its polygynic character was ascertained by the studies about its reproductive biology, with a great density of queens that were found isolated or in groups of 2 or more queens per nest, always with workers. From data obtained in increase in dry weight and fat contents from eclosion until mating time, it was demonstrated that *L. neglectus* queens can reproduce through dependent colony founding even though the value obtained from the proportion of the mesosome ratio from queens and workers (queen/worker thorax volume=23.83±0.15) coincides with the independent colony founding species. Workers were also obtained, at the laboratory, from newly mated queens (haplo- and pleometrotic independent foundations). From data in carbohydrate content, queens have intermediate values among the flying queens and those not capable to perform nuptial flights, even though for the relative area of the wings, *L. neglectus* queens are capable of flying. However it has never been documented a nuptial flight in *L. neglectus* until now. Queens reproduce by intranidal mating and these fertilized young queens are adopted by the old queens and workers into the same nest. This strategy avoids the possible predation of young fertilized queens after the nuptial flight, a critical moment for the expansion of the colony. Therefore they expand by budding or through passive human transport. The egg laying rates of old queens are two fold than that of young mated queens, with a total development time from egg to worker double than from egg to nanitic for young queens.

The most significant effect of the invasion of *L. neglectus*, on native arthropods abundance and species richness, was on coexisting native ants. In all the studied populations the presence of *L. neglectus* reduced dramatically the likelihood of finding native ants on tree canopies and soil. For the effect of *L. neglectus* on other non-ant arthropods the results were more variable: some arthropods like aphids were favoured by its presence and other orders as Arachnids, Hymenoptera and Diptera were negatively affected for the presence of the invading ant. However these effects, in native ants as well as in other arthropods, vary depending on the studied population and on the sampled habitat: tree canopy or soil surface.

In order to control this invasion, classical treatments with insecticides were applied to one of the supercolonies studied. All the possible expansion fronts of the invasion were attacked with a) an in-house baiting, b) a house perimeter injection treatment, c) tree trunk spraying, and finally d) canopy fogging in order to kill the aphids, which are the main food supply for *L. neglectus*. Thanks to the knowledge of the biological and ecological features of the supercolony of *L. neglectus* the results of these integrated treatments were satisfactory. They drastically reduced the probability of finding ants after the treatment and, for the next year after the first treatment, the initial ant levels were lower than those of the year before treating. This treatment was repeated for two years with promising results and great satisfaction of the homeowners.

Key words: biodiversity, biological invasions, Formicidae, Hymenoptera, invasive ants, *Lasius*, *Lasius neglectus*, pest control.

Llistat d'articles originals

Aquesta tesis es basa en els següents articles, els quals vindran referenciats al text per la seva referència bibliogràfica (els que ja estan publicats) i amb notació de nombres Romans els que encara estan per publicar.

I. Espadaler X., Rey S. 2001. Biological constraints and colony founding in the polygynic invasive ant *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Sociaux 48: 159-164.

II. Espadaler X., Rey S., Bernal V. 2004. Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*. Insectes Sociaux. 51: 232-238.

III. Rey S., Espadaler X. Effect of a *Lasius neglectus* (Hymenoptera, Formicidae) invasion on native ant communities in Northeast Spain. Acta Oecologica (submitted).

IV. Rey S., Espadaler X. Invasion of the garden ant, *Lasius neglectus* (Hymenoptera, Formicidae), in North-East Spain: impact on non- ant arthropod communities. Biological invasions (submitted).

V. Rey S., Espadaler X. 2005. Area-Wide Management of the Invasive Garden Ant *Lasius neglectus* (Hymenoptera: Formicidae) in Northeast Spain. Journal of Agricultural and Urban Entomology. Vol. 21: 99-112.

INTRODUCCIÓ GENERAL

Les invasions biològiques

Els invasors biològics són espècies que s'estableixen en un nou espai en el qual proliferen, s'escampen i persisteixen en detriment de l'ambient (Mack et al, 2000) i són importants agents del canvi global (Lövei G.L. 1997; Mack *et al.*, 2000; Lonsdale, 2004). Les espècies alienes invasores (IAS, en anglès), són destructives amb la biodiversitat i els interessos humans (invasores) i s'han mogut d'una àrea geogràfica a una altra en la qual prèviament no hi havien estat (alienes) (Lonsdale, 2004). Algunes vegades només es desplacen d'una regió a una altra, però normalment la majoria d'IAS s'han mogut d'un continent a un altre.

Les comunitats aïllades (com illes, zones protegides, etc), han estat clàssicament model d'estudi de les invasions biològiques (Elton 1958). Fins fa poc, els pitjors efectes de les invasions biològiques es consideraven restringits a illes oceàniques, les quals actualment encara s'enfronten a elevats nombres d'espècies invasores (de l'ordre del 50%), però no només aquests ecosistemes pateixen els efectes de les invasions biològiques. Actualment cada cop hi ha més espècies alienes invasores en zones continentals amb efectes igualment negatius i devastadors. Proporcionalment al total de la flora i la fauna d'un país concret, les espècies no natives poden arribar a ser de l'ordre d'un 20% del total (Vitousek el al., 1996) i per resultats de diferents estudis es considera que la magnitud de les invasions continentals és molt més gran de la suposada actualment.

L'home s'ha convertit en un factor de canvi molt important, essent actualment el principal agent de pertorbació ecològica existent al planeta (Vitousek, 1996; Mack et al., 2000). Les invasions biològiques representen processos naturals que han estat sempre part de la història evolutiva de les espècies. Malgrat això, el fet preocupant és que actualment la taxa d'invasions biològiques s'està incrementant degut a l'home i els seus efectes sobre els ecosistemes, que no només s'han incrementat en freqüència sinó també en la seva intensitat: fragmentació d'hàbitats, constant urbanització i degradació dels paisatges, canvis en els usos del sòl, transport involuntari d'espècies alienes, introducció voluntària de noves espècies amb finalitats comercials, etc (Vitousek 1996; Lövei, 1997) (Figura 1).

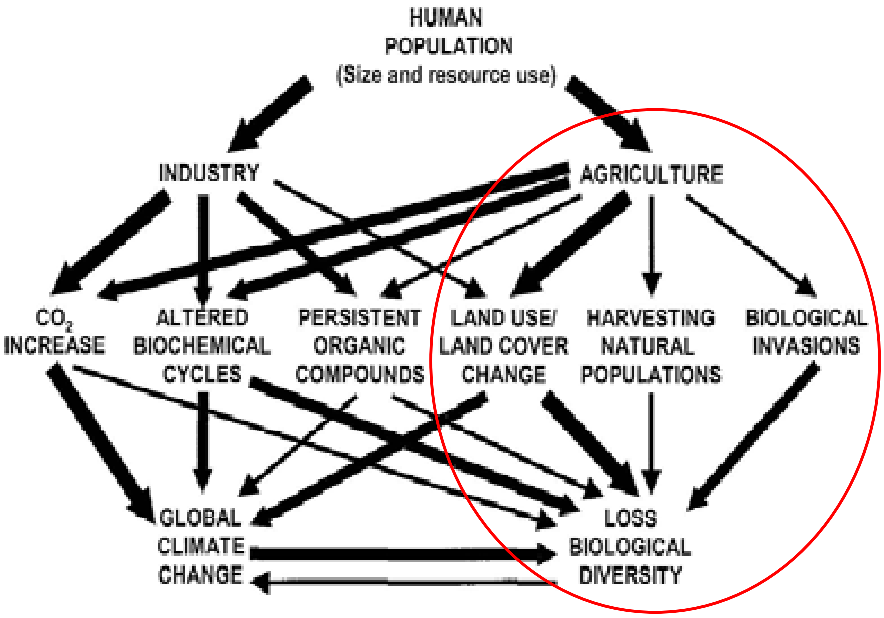

Figura 1. Principals components del canvi climàtic. Aquests components alteren, en diferent grau, el clima de la terra i redueixen la biodiversitat del planeta. El gruix de les fletxes dóna idea de la magnitud d'aquests efectes però les interrelacions i els efectes sinèrgics entre els sis components encara no estan completament estudiats (adaptat de Vitousek et al., 1997). Encerclat en vermell s'indica la ubicació dins el canvi global de les invasions biològiques, com a una de les vàries causes indicades, de la pèrdua de la biodiversitat.

No totes les espècies introduïdes s'estableixen, la gran majoria d'espècies exòtiques no arriben a la fase de colonització i moren (Figura 2, With 2002). Però tanmateix un tant per cent té èxit i s'estableix i dispersa en el nou hàbitat. Una aproximació usada sovint, és la que proposen Williamson i Fitter (1996), altrament coneguda com a "tens rule" que ve a dir que, de cada miler d'espècies que són transportades a un nou territori, un centenar s'acaben trobant als nous ecosistemes i d'aquestes, només una desena part s'estableix i només una d'elles acaba essent espècie invasora (veure figura 3).

Els habitats més vulnerables a les invasions biològiques són aquells que normalment presenten les següents característiques: aïllament històric o geogràfic (Elton, 1958; Brown, 1989); poca diversitat d'espècies natives (Elton, 1958; Case, 1990); nivells elevats de pertorbació o d'activitats humanes (Elton,1958; Case, 1996; Suarez et al., 1998); i absència d'animals co-adaptats, incloent enemics, competidors, depredadors, herbívors, paràsits i malalties (Elton, 1958; Colautti et al. 2004).

Les característiques més comunes que presenten les espècies invasores que finalitzen el procés invasor amb èxit són: amplis requeriment ecològics i toleràncies, algunes vegades reflectits en grans rangs geogràfics (Rejmánek, 1996); estratègies de reproducció r (Baker, 1965); associacions amb habitats pertorbats o humanitzats (Baker, 1965) i ser originaries de grans continents amb diversitat de biotes (Darwin, 1895).

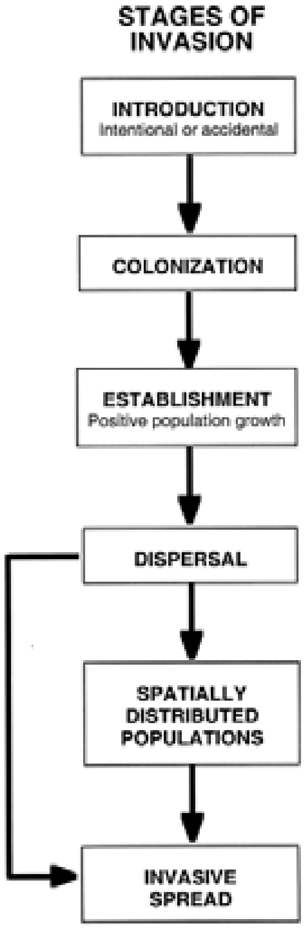

Figura 2. Estadis de la invasió (With, 2002)

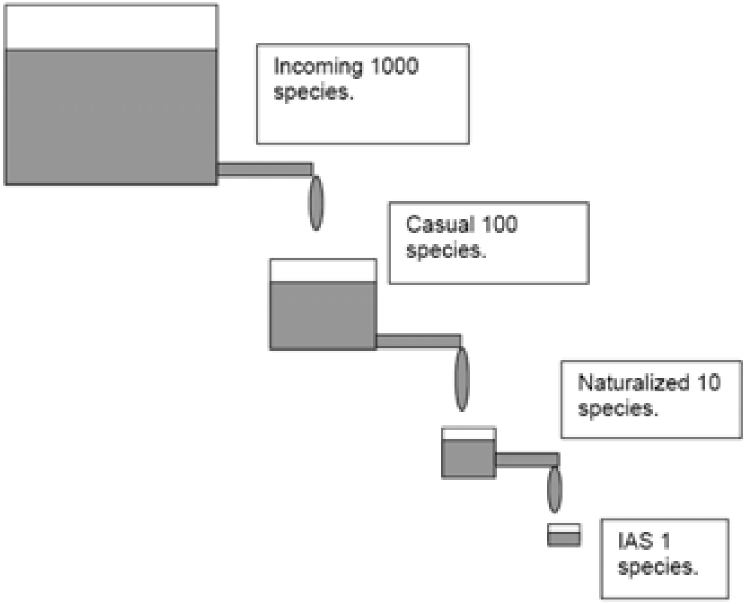

Figura 3. Etapes de transició de les IAS des que arriben fins que tenen un efecte negatiu (adaptat de Lonsdale, (2004) i basat en Williamson i Fitter (1996)).

Per tant l'habilitat de les espècies per a envair noves comunitats està influenciada pels trets dels invasors, el nombre d'invasors (pressió dels propàguls) i la susceptibilitat del medi a la invasió de noves espècies: la invasibilitat (Lonsdale, 1999).

Les principals conseqüències de les invasions biològiques segons Vitousek et al. (1996) i altres autors són:

1.-Impacte econòmic important ja que poden afectar cultius, boscos amb fins comercials, zones urbanes, etc. La despesa pels tractaments amb insecticides, lluita biològica o control de les plagues pot ser realment important (Pimentel et al. 2000).

2.-Els invasors alteren els processos dels ecosistemes com ara la productivitat primària, la descomposició, la hidrologia, geomorfologia, el cicle dels nutrients o els cicles naturals de pertorbacions (Mack i d'Antonio, 1998). Els efectes sobre els ecosistemes sovint també tenen conseqüències econòmiques.

3.-Les invasions redueixen la diversitat biològica i promouen l'extinció (Human i Gordon, 1996; Holway et al., 2002; Lonsdale, 2004).

4.- Promouen la facilitació: quan un invasor facilita l'entrada d'un altre (Simberloff i von Holle, 1999).

5.-Constitueixen un component significatiu del canvi global (Lövei, 1997; Mack et al., 2000).

Tanmateix moltes invasions són reflexes d'altres canvis i no agents canviants per elles mateixes (Vitousek, 1996).

Els insectes són un dels grups taxonòmics que més espècies invasores conté ja que tenen el potencial de dispersar-se a llargues distàncies (Simberloff, 1989). Dins dels Himenòpters, hi ha un grup de formigues, algunes d'elles considerades com de les pitjors espècies invasores descrites (Lowe et al., 2000) produint impactes molt negatius lluny dels seus medis naturals (Williams, 1994; Holway et al., 2002).

D'espècies transeünts de formigues a espècies invasores

Segons dades publicades, un total de 147 espècies de formigues en 49 gèneres han estat registrades fora dels seus hàbitats originals o nadius (McGlynn, 1999). Aquestes formigues exòtiques, que colonitzen hàbitats aliens, poden arribar a establir-se ("established ants") ens aquests nous hàbitats i dispersar-se. Una gran part d'aquestes formigues forma part de les anomenades formigues rodamón o transeünts ("tramp species", "espèces vagabondes"), comensals dels humans i que s'han escampat i, la majoria de vegades, establert en els hàbitats on han arribat. Aquestes formigues transeünts comparteixen una sèrie de característiques comunes que són les que els hi confereixen la seva gran plasticitat alhora d'ocupar i establir-se en nous hàbitats :

1.-Estan àmpliament escampades arreu del món, associades d’una manera o altre a l’home (comensals dels humans), ja sigui per utilitzar l’home com a medi de transport com per ocupar zones humanitzades en un o altre grau.

2.-Totes elles són de mida petita (<3.5mm) i, amb l’excepció de *Pheidole megacephala*, cap d’elles presenta polimorfisme (Passera, 1994) (veure figura 4).

3.-La seva biologia reproductiva és similar: totes elles són o presenten poblacions poligíniques i unicolonials (les formigues es comporten com a pertanyents a una mateixa colònia), que es reprodueixen per gemmació (“budding”) (Passera, 1994). No solen fer vols nupcials i per tant la fecundació té lloc dins el mateix niu on les noves reines fecundades són adoptades per la colònia existent.

Figura 4. Mida mitjana d’ obreres i reines d’algunes formigues transeünts. Les fletxes vermelles indiquen les que també són espècies invasores (adaptat de Passera, 1994).

4.-La mitjana de vida de les reines d’espècies poligíniques sol ser més curta que la de reines d’espècies monogíniques. En el cas de les formigues transeünts la seva esperança de vida pot ser extremadament curta: de setmanes en el cas de reines de *Tapinoma melanocephalum* a poc més d’un any en el cas de reines de *Wasmannia auropunctata*. No hi ha dades per totes les espècies però, tenint en compte les que hi ha, sembla que cap excedeix un any de vida. En quant als cicles reproductius de les reines, sembla que les reines d’espècies de formigues transeünts s’ajusten als cicles estacionals de la zona que ocupen.

5.-Les obreres de les espècies de formigues transeünts són, en tots els casos estudiats, estèrils.

6.-Formen supercolònies ja que perden la capacitat de reconèixer individus d'altres colònies i per tant, el comportament agressiu que això desencadena. Es tracta de societats "obertes" en el sentit de Le Masne (1952). En canvi l'agressivitat interespecífica és en general molt forta en aquestes espècies, tant directament com indirectament per competència pels recursos i el territori. Poden arribar a ocupar territoris exclusivament, sense coexistir amb pràcticament cap altre espècie de formiga (Haskins and Haskins, 1965 amb *Pheidole megacephala*; Van Loon et al, 1990 amb *Lasius neglectus*; Holway 1999 amb *Linepithema humile*)

De formigues transeünts n'hi ha moltes de descrites (McGlynn, 1999). Algunes d'elles també s'han descrit com a espècies invasores (marcades en vermell a la gràfica). No sempre les formigues transeünts es converteixen en invasores, sembla que calen una sèrie de factors desencadenants perquè formigues transeünts passin a ser invasores i per tant, a causar danys sobre altres espècies animals i/o vegetals, malestar a l'home i impacte econòmic. Tampoc, en una mateixa regió geogràfica, totes les espècies transeünts esdevenen invasores. Hi ha certes poblacions en llocs concrets que es consideren invasores i altres que no (veure Figura 2: estadis de la invasió).

Tant el terme invasor com el de plaga tenen una connotació humana. Som els homes qui les considerem com a tal en el moment en que causen un impacte econòmic o bé social en la zona que ocupen. Altres efectes sobre la biota i la biodiversitat de la zona es consideren, normalment, *a posteriori.*

***Lasius neglectus* com a espècie de formiga invasora**

Lasius neglectus es va descriure per primera vegada com a nova espècie invasora a Europa continental, a Budapest (Hongria) (Van Loon et al., 1990). La seva biologia era desconeguda i pels primers estudis descriptius, genètics i de comportament ja es va intuir la seva condició de nova espècie de formiga invasora tant a Budapest com a centre Europa i a la regió Mediterrània, concretament a l'Est de la Península Ibèrica (Van Loon et al., 1990; Boomsma et al., 1990; Passera 1994; Espadaler 1999, Seifert 2000; Dekoninck et al. 2002). Les obreres eren morfològicament semblants a les de *Lasius alienus,* amb la qual se la va confondre en les primeres identificacions, (mida mitjana d'obreres: 2.5-3.5 mm) però tant les reines com els mascles eren clarament inferiors (mida mitjana de reines: 6mm; mida mitjana de mascles: 2.5-3.5 mm). Pels anàlisis allozimàtics es va confirmar el seu status com a espècie diferent de *L. alienus* (Boomsma et al. 1990).

Es tracta d'una formiga poligínica i polidómica que forma enormes supercòlonies sense límits aparents i poca o nul·la agressivitat intraespecífica. No s'han detectat vols nupcials i s'ha observat l'aparellament dins dels nius (Van Loon et al., 1990). En aquest aspecte *Lasius neglectus* té certs trets semblants a l'espècie japonesa de formiga *Lasius sakagamii* (Yamauchi et al. 1981) i a l'altra espècie invasora que s'ha expandit per la vessant mediterrània d' Europa: la formiga argentina *Linepithema humile* (Mayr 1868). L'expansió de la formiga sembla que sigui per gemmació, degut a la manca de vols nupcials. L'home afavoreix aquesta expansió per moviment i transport de terres i també, com es suggeria per la colònia de Budapest, gràcies als pans de terra que acompanyen a les plantes dins els testos (Van Loon et al., 1990).

Per les mostres recollides en l'elaboració del treball de revisió de Seifert sobre formigues paleàrtiques del gènere *Lasiu*s (Seifert, 1992) hi ha evidències que *L. neglectus* va envair Europa des de l'Oest d'Àsia expandint-se de manera considerable durant els últims 25 anys (Seifert, 2000). El rang geogràfic que ha colonitzat és enorme: tota la vessant Mediterrània fins a Centre Europa (veure http://www.creaf.uab.es/xeg/Lasius per al mapa actualitzat de localitats d'Europa i Àsia Continental on s'ha citat *L. neglectus*) amb una localitat extrema a les Illes Canàries. Aquest ràpid progrés de *L. neglectus* juntament amb la seva habilitat per desplaçar altres formigues autòctones de la zona és comparable al que fa *L. humile* (Van Loon et al., 1990; Seifert, 2000), l'altre formiga invasora que afecta Europa. Tanmateix les limitacions de temperatura que afecten a la formiga argentina sembla que no afecten en el mateix sentit a *L. neglectus* ja que aquesta pot establir colònies permanents en regions amb una mitjana de temperatura al Gener de -5ºC (situació inadequada per *L. humile*). Per tant la posibilitat d'envair regions més fredes d'Europa fa que *L. neglectus* tingui la potencialitat de convertir-se en la formiga invasora més important de tota l'Europa no mediterrànea (Seifert, 2000).

Pels estudis al.lozimàtics realitzats en la població de *L. neglectus* a Budapest, es va detectar certa viscositat (taxes baixes de fluxe genètic en la població). La mitjana de relació genètica intranidal de *L. neglectus* es va trobar molt baixa, tant entre obreres (0.14) com entre obreres i mascles (0.014), tot i que no hi ha indicis que les reines siguin fecundades més d'una vegada. Per l'anàlisi dels genotips de les obreres, es va trobar un efecte significatiu d'entrecreuament, possiblement degut a un coll d'ampolla genètic tal i com s'explica per *Linephitema humile* (Giraud et al, 2000; Tsutsui et al. 2003).

Lasius neglectus s'alimenta principalment de la melassa dels áfids arboricoles. Ja en els primers estudis es va suggerir que *L. neglectus* pràcticament excloïa qualsevol altre formiga autòctona existent en la seva zona d'alimentació i nidificació (Van Loon et al., 1990). Sempre es troba en hàbitats semi-naturals o bé humanitzats completament (parcs, urbanitzacions, jardins públics, jardins botànics, ciutats, pobles, etc), com a espècie transeünt es considera comensal humana i utilitza l'home com a transport passiu per realitzar la seva expansió i també per establir-se en les zones colonitzades. Mai, fins ara, se l'ha descrit en cap hàbitat completament natural.

Estudis d'agressió al laboratori realitzats entre *Lasius neglectus* i tres altres espècies del gènere *Lasius* presents en la zona perifèrica d'una població (*L. grandis* Forel, 1909; *L. emarginatus* Olivier, 1792 i *L. cinereus* Seifert, 1992) indiquen que *L. neglectus* presenta una elevada agressivitat interespecífica amb una taxa d'atacs més alta que les altres *Lasius* contra ella, les quals feien més comportaments d'evitació o fugida (Cremer et al., 2006).

Àrea d'estudi

Aquest estudi s'ha dut a terme en tres zones ocupades per colònies de la formiga *Lasius neglectus*, totes localitzades al Nord-Est de la Península Ibèrica (Figura 5, mapa de distribució de *L. neglectus* a Catalunya).

Població A: El Muntanyà, a Seva (41º48'N, 2º16'E; 650m alçada). Es tracta d'una urbanització molt propera a nuclis urbans i envoltada de vegetació Mediterrànea: principalment boscos d'alzines (*Quercus ilex*) i pi blanc (*Pinus halepensis*). També es troben zones més esclarissades amb matolls i arbustos (brucs, ginestes, ginebrons, garrics) així com camps de conreu. Les cases de la urbanització tenen jardins amb sistemes de reg (manual o bé automàtic) i alguns contenen plantes exòtiques i tancats fets amb l'arbust *Prunus laurocerasus* envoltant-les. Hi ha gran abundància d'aigües freàtiques en aquesta zona així com diversos torrents estacionals al voltant de la urbanització. Plou una mitjana anual de 775mm i la temperatura mitjana anual és de 11.5ºC. Aquesta zona ha estat altament envaida per *Lasius neglectus* on trobem una supercolònia que ocupa unes 17hectàrees (Figura 6A; mapa de Muntanyà). Aquesta és la població que s'ha estudiat més i de on més dades s'han extret pel nostre treball.

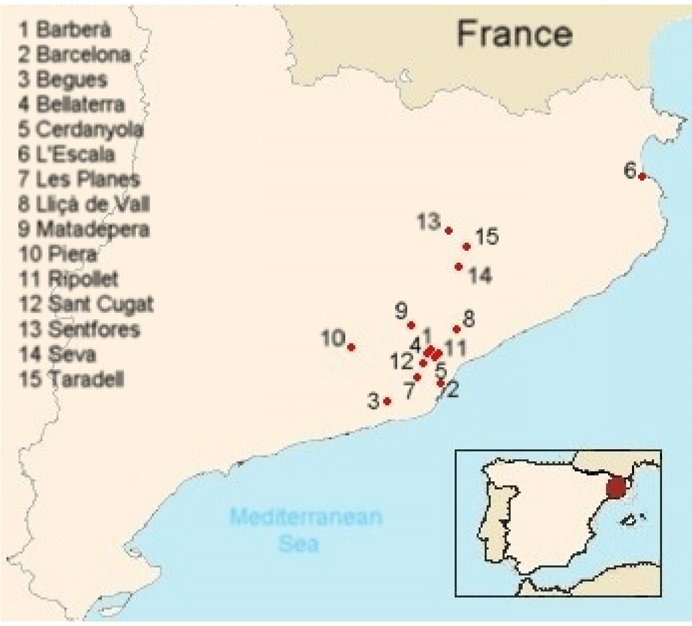

Figura 5. Mapa de distribució de *Lasius neglectus* a Catalunya. Els punts indiquen poblacions conegudes de *L.neglectus*. Tan sols la 9,14 i 15 es consideren plagues.

Població B: Matadepera (41º37'N, 2º2'E; 570m d'alçada). També una zona urbanitzada, amb cases escampades i totes enjardinades i amb diferents sistemes de regs. Els jardins contenen varietats de plantes exòtiques i autòctones. Els voltants de la urbanització són zones més o menys naturals en constant canvi (moviments de terres, neteja de boscos, etc). La vegetació que envolta la urbanització és també de tipus mediterrani amb alzines i pi blanc com a arbres més abundants. Hi ha diferents torrenteres de caire estacional que creuen la urbanització. La temperatura mitjana anual és de 13.5ºC i la precipitació mitjana anual es de 775mm. La zona ocupada per la supercolònia de *Lasius neglectus* a Matadepera és de 28 ha (Figura 6B;mapa de Matadepera).

Població C: La Roca, de Taradell (41º88'N, 2º30'E; 650m alçada). També es tracta d'una zona urbanitzada però la superfície ocupada per la formiga invasora en aquest cas és menor: només 6 hectàrees (Figura 7; mapa de Taradell). La zona urbanitzada es troba altament pertorbada amb cases molt juntes i pocs espais verds. Les zones enjardinades són més petites que en les altres poblacions estudiades però tanmateix la vegetació que envolta

A B

Figura 6. Mapa de Muntanyà (població A) i Matadepera (població B). Les linies en vermell delimiten aproximadament la zona ocupada per les supercolonies de *Lasius neglectus*.

la urbanització es troba força conservada (vegetació mediterrània i de ribera). Hi ha una riera que travessa pel costat Sud de la urbanització i que porta aigua gairebé tot l'any. Les formigues es troben sobretot en els jardins i les voreres de les cases i en tots els solars no construïts. El nivell d'invasió en aquesta urbanització sembla que no és tant alt com en la població A i més semblant a la de la població B. Plou una mitjana de 775mm anuals i la temperatura mitjana anual és de 14ºC.

Com es pot veure les condicions climàtiques i d'altitud varien poc entre les tres poblacions. Dues de les poblacions són més interiors: Muntanyà i Taradell (A i C) i la població B de Matadepera és més propera a la costa. La característica comuna és la presència de la formiga com a plaga en tres hàbitats altament humanitzats, pertorbats i en constant canvi. Només a la població A es té constància de la formiga des de l'any 1985, quan ja va començar a provocar molèsties als habitants de la casa on hi havia la colònia de *Lasius neglectus*. Suposem que des d'allà es va anar expandint cap a la resta de la urbanització, o per múltiples focus.

Lasius neglectus a més d'afectar els arbres, les plantes i la resta d'artròpodes, també s'introdueix dins d'aparells elèctrics com alarmes, bombes d'aigua, filtres de les piscines i caixes elèctriques, fent-los malbé i provocant curt-circuits. També provoquen malestar a les cases que envaeixen ja que n'hi ha tantes que qualsevol resta de menjar acaba convertint-se en un munt de formigues en pocs minuts.

Figura 7. Mapa de Matadepera (població C). Les línies en vermell delimiten aproximadament la zona ocupada per la supercolonia de *Lasius neglectus*.

Les formigues, a més a més, utilitzen el cablejat de la casa com a pas per entrar i sortir i també, suposem, per niar en cas de necessitat. D'aquesta manera les caixes dels cables es converteixen en llocs d'abandó de les formigues mortes i acaben omplint-se d'elles contínuament (veure figura 8). Aquesta atracció per les conduccions elèctriques o potser pels camps electromagnètics que es generen, ja s'ha descrit per una altra espècie de formiga invasora: *Solenopsis invicta* (la formiga de foc) als Estats Units (Vinson i Mackay, 1990) i els danys que aquesta espècie provoca són similars als de *L. neglectus* a Europa.

Mai s'ha descrit *L. neglectus* en un habitat completament natural. Sempre es troba associada d'una manera o altre a l'home, ja sigui directament ocupant habitatges o bé en zones altament humanitzades o urbanitzades. No sempre les colònies de *L. neglectus* tenen un caràcter invasiu, ja sigui perquè es troben en estats molt inicials de l'expansió o bé perquè hi ha limitacions pel seu òptim desenvolupament: geogràfiques, climàtiques, hídriques o biològiques. Sembla que la principal limitació a la que s'enfronta *L. neglectus* és la presència d'aigua en la zona que colonitza. En altres espècies de formigues amb característiques similars com és el cas de la formiga argentina (*Linepithema humile*) ja s'ha descrit el mateix fenòmen (Holway, 1998; Holway i Suarez, 2006).

Figura 8. Fotografia corresponent a un endoll d'una de les cases situades al centre de la zona ocupada per *Lasius neglectus* a la població B (Matadepera). Les formigues mortes s'acumulen dins les caixes i les conduccions elèctriques, suposadament transportades per les obreres (Fotografia cortesia de Monserrat Jorba, Matadepera).

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Objectius

Els objectius concrets d'aquest treball de tesis doctoral són:

1.-Estudiar la biologia reproductiva de la formiga *Lasius neglectus* tant al laboratori com al camp. Establir les taxes de posta davant diferents escenaris així com els paràmetres fisiològics i morfològics que determinen la seva biología reproductiva.

2.-Estimar el nombre de reines i el nivell de poligínia en una supercolònia de *Lasius neglectus* del Nord-Est de la Península Ibèrica.

3.-Estudiar l'efecte de la plaga de *Lasius neglectus* sobre altres comunitats de formigues autòctones en quant a abundància, riquesa i biomassa. Calcular un índex de diversitat per les diferents poblacions i estatus (infestat i no infestat) estudiats.

4.-Estudiar l'efecte de la plaga de *Lasius neglectus* sobre altres comunitats d'artròpodes (no formigues) presents. Determinar els efectes sobre l'abundància i el nombre d'ordres presents en zones infestades i no infestades.

5.-Fer un assaig de control de la plaga de *Lasius neglectus* en una supercolònia mitjançant insecticides.

RESULTATS I DISCUSSIÓ GENERAL

Biologia reproductiva de *Lasius neglectus*

El gènere *Lasius* és de distribució holàrtica, amb un caràcter nord temperat (Wilson 1955). Les espècies que conformen el gènere *Lasius* es troben en tres regions zoogeogràfiques: Paleàrtic, Neàrtic i Oriental (Bolton 1995). Pràcticament totes les espècies que conformen el gènere *Lasius* són monogíniques, amb l'excepció de la formiga japonesa *Lasius sakagamii* (Yamauchi et al, 1981) i de la formiga invasora *Lasius neglectus*, les quals presenten força similituds en la biologia reproductiva, l'ecologia i el comportament (Van Loon et al., 1990; Boomsma et al, 1990). Tanmateix és força improbable que aquestes dues espècies estiguin molt emparentades ja que estan geogràficament molt separades i hi ha diferències substancials en morfologia i comportament alimentari (Van Loon et al., 1990). Seifert (2000) la va comparar amb la formiga europea del gènere *Lasius* s. str., *L. turcicus,* al·legant una possible co-especificitat entre elles, però per les dades obtingudes va concloure que en tot cas *L. neglectus* havia divergit de *L. turcicus* per un canvi radical en el comportament de l'aparellament (de vols nupcials a l'aparellament intranidal) i això havia portat, segons ell, a una ràpida expansió de *L. neglectus* gràcies al seu alt poder competitiu.

Lasius neglectus s'ha descrit com a formiga poligínica (més d'una reina per niu) i polidómica (una única colònia que ocupa més d'un niu) en totes les poblacions estudiades fins ara dins l'Europa Continental (Van Loon et al., 1990; Boomsma et al., 1990; Passera 1994; Espadaler 1999, Seifert 2000; Dekoninck et al. 2002). Tanmateix no disposem de dades sobre la biologia reproductiva de *L. neglectus* en el seu lloc d'origen, i pot molt ben ser que allí, sigui una formiga que segueixi el mateix patró que la resta de *Lasius* coneguts, com ja passa amb altres formigues exòtiques establertes fora del seu lloc d'origen i que també són plaga o IAS (Ivasive Alien Species) com ara la formiga argentina (*Linepithema humile*). De la formiga argentina si que es coneix la seva biologia reproductiva i estructura social en el seu lloc d'origen i sembla que es força diferent de la dels llocs que colonitza (Tsutsui et al. 2003). Tanmateix podria ser que mantingués certs trets de la seva biologia reproductiva com ara la poligínia però que d'altres com ara la capacitat d'aparellar-se mitjançant vols nupcials, l'hagués perdut o canviat per raons desconegudes fins ara.

Pels nostres resultats en l'increment en pes sec i contingut en greixos des de l'emergència a l'aparellament (veure taula 1) sembla que les reines tenen característiques similars a les d'aquelles espècies que només es poden reproduir mitjançant fundació dependent (Passera, 1990).

	Pes sec (mg) (24 h a 70º C)	Contingut en greixos (%)
Emergència	3.1±0.5 (n=23)	29.6±9.7 (n=23)
Aparellament	4.8±1.0 (n=10)	43.3±9.5 (n=10)
Increment(%)	54.8	46.2

Taula 1. Estat fisiològic (mitjana±SD) de reines de *Lasius neglectus* en el moment de l'emergència i de l'aparellament. Les dades es basen en la població de Muntanyà (A).

Tanmateix el valor obtingut de la proporció del mesosoma de reines i obreres (relació entre el volum del tórax de reines i obreres=23.83±0.15) és típica d'espècies que fan fundacions independents (Taula 2; dades de Stille, 1996). Sembla que al camp, les reines només es poden reproduir mitjançant fundació dependent o bé per gemmació, tot i que al laboratori es va aconseguir que produïssin obreres mitjançant fundació independent (fundacions haplo- i pleometròtiques) (veure resultats de fundacions i taxes de posta a Espadaler i Rey, 2001). És a dir, la capacitat per a fer fundacions independents no l'han perdut (ratio tòrax/volum >20) tot i que no s'han detectat mai a la natura fins ara. És possible que en cas de necessitat encara siguin capaces d'utilitzar-la. Sembla, doncs, que per les dades fisiològiques i morfològiques obtingudes de les reines estudiades *L. neglectus* té el millor dels dos mons: les reines joves encara conserven el tòrax gran típic de les espècies amb fundació independent però, en canvi, les obreres les adopten. Tanmateix l'increment en el contingut de greixos des de l'emergència fins a l'aparellament indica que les sexuades de *L. neglectus* han iniciat el canvi evolutiu cap a la fundació dependent.

Fundació claustral independent (n=23; 3 géneres) a Stille,1996	*Lasius neglectus*	Fundació dependent (n= 39; 4 géneres) a Stille, 1996
24.1-31.6	23.83	2-9.5

Taula 2. Valors mitjans de la proporció del mesosoma de reines i obreres per reines del género *Lasius* que fan fundació claustral independent i reines que fan fundacions dependents. Dades de Stille, (1996) i de *Lasius neglectus,* (Espadaler i Rey, 2001).

La capacitat de fer vols nupcials, tot i que mai se n'ha detectat cap i només en una observació es van veure possibles evidències de vol nupcial (Seifert, 2000), no l'han perdut del tot. Per les dades en contingut de carbohidrats es demostra que les reines tenen valors entremitjos entre les reines que fan vols nupcials i les que no són capaces de volar (veure taula 1), tot i que per l'àrea relativa de les ales les reines de *L. neglectus* han de ser capaces de volar (veure resultats a Espadaler i Rey, 2001). Seifert (2000) també va arribar a les mateixes conclusions a través dels seus resultats. Ell, a més a més, va estudiar els muscles de vol que segons les seves observacions representaven el 85% del volum del mesosoma, la qual cosa indica que estan prou desenvolupats per a ser utilitzats.

Les obreres són estèrils i monomòrfiques. Mai es va observar posta d'ous per part d'obreres en cap de les diferents condicions establertes al laboratori. Això era d'esperar ja que en cap cas s'ha observat en formigues poligíniques que les obreres fossin fèrtils. L'esterilitat de les obreres també és una de les principals característiques de les espècies transeünts (Passera, 1994).

Viuen en supercolònies obertes que no presenten agressivitat intraespecífica (observació personal). Les reines mai es troben soles, sempre van acompanyades d'una o més obreres o bé en companyia d'altres reines (veure distribució de freqüències del nombre de reines, figura 2 a Espadaler et al. 2004). El nombre de reines del centre de la colònia i de la perifèria sembla que és similar, és a dir, el grau de poligínia de la colònia no mostra variació espaial. No es van trobar diferències significatives que indiquessin la possibilitat que les reines dels límits de la colònia es reproduïssin mitjançant fundació independent (veure resultats de densitat de reines a Espadaler et al. 2004). Les taxes de posta en nius artificials de les reines velles eren superiors que les de les reines joves (d'una mitjana de 6.8±2.4 ous/reina/dia en reines joves i fundació independent a 12.9±6.3 ous/reina/dia en reines velles amb fundació dependent; en ambdós casos només hi havia una reina per niu; veure taula 3).

Tanmateix el temps de desenvolupament d'ou a nanítica en el cas de reines joves era menor (36 dies) i significativament diferent del desenvolupament d'ou a obrera de les postes de reines velles (70.9 dies). No es van trobar diferències significatives en les taxes de posta entre reines del centre de la colònia i de la perifèria, ni en els seus pesos frescos (indicadors de la capacitat reproductiva). Tan sols es van detectar diferències significatives en el nombre de larves, que eren més abundants al centre que a la perifèria de la colònia. Tot això fa indicar que la manera com la supercolònia estudiada s'expandeix habitualment és per gemmació (budding). La supercolònia de *L. neglectus* en que es va fer aquest estudi del nombre de reines ocupa actualment 17 hectàrees (Població A, Muntanyà; veure figura 6 de la Introducció General). La magnitud del nombre de reines estimat, tenint en compte les dades obtingudes de densitat de reines per colònia, és enorme: 360.000 reines per supercolònia.

	Fundació independent Reines joves (sense obreres)		Fundació dependent Reines velles (amb obreres)	
	Haplometrótica -1 reina sola- (n=20)	Pleometrótica -2 reines per niu- (n=20)	Reines+obreres(30) (n=5)	Reines+obreres(60) (n=5)
Taxes posta (ous/reina/dia)	6.8±2.4	6.1±3.0	12.9±6.3 (n=10)	
Temps incubació ou (dies)	12.1±2.3		31.2±8.8 (n=10)	
Desenvolupament larval (dies)	9.3±2.1		26.4±2.5 (n=5)	
Maduració pupa (dies)	14.5±2.5		13.3±1.1 (n=3)	
Temps mitjà de desenvolupament total (dies)	36±4		70.9	
Nombre total d'obreres nanítiques obtingudes	11.5±4.8	19.2±7.5	Eclosions d'obreres (nombre desconegut)	

Taula 3. Desenvolupament de les postes de reines de *Lasius neglectus* en diferents escenaris (mitjanes±SD).

El gran nombre de reines presents al niu fa que la quantitat d'obreres produïdes al llarg de l'any sigui enorme (nº d'obreres estimat per la colònia sencera,població A: $1.12x10^8$), competint amb qualsevol altra espècie de formiga existent a la zona ocupada per *L. neglectus* tant directament (agressió interespecífica) com per competència directa o indirecta pels recursos. El nombre d'obreres sota pedres tampoc era estadísticament diferent entre centre i perifèria de la colònia. Es va observar que hi havia dues cohorts d'obreres durant l'època d'activitat de la formiga (veure taula 1 d' Espadaler i Rey, 2001).

Pels resultats obtinguts en aquest treball (contingut en carbohidrats a l'aparellament i gran nombre de reines dins del niu) i altres que han obtingut dades genètiques i morfològiques (Boomsma et al, 1990; Seifert 2000), es confirma que les reines de *L. neglectus* no realitzen vols nupcials i tan sols s'aparellen dins del niu. Aquesta estratègia els confereix una avantatge respecte les formigues que fan vol nupcial en quan a que eviten els depredadors que aprofiten aquests vols nupcials per alimentar-se de mascles i reines alades. L'adopció de les reines joves fecundades dins el niu incrementa la seva supervivència. Tanmateix el fet de no realitzar vols nupcials els impedeix el procés de dispersió a grans distàncies i tan sols és per transport passiu a grans distàncies, normalment afavorit per l'home (moviments i transports de terres), que poden colonitzar nous habitats.

Efecte de *Lasius neglectus* sobre altres artròpodes

L. neglectus s'alimenta principalment de la melassa que excreten els pugons, també de la melassa dels arbres i, molt poques vegades, s'han observat obreres portant preses entre les mandíbules. El seu cicle d'activitat s'inicia al moment que acaba la hibernació que, depenent dels anys, pot ser entre abril i maig i s'acaba quan les temperatures ja no els permeten sortir, entre novembre i desembre (Figura 1, cicle d'activitat anual). Per tant té un cicle anual llarg. El seu cicle d'activitat diària, en els mesos de màxima activitat: entre l'abril i l'octubre, és de 24h (observacions personals).

El present estudi es va centrar en efectes de la supercolònia de *L. neglectus* sobre la resta d'artròpodes coexistint en la zona infestada comparant-los amb els de la no infestada. En les tres poblacions esmentades abans es van comparar dades d'abundància i de diversitat: riquesa d'espècies en el cas de les formigues natives i d'ordres en el cas dels altres artròpodes. Es van agafar dades (mitjançant trampes de caiguda, safates i esquers) de les formigues del sòl i dels arbres, per tal d'estudiar tant els artròpodes epigèics com els arborícoles. Per l'estudi dels efectes sobre formigues natives també es va calcular la biomassa i l'índex de diversitat de Shannon (Magurran 1988) de cada zona estudiada (infestada o no infestada). Només en aquest estudi es van fer servir esquers per detectar les formigues natives i les invasores i el grau de coexistència.

L'efecte més significatiu de la invasió de *L. neglectus,* tant a nivell d'abundància com de riquesa d'espècies, es va trobar sobre la resta de formigues natives. En totes les poblacions estudiades la presència de *L. neglectus* reduïa dramàticament la probabilitat de trobar espècies de formigues natives tant al sòl com als arbres. En la població A, pràcticament les excloïen completament i en les altres dues poblacions poques formigues arribaven a coexistir amb *L. neglectus*, i si ho feien era perquè els hàbitats no es solapaven (formigues hipogees) o bé tan sols estaven de pas per la zona infestada (veure taula 1, 2 i 3; Rey i Espadaler III). Es va detectar una zona de transició entre zona infestada i no infestada que es va anomenar "vora" (border) i on la nidificació de *L. neglectus* no era condició necessària però si la seva presència. *L. neglectus* coexisteix en aquesta zona de vora amb altres espècies de formigues natives. L'efecte vora ja s'ha detectat amb altres formigues invasores (Human i Gordon, 1996) i presenta característiques transicionals entres zones envaïdes i no envaïdes (veure resultats d'abundància i riquesa d'espècies a Rey i Espadaler III; Taula 1, 2 i 3).

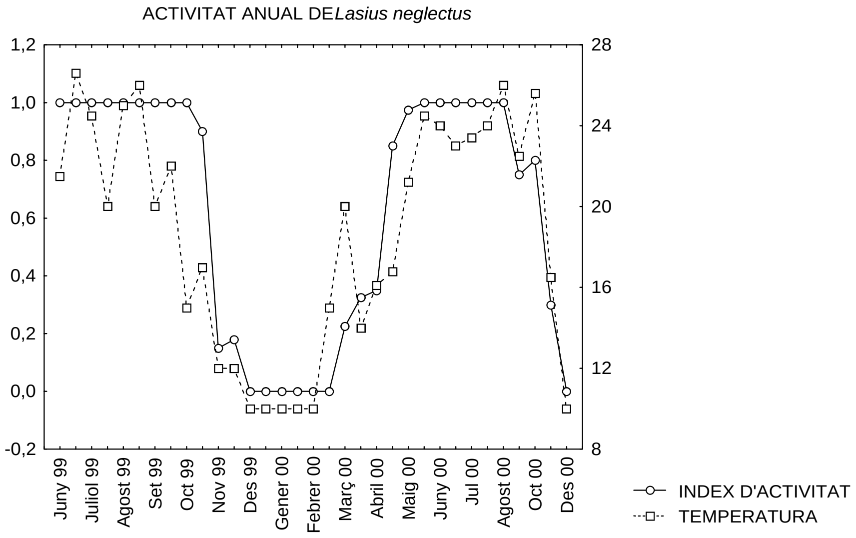

Figura 1. Cicles d'activitat de la formiga plaga *Lasius neglectus* al Muntanyà (població A). La gràfica comprèn el període del 15/6/1999 fins al 5/12/2000. L'índex d'activitat es calcula dividint els arbres amb formigues pel nombre d'arbres totals (n=40) (Dades inèdites).

Aquesta vora, podria actuar com a tampó, limitant l'expansió en zones on llinda amb zones naturals tal i com s'ha descrit per *Linephitema humile* (Holway, 1998). Tanmateix en el moment en que una pertorbació afecta aquestes zones naturals, la vora pot fer l'efecte contrari: seria el punt d'expansió de la colònia, les formigues invasores que normalment es troben allà en coexistència amb les altres formigues natives, en trobar un habitat buit (degut a la pertorbació) l'ocuparien ràpidament i establirien nous nius. Aquesta vora es va considerar alhora de fer els anàlisis sobre l'efecte de *L. neglectus* sobre altres espècies natives de formigues però no per analitzar les dades d'artròpodes, ja que no arribàvem a analitzar a nivell d'espècie sinó d'ordre i les subtileses de la zona de vora no semblava que es poguessin detectar a aquest nivell. Tampoc hi havia dades suficients per poder fer els anàlisis correctament.

L'abundància de formigues de la zona infestada per les tres poblacions i pels mètodes de mostreig de trampes de caiguda i safates als arbres, sempre era superior que la de la zona no infestada i significativament diferent (veure resultats Rey i Espadaler III). Aquests resultats sempre es devien a la gran quantitat d'obreres de *L. neglectus* que hi havia, tant al sòl com als arbres, en les zones ocupades per la supercolònia. En el cas dels esquers només es van trobar diferències significatives per una població (A) però era d'esperar ja que es tracta d'un mètode de mostreig que prioritza la diversitat d'espècies i no la seva abundància.

El nombre total d'espècies trobades tant en zones infestades com no infestades varia depenent de la població estudiada. La població A va resultar ser la més diversa, amb un total de 30 espècies (tenint en compte *L.neglectus* com a única NIS:"Non-Indigenous Species" trobada), la població B comptava amb 18 espècies diferents (dues eren NIS: *L. neglectus* i *Linepithema humile*) i a la població C es van trobar 27 espècies de formigues diferents i la única NIS era *L. neglectus* (veure taules 1, 2 i 3 de Rey i Espadaler III).

La comparació de les dades de biomassa també van donar significativament diferent en zones infestades i no infestades a la població A i C. Malgrat que en la població B no hi va haver significació estadística sempre ens donaven unes dades de biomassa major en les zones infestades, i sempre degut a la gran quantitat d'obreres de *L. neglectus* que hi havia (veure taula 4 de Rey i Espadaler III). La biomassa indica la importància relativa de la comunitat de formigues respecte la resta de l'ecosistema. En aquest cas l'impacte de *L. neglectus* en quan a la resta de la comunitat de formigues a nivell de valors de biomassa és destacable. *L. neglectus* monopolitza una part important dels recursos (per no dir la majoria) de l'ecosistema, afectant negativament a la resta de la comunitat de formigues que utilitzen els mateixos recursos alimentaris i de territori.

Amb les dades de riquesa d'espècies també es va obtenir un índex de diversitat, perquè detectàvem poca significació amb els tests estadístics, tot i que tots ens van donar significativament diferents per l'estatus (menys els obtinguts amb el mètode de mostreig d'esquers per les poblacions B i C). Al no entrar el nombre acumulatiu d'espècies diferents sinó les mitjanes, la diferència entre zones infestades i no infestades s'emmascarava i no es feia tant palesa. Aleshores es va decidir calcular un índex de diversitat (es va triar l'índex de Shannon: Magurran 1988) per tal que quedés més clar l'efecte de *L. neglectus* sobre la biodiversitat d'espècies de formigues natives. Aquest va donar sempre més alt en zones no infestades de les tres poblacions estudiades (veure taula 5 de Rey i Espadaler III, pels resultats dels índexs de Shannon per les tres poblacions estudiades i els diferents estatus).

Per la resta d'artròpodes es van analitzar totes les dades conjuntament (població A,B i C) i no es va tenir en compte el factor vora. L'anàlisi de les dades és més groller ja que tan sols es van determinar els individus obtinguts a nivell d'ordre. Tanmateix s'observa una disminució general de l'abundància d'artròpodes en les zones infestades del sòl de les tres poblacions i un increment en els arbres de la zona infestada (veure figura 2 de Rey i Espadaler, IV). Malgrat pugui semblar una contradicció l'explicació és senzilla: la causa de que el nombre d'artròpodes arborícoles augmenti es deu a la gran quantitat d'àfids que es troben en els arbres de les zones infestades. Ja que *L. neglectus* s'alimenta principalment de la melassa que excreten els àfids i els protegeix d'enemics naturals (González-Hernández et al.,1999), era d'esperar que aquests augmentessin en nombre degut al gran nombre de formigues existents. Sembla que la presència de formigues invasores beneficia aquest ordre (Homòpters) i a vegades, sobretot en cultius, provoquen grans explosions d'àfids (revisat a Holway et al., 2002).

La resta d'ordres coexistint amb *L. neglectus* afectats, normalment ho eren en forma negativa (veure taula 1 i 2 de Rey i Espadaler, IV). En arbres tant els depredadors (Aràcnids i Himenòpters) com els psocòpters disminuïen significativament en zones infestades (no sempre era significatiu en totes les poblacions però si que es mantenia la tendència). Uns altres ordres que es va veure que disminuïen (encara que no significativament) eren els dípters i els lepidòpters. En el cas dels depredadors es suposa que seria per competència directa per les preses o bé indirecta com a exclusió competitiva. En el cas dels psocòpters, els dípters i els lepidòpters, com a preses potencials (observacions personals), poden disminuir degut al gran nombre d'obreres alimentant-se. Tot i que *L. neglectus* s'alimenta bàsicament de les excrecions dels homòpters, també se l'ha vist transportant preses cap al niu i la major part d'elles (Paris, 2005) eren psocòpters, tot i que també s'han vist dípters i lepidòpters. Molts dels lepidòpters determinats eren larves i aquestes són molt més susceptibles a ser depredades (degut a la seva baixa mobilitat) que altres artròpodes.

Al sòl només els dípters es trobaven significativament menys abundants en zones infestades que en no infestades. Sembla que sigui per depredació dels ous, juvenils i adults, tal i com es troba en arbres (Human i Gordon, 1997). Els col·lèmbols semblaven afectats negativament tot i que la variació era tan gran que no va donar significatiu. Els col·lèmbols són artròpodes molt sensibles a les pertorbacions (Springet 1976, Deharveng 1996) i les formigues invasores sovint viuen en habitats pertorbats. Per tant potser una disminució en el nombre de col·lèmbols sigui deguda al tipus d'hàbitat on *L. neglectus* es desenvolupa i no a l'efecte de la formiga invasora en si. Tanmateix s'han observat obreres de *L. neglectus* depredant col·lèmbols (observacions personals), tot i que no tenim dades sobre la importància relativa d'aquesta presa en la dieta de *L. neglectus.*

Assaig de control sobre una colònia de *Lasius neglectus*

Tal i com s'ha explicat abans, *L. neglectus* és una formiga que a més d'afectar la biodiversitat de la zona que ocupa, també provoca gran malestar en els habitants de les zones infestades, sobretot al interior de les cases, on la seva presència és més molesta ja que apareix en tots els indrets on hi ha menjar i també s'introdueix per les conduccions elèctriques i les malmet. Sembla que té una certa afinitat pels circuits elèctrics, semblant a la que ja s'ha descrit per *Solenopsis invicta* i produint efectes semblants (Vinson i Mackay, 1990). El grau d'invasió de la formiga al interior de les cases és molt variable, estacional i coincideix amb els seus cicles d'activitat anuals a l'exterior. Gràcies als estudis previs sobre la seva biologia reproductiva, els cicles d'activitat, la seva ecologia i trets del seu comportament es va poder planificar un assaig de control de la supercòlonia de *L. neglectus* que afectava a la població del Muntanyà (població A).

Per tal d'atacar tots els fronts de la invasió es van fer diferents tractaments que consistien en: a) Aplicar a les capçades dels arbres un insecticida que matés els àfids (principal font d'aliment de *L. neglectus*). b) Aplicar als troncs dels arbres un insecticida de contacte persistent mitjançant un esprai, per tal de matar les formigues que pugen i baixen dels arbres per alimentar-se. c) Injectar els perímetres de les cases amb un insecticida residual per crear una barrera i evitar que les formigues de l'exterior puguin entrar al interior de les cases i a l'inrevés, a buscar menjar. d) Col·locar trampes a l'interior de les cases que ho necessitin. Aquestes trampes contenen un insecticida i un atraient de formigues dins d'un contenidor de plàstic amb obertures per on les formigues hi poden accedir. Això es va fer amb la intenció de matar les formigues que poguessin quedar al interior de les cases (veure taula 2 de Rey i Espadaler, 2005 per tipus, dosis i mètodes d'aplicació concrets dels insecticides utilitzats).

Aquest assaig de control és el primer intent de control que s'ha fet sobre una supercolònia de la formiga invasora *L. neglectus*. Aquest tractament complert es va fer per tota l'àrea afectada durant la primavera i l'estiu de l'any 2001 i del 2002. El nivell d'infestació va disminuir immediatament després de l'aplicació combinada dels tractaments amb insecticides i es va mantenir baixa almenys durant els dos anys posteriors (veure resultats a Rey i Espadaler, 2005). Al segon any de tractament, els nivells inicials ja eren més baixos que l'any anterior abans de tractar. Per les opinions favorables dels propietaris de les cases afectades, sabem que en els interiors de les cases també va disminuir el nombre de formigues. Per tant, aquest tractament combinat sembla que és efectiu a curt termini en quan a disminuir considerablement el nombre de formigues i a llarg termini, controlant i alentint l'expansió de la plaga. Desconeixem l'estat actual d'aquesta supecolònia, pel que fa al seu efecte en les cases ocupades.

La prevenció sembla ser la millor arma contra l'expansió de *L. neglectus*. El constant moviment de terres i el seu transport d'un lloc a altre afavoreix l'expansió de la plaga. És important que hi hagi un coneixement per part del públic en general i un control quan es produeixen aquests moviments de terres.

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Conclusions

1.-*Lasius neglectus* és una formiga poligínica amb el mateix grau de poligínia tant a l'interior com a la vora de la colònia. Pels continguts en greixos i l'increment de pes sec des de l'emergència fins a l'aparellament aquesta espècie té un perfil similar al de les que només poden fer fundacions dependents. Conserven però, la capacitat per fer fundacions tant dependents com independents al laboratori. Tanmateix la proporció del volum del mesosoma de reines relatiu al d'obreres cau dins el rang de formigues que fan fundació independent.

2.- Les reines i els mascles de *L. neglectus* no fan vols nupcials. Ho constatem per les observacions al camp (no s'han detectat mai enlloc) i els continguts de carbohidrats des de l'emergència fins a l'aparellament (insuficients energèticament per fer els vols). Tanmateix l'àrea relativa de l'ala ens indica el contrari, es a dir que la morfologia encara no s'ha modificat.

3.- Les obreres de *L. neglectus* són estèrils. La fecundació és intranidal i les noves reines fecundades són adoptades per la colònia originària. Hi ha dues cohorts d'obreres durant el període d'activitat de la colònia i tan sols una de sexuats.

4.- Tant la morfologia com la fisiologia de la reina en fase adulta ens indiquen que el perfil de *L. neglectus* és un entremig entre el d'una espècie monogínica, amb fundació independent, no invasora i el d'una espècie de formiga invasora poligínica (moltes reines). L'espècie estudiada està en transició entre les formigues que necessiten fer fundacions dependents i les que fan fundacions independents.

5.- El nombre de reines dins la colònia és enorme i sempre es troben acompanyades d'obreres i sovint d'altres reines. No s'observa un grau de poligínia major al centre que a les vores de la colònia. La colònia només es pot expandir per gemmació o bé per transport passiu.

6.- L'efecte de la formiga invasora sobre les altres formigues natives és devastador: dins la supercolònia de *L. neglectus* cap altre espècie de formiga pot establir-se i prosperar ja que l'elevat nombre d'obreres competeixen amb elles pels recursos i l'espai. Es detecten efectes a nivell d'abundància d'individus i també de riquesa d'espècies, éssent sempre les zones ocupades les més abundants en nombre d'individus (degut a les obreres de *L. neglectus*) i les més baixes en nombre d’espècies. També s’ha constatat una elevada agressió interespecífica. En canvi hi ha una mancança absoluta d’agressió intraespecífica i de reconeixement, d’aquí que *L. neglectus* sigui unicolonial.

7.- Els altres artròpodes coexistint amb *L. neglectus* presenten respostes diferents depenent de l’ordre estudiat. En general la presència de la formiga invasora disminueix el nombre d’artròpodes del sòl i la riquesa en nombre d’ordes, afectant així a la comunitat d’artròpodes amb qui coexisteix. En canvi a les capçades dels arbres, tot i que també afecta el nombre d’ordres que trobem i el disminueix, l’abundància d’individus és major que a la zona no infestada. Això és degut al gran nombre d’àfids que es troba als arbres de les zones infestades, ja que *L. neglectus* s’alimenta de les secrecions dels pugons i sembla que afavoreix aquest ordre i en provoca una explosió.

8.-Si s’utilitza un tractament simultani de diversos insecticides i s’ataquen els aspectes clau de la biologia de la formiga (alimentació i fenologia) combinat amb l’objectiu d’eliminar les formigues dels interiors de les cases afectades, el nombre de formigues es redueix significativament i al següent any encara es noten els efectes. Això serviria, potser no per eliminar totalment la plaga, però si per controlar-ne l’expansió.

AGRAÏMENTS

Primer de tot voldria agrair al meu director de tesis, el Dr. Xavier Espadaler per la seva ajuda i paciència. Per ser un bon mestre i un bon amic. Al Sr. Mariano Rojo pels seus consells i el seu coneixement. A l'alcalde de Seva, Sr. Josep Palmarola, pel suport logístic. Al Victor Bernal per la seva ajuda amb les mostres d'artròpodes. A l'Iris, l'Emma i l'Eva Farga per l'ajuda al camp, contant formigues. Al Simon Mackenzie per l'ajut amb els recomptes de 24 hores. A la Carolina Paris per l'última empenta i un futur ple de projectes. A l'Eva Muñoz que des de Londres m'ha dissenyat la portada.

També a tots els meus companys i amics de despatx, entre ells la Lídia Serrano i l'Eduard Pla, pels bons moments que hem compartit. A tots els altres companys i amics de feina i passadissos per fer la meva estància molt agradable durant la realització de la tesis: Anabel, Angie, Anna Pou, Marc, i un llarg etc. A la Marta Barceló per la seva complicitat, suport i amistat en tot moment.

Voldria agrair a tots els i les mestres de l'escola Casals-Gràcia el seu suport emocional i la seva confiança en que un dia acabaria allò que vaig començar. I també agrair tot el que d'ells i d'elles vaig aprendre. Sobretot la Margarita Burgaya, que sempre em burxava perquè no ho deixés...i no ho he fet. També voldria esmentar els meus alumnes, que em van fer adonar de quant m'estimo la Ciència i de quant m'agrada ensenyar-la. Sense aquesta experiència la tesis no hauria estat el mateix. Gràcies.

Un esment per tots els meus amics, antics i nous, que han aguantat les interminables explicacions sobre formigues estoicament, i fins i tot animant-me a continuar. A les nenes del pis, als amics del Trull, a la colla de La Garriga, sobretot a l'Ester pels dinars i l'amistat en tot moment, als dels sopars de masies, als veïns de la Vall de la mandra i sobretot als de can Xicola i al Paco, l'Emma i la Iris (la nostra família d'adopció) per ser sempre al nostre costat.

I finalment, estic molt agraïda a la meva família, que ha anat augmentant feliçment al llarg de la realització d'aquesta tesis. Agrair a la meva mare que, malgrat les seves pors, m'hagi deixat anar fent camí i sentint-me lliure. Gràcies al Simon Mackenzie pel seu suport incondicional, per les llargues converses sobre Ciència i sobretot, pel seu amor. Als nostres fills (Jofre, Arran i Tommy) només dir-los que sense ells això no s'hauria allargat tant, però segurament no ho hauria viscut amb tanta intensitat.

Aquesta tesi s'ha fet en el marc dels projectes següents: 1) Biología, ecología y comportamiento de la hormiga invasora *Lasius neglectus* y sus efectos en la biodiversidad local (REN2000-0300-C02/GLO) finançat per la DGESIC i 2) Biología e interacciones multitróficas entre hormigas –exóticas o nativas- y plantas en ecosistemas mediterráneos (GGL2004-05240-C02-01/BOS) finançat pel MCYT.

Articles Originals

Article I

Insectes soc. 48 (2001) 159–164
0020-1812/01/020159-06 $ 1.50+0.20/0

Insectes Sociaux

Research article

Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera, Formicidae)

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Received 19 December 2000; revised 15 February 2001; accepted 19 February 2001.

Summary. The polygynous invasive ant *Lasius neglectus* was described from Budapest, Hungary, as an unicolonial species, with no apparent colony barriers, and inferred intranidal mating without a nuptial flight. Here we analyze additional morphological characteristics of gynes, their physiological condition at emergence and at the time of mating and we describe the productivity of different types of colony founding in the laboratory. A low increase in dry weight and in fat content from emergence to mating indicates that gynes can only succeed in dependent colony founding. However, the queen-worker thorax volume ratio is typical of a species with independent colony founding and we were able to demonstrate independent colony founding in the laboratory (both haplo- and pleometrotic). Brood development in independent founding is rapid and the number of nanitics higher than in other *Lasius* species. Both colony budding and dependent colony founding could also be demonstrated in the laboratory. Worker oviposition was absent. The carbohydrate content of newly mated queens is consistent with the observed loss of mating flight of this species. However the relative wing area clearly indicates that *L. neglectus* queens should be able to fly. Therefore, both queen morphology and physiological state at maturity show that *L. neglectus* is intermediate between a monogynous, free-living, non pest ant and a multiqueened (polygynic) invasive tramp ant. This neglected ant thus offers interesting opportunities to study the origin of unicoloniality and the spread of this species in northeast Spain.

Key words: Carbohydrate content, colony founding, egg-laying rate, fat content, *Lasius neglectus*.

Introduction

The degree of size dimorphism between queens and workers and the physiological condition of queens at emergence and at the time of mating are associated with the mode of colony founding (Hölldobler and Wilson, 1977). In independent colony founding the newly mated queen starts a new colony by herself, without the help of workers and she rears the first brood alone. Usually, such queens are claustral and do not forage during their confinement. In a few cases, however, the queen leaves the nest to forage, which has been referred to as non-claustral independent or semiclaustral colony founding. Alternatively, when a group of workers help the queen to found a colony (budding) or when new queens are accepted in the mother nest after mating (queen adoption), colony founding is dependent. This mode of reproduction is common in polydomous systems and some parasitic species.

In species employing independent claustral colony founding, the queens rely on their fat reserves and the proteins released and the energy provided by the histolysis of the flight-muscles (Janet, 1907). The relative fat content of such queens has been shown to be higher than in dependent founding queens (Keller and Passera, 1989). Stille (1996) showed a congruent difference in queen/worker thorax volume ratio between dependent and independent founding species, in that dependent founding queens had proportionally smaller ratios than independent founding queens.

A mating flight is usual in ants (Hölldobler and Wilson, 1990). Some species, however, do not have such a flight but mate directly inside the nest (some socially parasitic species) or in the vicinity of the nest (Buschinger, 1986; Felke and Buschinger, 1999). The glycogen content of ant sexuals appears to be related to their flight ability: non-flying species have a lower content of carbohydrates although their wing surface is not proportionately smaller than that of flying species (Passera and Keller, 1990).

Lasius neglectus is a poorly known species living in huge supercolonies with no apparent within-colony boundaries, and with a highly polygynous kin-structure (Van Loon et al., 1990; Boomsma et al., 1990). It has been considered an invasive pest ant by recent authors (Passera, 1994). Information concerning continental Europe (Seifert, 2000) and its status in Spain (Espadaler, 1999) indicate that this species has the potential of becoming a serious problem, similar to the argentine ant. We have undertaken a research program to characterize the basic biological characteristics of *L. neglectus* in Northeast Spain. Here we examine the physiological status and a number of morphological characteristics of the gynes of *Lasius neglectus* at the time of mating to ascertain whether these data indicate a dependent or an independent colony founding and the presence or absence of a mating flight. Specifically, we have determined the relative fat content of queens and the queen/worker thorax volume ratio to have a good approximation of the most likely mode of colony founding. We have further used the carbohydrate content and the relative wing area to obtain an independent estimate of the likelihood of mating flight in *Lasius neglectus*. Finally, we have tested the success of dependent and independent founding in the laboratory and we have determined worker egg-laying capacity.

Table 1. Presence of castes, sexes and developmental stages of *Lasius neglectus* in the population of Seva, Barcelona (data from April 1999 to November 2000)

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Eggs				X	X	X	X	X	X	X		
Larvae	X	X	X	X	X	X	X	X	X	X	X	X
Worker pupae					X	X			X	X		
Sexual pupae					X	X						
Young workers					X	X				X	X	
Sexuals					X	X						

Materials and methods

The species and colony used in the analysis

Lasius neglectus was recently described from Budapest, Hungary (Van Loon et al., 1990) and further data on distribution are provided by Seifert (2000). The huge, highly polygynous colony of *L. neglectus* studied here occupies at present a continuous surface of 14 hectares in a suburban zone in northeast Spain, at 650 m a.s.l. The climate is Mediterranean, with a wet spring and fall and a dry winter and summer. Mean annual temperature is 11.5 °C and annual rainfall ranges from 700 to 750 mm. Throughout the area colonized, queens are found isolated or in small group (2–5 queens) under medium-sized stones. Houses are widely spaced and many lots have so far remained without human construction and have a mildly degraded natural vegetation. Irrigated gardens have both native trees and exotic bushes and trees. Monthly routine checks of life stages (eggs, larvae, pupae and adults) were performed during the activity period (April 1999 to November 2000). The life history for this colony is summarized in Table 1. Polygyny was ascertained through queen dissection and looking for a full spermatheca and developed ovaries. Within the limits of the 14 ha colony, the area is exclusively occupied by *L. neglectus* (pers. obs.). Two other populations have been reported in north-east Spain (Espadaler, 1999) and four more have since been detected (see Table 2). So far, *Lasius neglectus* has never been observed in natural habitats in Spain but only in disturbed areas from where the original vegetation has been cleared, substituted or heavily modified. B. Seifert (pers. comm.) is currently updating the distribution of the species. In four of the seven populations polygyny has been ascertained. The pest status of this ant is clear from the first reports (Boomsma et al., 1990; Tartally, 2000). In Spain, affected inhabitants of private property invaded by *Lasius neglectus* report extensive presence inside homes, occupation of electrical conduction with damage to electrical outlets, phones and electric swimming pool engines. Outside, ant aphid-attendance, in enormous numbers has caused the death of some of the affected trees. The ants were first detected, merely as a mild nuisance, in 1985.

Determination of fat content, queen/worker thorax volume ratio, carbohydrate storage and relative wing area

To measure the lipid content of queens we followed the same protocol as Keller and Passera (1989), based on Peakin (1972). In short, individual queens were weighed fresh, then dried for 24 h at 70 °C and the dry weight determined. Petroleum ether (boiling point 40–60 °C) and a Soxhlet apparatus were used to extract fat during 24 h from queens, which were subsequently dried again and weighed to the nearest 10^{-4} g, to obtain the lean weight. Fat content is expressed as a % of dry weight. Fat content was measured from queens at emergence (n = 24) and from queens at the time of mating (two weeks old; n = 10). Mating was observed at the laboratory and newly inseminated queens were used. Throughout the paper means are presented ± s.d. All statistical tests are two-tailed.

Queen/worker thorax volume ratio was determined as in Stille (1996). Thorax volume was estimated as length × width × height. We used a mean of two-three queens and three workers each from five distinct populations.

Carbohydrates (glycogen and free sugars) were determined following Van Handel (1985) and Passera and Keller (1990) with modifications to optimize the procedure. Queens were dried at 90 °C for 45 min., weighted and analyzed individually. A polytron was used, for a minimum of 30 s, to crush and homogenize each ant in water (400 µl per ant). The homogenate was centrifuged (30 s at 1500 rpm) in a bench top microfuge to eliminate the exoskeletal chitin to avoid interference in spectrophotometric analysis. Only the supernatant was used to analyze the carbohydrate content. Two replicates for each sample (250 µl of supernatant for each replicate) were obtained. Subsequently, the protocol of Van Handel (1985) was followed utilizing a Spinvac centrifuge (2 h at 50 °C) to evaporate the methanol. Anthrone (Sigma A1631) was

Table 2. Geographical and climatic characteristics of several known populations of *Lasius neglectus* in Northeast Spain

Locality	Longitude/Latitude	Elevation (m)	Rainfall*	Temperature**
Seva	41°48′N, 2°16′E	650	775	11.5
Bellaterra	41°30′N, 2°6′E	90	675	14.5
Barcelona	41°23′N, 2°9′E	20	575	16.5
San Cugat	41°28’N, 2°5’E	130	725	13.5
Les Planes	41°26′N, 2°6′E	230	725	14.5
Matadepera	41°37′N, 2°2’E	570	775	13.5
Taradell	41°53′N, 2°18′E	650	775	13.5

* Mean annual precipitation (mm.), ** Mean annual temperature (°C).
Extracted from Atles Climàtic de Catalunya. Termopluviometria (1997). Institut Cartogràfic de Catalunya (www.icc.es).

used as the color reagent. Optimal wavelength for spectrophotometric analysis of the green-blue reaction product is 625 nm. Optical Density (OD) was determined, and glycogen and sugar content read directly from the glucose calibration line.

Surface area of the wings was determined as in Passera and Keller (1990) by sketching the wing profiles with a drawing tube, cutting the profile, weighing the cuts and inferring the wing area from the weight of a known area of the same paper. This area was subsequently related to the fresh weight.

Testing independent colony founding

Queens and males were captured in the field in June. In the laboratory they were allowed and observed to mate freely in a large plastic box. On the following morning, dealate queens were assumed to be mated. Twenty replicates of single queens (haplometrotic) and twenty of pairs of queens (pleometrotic), without workers, were placed in vials (7 cm length, 1 cm diameter) with a water reservoir behind a cotton plug and no food. Egg-laying rates were obtained by a daily count of eggs during the first three days. Thereafter, vials were checked two or three times per week for the appearance of larvae, pupae and up to the eclosion of the first nanitic workers. The total number of workers produced (dead + alive) were counted until the original field colony started hibernation (November). Laboratory temperatures ranged from 22.5 °C minimum at night to 30 °C during daytime.

Testing colony budding and dependent colony founding

We use colony budding in the sense of Bourke and Franks (1995): ”colony foundation by a queen (or queens) plus workers leaving an established colony”, in which the age of queens is not defined. Old queens were captured in the field before the appearance of the new queen generation in June (Table 1). As the number of attending workers may influence the output of queens and the rearing of the new brood (Keller and Passera, 1990; Cassill and Tschinkel, 1999), two nest compositions were used. Single queens were placed with either 30 or 60 workers in vials (n = 5 replicates each) and fed with Bhatkar's artificial diet (Bhatkar and Whitcomb, 1970) once a week. Egg-laying rates were recorded as before and vials were maintained until the appearance of pupae and workers. Dependent colony founding was not systematically studied but for three artificial nests with >15 freshly mated queens and >100 workers. Egg laying and larval development until workers eclosion was ascertained.

Testing worker oviposition

Male production by worker ants has been reported for *Lasius niger* (Van der Have et al., 1988). To test the possibility of worker egg-laying, two groups of 100 workers older than seven months and two groups of ca. 50 worker pupae plus 100 old workers were established to check for possible egg-laying in old or in young, freshly eclosed workers. Also these colonies were maintained in vials, and fed Bhatkar's artificial diet twice a week. Freshly eclosed workers of one, two and three weeks old and old workers were dissected in Ringer' insect solution to study ovary development. The stage of development of the ovarioles and the presence of yellow bodies was examined under the microscope.

Results

Fat content, body-size relationship and carbohydrate content

The mean fat content of queens at the time of mating was of 43% (Table 3). Relative fat content of queens showed an increase of 46% from emergence to mating with an increase in total dry weight of 55%. Queen and worker thorax volumes were 3.62 ± 0.24 and 0.15 ± 0.19 mm^3 respectively. The mean queen/worker thorax volume ratio obtained for five populations of *Lasius neglectus* was 23.83 ± 3.59.

Table 3. Physiological status (mean ± SD) of *Lasius neglectus* gynes at the respective times of emergence and mating. Data are based on one Iberian population (Seva, Barcelona)

	Dry weight (mg) (24 h at 70 °C)	Fat content (%)
Emergence	3.1 ± 0.5 (n = 23)	29.6 ± 9.7 (n = 23)
Mating	4.8 ± 1.0 (n = 10)	43.3 ± 9.5 (n = 10)
Increase (%)	54.8	46.2

Glycogen content of mature queens was 225 ± 92.2 µg, and the weight of free sugars was 69.2 ± 20.8 µg. The mean dry weight of *Lasius neglectus* mature queens after 45 min at 90 ºC was 5.8 ± 1.2 mg (n = 10). Therefore the values of the glycogen and free sugars as percentage of dry weight were 3.84% and 1.26%, respectively. The amount of total carbohydrates (glycogen + free sugars) expressed as percentage of dry weight was 5.1 ± 1.2%.

The forewing area of gynes was of 20.38 ± 0.68 mm^2 (n = 7) and the ratio of wing area to body fresh weight (mean fresh weight at mating: 11.35 mg) was 1.74 ± 0.15.

Independent colony founding

Lasius neglectus queens are able to found new colonies independently. In almost all foundations (38 of 40), both pleometrotic and haplometrotic, egg laying began on the first day. The egg laying rates for young queens founding independently in laboratory nests were 6.8 ± 2.4 eggs/queen/day (n = 20; single queen) and 6.1 ± 3.0 eggs/queen/day (n = 20; two queens). Egg-laying rates did not differ between neither haplo- and pleometrotic foundations (ANOVA, $F_{1,38}$ = 0.6; P = 0.4) nor was there any difference in duration of egg, larval and pupal stages (MANOVA, Wilks' Lambda = 0.98; P = 0.95). The mean time of egg incubation was 12.1 ± 2.3 days; larval development lasted for a mean of 9.3 ± 2.1 days and the pupal maturation was 14.5 ± 2.5 days. The total development time from egg to a nanitic worker in *L. neglectus* was 36 ± 4 days. The mean egg size was 0.55 × 0.33 mm (n = 21).

After four months (from 13/6/00 to 9/10/00) haplometrotic foundations (n = 20) produced 11.5 ± 4.8 nanitics and pleometrotic foundations (n = 16) produced 19.2 ± 7.5 nanitics. Totals were different (ANOVA, $F_{1,34}$ = 14.6; $P < 0.001$) but no differences were found between the rates of nanitics produced per queen (11.5 ± 4.8 vs. 9.6 ± 3.7) for the two modes of colony founding (ANOVA, $F_{1,34}$ = 1.2; P = 0.26). Mortality before the appearance of the first workers was 2/20 (haplometrotic) and 1/20 (pleometrotic). No aggression was ever detected between pleometrotic queens before or after the appearance of nanitics.

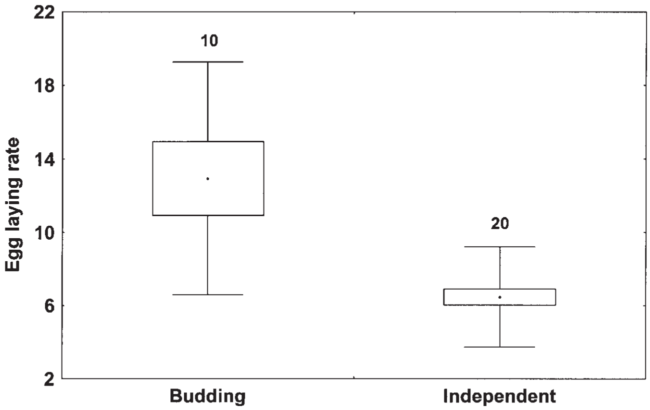

Figure 1. Egg laying rates (mean number of eggs/day/queen; three days) produced by old queens + workers (budding) and independent founding queens of *Lasius neglectus* in laboratory nests. Central point: mean; box: S.E.; whiskers: S.D. Number of replicates is given above each box

Colony budding and dependent colony founding

Although the number of workers between the nests differed (30 or 60) there were no differences in egg laying rate between treatments (ANOVA, $F_{1,8} = 0.0039$; $P = 0.951$). The egg-laying rate for old queens was 12.9 ± 6.3 eggs/queen/day (n = 10). All nests contained only one old queen.

The egg-laying rate for old queens is significantly higher than the egg-laying rate for the independent founding young queens (ANOVA, $F_{1,54} = 25.66$; $P < 0.001$; Fig. 1). The mean time of egg incubation was 31.2 ± 8.8 days (n = 10); larval development lasted for a mean of 26.4 ± 2.5 days (n = 5) and the maturation of the nymph lasted for a mean of 13.3 ± 1.1 days (n = 3). The mean development time from egg to worker in eggs laid by old queens was 70.9 days, much longer than in the independent foundations (MANOVA, Wilks' Lambda = 0.15; $P < 0.001$). Queen mortality of those >1 year old queens, before new workers had eclosed was three out of ten, a higher mortality than in independent foundations (three out of 40; <1 year old queens). This difference approached significancy ($p = 0.056$; difference between two proportions). Pupae and young workers appeared in the three artificial nests simulating dependent founding.

Worker oviposition

Worker dissection showed that young workers have two developed ovarioles. In workers of 5 to 10 days old, one egg was usually present in one of the ovarioles. Its aspect, however, was rounded and no defined chorion was ever seen. The size of the meroistic ovarioles ranged from 1 to 1.60 mm length. The size of the developed egg was 0.4 × 0.3 mm. Older workers had regressed or completely degenerate ovarioles (0.5 to 0.8 mm length) with 2 yellow bodies present (n = 25). After 4 months the ovarioles always appeared completely degenerate, similar in aspect to those described in *Formica* (Billen, 1984), *Myrmica* (Minkenberg and Petit, 1985) or *Ectatomma* (Fénéron and Billen, 1996). No eggs ever appeared in nests of old workers nor in nest of young + old workers during the two months of observation. Worker egg laying is thus probably restricted to the production of relatively few trophic eggs.

Discussion

The physiological status of *L. neglectus* gynes at emergence and at mating time shows a puzzling mixture of characteristics. The increase in total dry weight from emergence to mating and the relative fat content at mating of young queens of *Lasius neglectus* falls within the range of data obtained for ants with a dependent mode of colony founding (Keller and Passera, 1989). The values of relative fat content at mating (43.3%) are intermediate between the values for independent and dependent colony founding species. The increase in fat content from emergence to mating time (46.2%) is definitely within the values for the dependent founding species.

In contrast, thorax-volume ratio of queens to workers (23.8) shows that *L. neglectus* has still retained the morphology corresponding to independent colony founding. The ratios obtained for other *Lasius* species (Stille, 1996) based on the type of foundation were very distinct: on independent founding species, ratios varied between 24.1 for *L. niger* and 31.6 for *L. psammophilus* Seifert. For the dependent or temporary parasitic *Lasius* species, thorax ratios were from 2.0 to 9.5. In some other genera the division into groups of dependently and independently founding species was not as distinct as in *Lasius* (Stille, 1996). In the population studied we have not checked the intranidal mating in the wild and probable subsequent budding process, that has been attributed to this species (Van Loon et al., 1990; Boomsma et al., 1990). At the population studied, inhabitants have never detected masses of sexuals at swimming pools in spite of those swimming pools being surrounded by lawns invaded, exclusively, by *L. neglectus*. Boomsma et al. (1990) inferred that *L. neglectus* has intranidal mating; as we have observed matings in boxes, without a nuptial flight, this is an additional evidence for intranidal mating. If we could eventually confirm intranidal mating at the field, it would imply that *L. neglectus* has the best of both worlds: young queens have still the relative large thorax typical for independent founding species but, instead, the workers adopt them. However, the increase in fat content from emergence to mating time shows clearly that *L. neglectus* gynes have initiated the evolutionary development towards dependent founding.

With regard to the total carbohydrate content (5.1 ± 1.2%) *Lasius neglectus* gynes have an intermediate value between the flying (7.7 ± 2.2%) and the non-flying (2.8 ± 0.9%) gynes (Passera and Keller, 1990). Glycogen content was far higher than that of free sugar in *Lasius neglectus* gynes. Wing area development in relation to fresh weight of *L. neglectus* gynes is similar to values obtained for the gynes of a non-flying species, *Linepithema humile* (1.70 ± 0.13) by Passera and Keller (1990). By its wings *L. neglectus* does not show any impediment to be able to make a mating flight.

This mating flight has never been observed in nature for this species and is probably absent (Van Loon et al., 1990; but see Seifert, 2000). Independent colony founding is possible by both single queens and groups of *L. neglectus* queens in the laboratory; thus, a propagule size of 1 young, fertilized queen is sufficient for colony initiation. This was already stated in short by Boomsma et al. (1990). Apparently, mated queens of *L. neglectus* have enough body reserves to lay eggs and rear the first young without any external food during four weeks. Boomsma et al. (1990) suggested a lesser degree of polygyny towards the edges of the distribution; it is likely that it is mostly there that independent foundings take place in the field. That pleometrotic foundations produce a larger initial brood in *L. neglectus* is not unexpected, as this is well documented for other species (refs. in Sommer and Hölldobler, 1995). Colony budding in the form of old queens with the help of workers, were also able to start new colonies and their egg-laying rate was higher than that of young queens. In this last aspect, *L. neglectus* behaves more like an independent founding species than a dependent founding species. Keller and Passera (1990) showed that queen fecundity was higher in old queens of independent founding species (*Lasius niger, Camponotus herculeanus, C. ligniperda*) but was similar for both old and young queens in dependent founding species (*Plagiolepis pygmaea, Linepithema humile*). As could be anticipated, dependent colony founding of young queens with workers is also successful in *L. neglectus*. The absence of reproductive egg laying in workers shows also an evolution towards the tramp species life history, one of whose characteristics is this absence of worker oviposition (Passera, 1994).

According to our laboratory results, that nanitics produced by isolated queens develop in roughly a month, we should expect to find pupae during July and August. However, we did not observe pupae during those months in the field (Table 1); instead, it seems that there are two distinct cohorts of workers. A possible explanation may be that there is an arrest of egg eclosion and/or larval development (due to high temperature?); this is to be ascertained by mantaining brood under different temperatures at the laboratory. Alternatively, the food provided to larvae of recently mated queens could be different from that given to larvae of mature queens, which might, in turn, influence the rate of brood development. To understand the absence of worker pupae in the mid of summer, more work is required and should be confirmed in other populations. The development time of nanitics, from young queens, was much shorter than that of normal size workers from old queens. A short development time of the immature brood has been invoked as a factor intervening in the rapid spread of a social insect, *Polistes dominulus* (Cervo et al., 2000); although not directly comparable, this could be among the factors promoting the invasiveness of *L. neglectus.*

Polygyny is expected to result in the reduction of fecundity per queen (Tsuji and Tsuji, 1996). Although the per queen output (eggs to nanitics) in *L. neglectus* was higher in haplometrotic foundations neither the egg laying rates nor the number of nanitics per queen were statistically different. Brood development time in *L. neglectus* was shorter than in other *Lasius* (s.str.) species. In *L. niger*, the first eggs, larvae and pupae appeared later than in *L. neglectus*: in the 5th week (Keller and Passera, 1990) or after a mean of 8 weeks (Sommer and Hölldobler, 1995). A *L. alienus* queen laid a first egg one month after the mating flight (Černá, 1978) and for *L. pallitarsis* the first workers emerged after 13 weeks (Nonacs, 1990). The number of first workers also seems to be higher than in other *Lasius* (s.str.) species: 3 workers/queen after 60 days in *L. niger* (Sommer and Hölldobler, 1995) or one worker/queen after 17 weeks in *L. pallitarsis* (Nonacs, 1990). Those comparisons are not quite accurate as much of it depends on the temperature and different authors may not have reared their queens at the same temperature. It remains to be proved if solitary or grouped queens can found new colonies in the field.

A single other species of *Lasius* (s.str), *L. sakagamii*, from Japan, is known to be polygynous (Yamauchi et al., 1981). This seems to indicate two independent origins of unicoloniality in *Lasius* (s. str.). Otherwise, it seems that these evolutions towards high polygyny are rare in *Lasius*, compared to other genera (e.g. *Formica*). Our results indicate that *L. neglectus* evolved rather recently, because many adaptive behaviours for polygyny are in place, but the morphology has not yet followed. In short, *L. neglectus* has a series of highly interesting characteristics as an evolutionary intermediate between the standard *Lasius* type and a real unicolonial ant such as the argentine ant. In view of its dangerous pest potential we emphasize the urgent need for a careful identification of suspected colonies, perhaps wrongly attributed to other *Lasius* species and of biological studies directed towards a thorough knowledge of the biology of *Lasius neglectus*.

Acknowledgments

To Simon MacKenzie for definitive help in the carbohydrate analysis and language correction; to Marcial Moreno and Maria Lumbierre in lipid analyses. To Mr. Josep Palmarola (Seva) and Josep Mª Vives (DARP, Generalitat de Catalunya) for logistic support. J.J. Boomsma (Copenhagen), L. Passera (Toulouse), H. Cagniant (Toulouse) made excellent suggestions that greatly improved the manuscript. This study was also supported by a D.G.E.S.I.C. fund (REN2000-0300-C02-01/GLO).

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Article II

Insect. Soc. 51 (2004) 232–238
0020-1812/04/030232-07
DOI 10.1007/s00040-003-0732-y

Insectes Sociaux

Research article

Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*

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Received 10 September 2003; revised 1 December 2003; accepted 9 December 2003.

Summary. We have analysed the distribution of queens under stones at the core and at the periphery of a supercolony of *Lasius neglectus* that occupies 14 ha at Seva (NE Spain). Queens were not found alone, but rather within worker groups. Density at the center (mean ± s.d.: 1.38 ± 2.87 queens/stone; n = 100 stones; range 0–14) was not different from density at the periphery (1.18 ± 2.38; range 0–12). The estimate of the number of queens found under stones for the whole colony is about 35500. Egg-laying rates for queens from these two zones were obtained in the laboratory, at three different temperatures, and there were no differences detected. The presence of brood stages, from eggs to cocoons, was also similar in both zones. The homogeneous distribution of colony components may indicate that the area occupied by *L. neglectus* has already reached saturation. With a different technique – soil core extraction – we could estimate the density of workers in the soil: 800 workers per m^2 . Soil cores had 6.28 ± 20.0 workers/core (range: 0–173), giving a rough estimate of 1.12×10^8 workers in the soil, for the entire colony. Though few, some queens were also recovered from soil cores. Queen numbers for the supercolony, based on queens found in the soil, reaches the astounding level of 360000. Numbers are consistent with previous predictions.

Key words: Egg-laying rate, *Lasius neglectus,* polygyny, queen distribution, worker density.

Introduction

The number of queens is a key biological parameter in the sociobiology of ants (Keller, 1993). Unicolonial ants are characterized by high levels of polygyny (Bourke and Franks, 1995) that lead to extremely low within-nest genetic relatedness of workers and queens, posing a challenge for the kinship theory (Bourke and Franks, 1995; Keller, 1995). Unicoloniality – and its origin – has received considerable attention recently, especially in search for an evolutionary explanation of one of the most astonishing facets of this social structure, the absence of aggression between members of the colony, even from widely separated nests. Proximate mechanisms underlying this reduced aggression are still disputed (Giraud et al., 2002; Tsutsui et al., 2003). One system that has potential to help unravel the origin of unicoloniality is a recent invasive pest ant, *Lasius neglectus* (Seifert, 2000; Dekoninck et al., 2002; Espadaler and Bernal, 2003). This unicolonial species has many of the typical tramp ant characteristics (Passera, 1994). It is found in disturbed or highly degraded habitats, has many queens, disperses by budding, and has relatively small workers (Van Loon et al., 1990; Tartally, 2000; Seifert, 2000) in which reproductive egg laying cannot be detected (Espadaler and Rey, 2001). At three known NE Spanish populations ants are invading urban premises, and have attained pest status (locations: Matadepera, Seva, Taradell). According to what is known about several supercolonies of *Lasius neglectus*, a nuptial mating flight is absent and colonies grow by accepting their own young queens after insemination by local males inside the nest (Van Loon et al., 1990; Espadaler and Rey, 2001; Seifert, 2000), although this last author reported that some individuals were found in a spider web. No nuptial flight has ever been recorded in North East Spain. Private property owners do not remember having ever seen drowned sexuals in their swimming pools surrounded by lawns occupied by *L. neglectus*. Thus, the absence of a dispersal flight means that the colony can only develop by a slow expansion of the edges and by budding. A further source of infestation and/or expansion of the supercolony could be by the exchange of infested flower pots between neighbours; thus, a human mediated influence cannot be dismissed.

Boomsma et al. (1990) studied the Budapest colony with an allozyme analysis, and detected very low colony relatedness. From their analysis they deduced: i) there is probably a very huge number of queens in the center of the colony (their Fig. 2) and, due to a differential spatial distribution of allele frequencies, ii) a lesser degree of polygyny towards the edges of the distribution. Here we have tested this in more detail in a Spanish population. A first step was to assess if there were any differences in the presence of queens and the number of queens in the core of the colony and the periphery. This test is relevant because we now know that young queens could, in principle, migrate from their eclosing sites, to carry out short distance nest buds or to independently found new colonies (Espadaler and Rey, 2001), thus providing eggs and new workers for the slowly expanding colony edges. We further explored this possibility by comparing the egg-laying rates of queens from the core and periphery of the colony. Older queens have a higher egg laying rate (Espadaler and Rey, 2001); hence measuring egg laying here is an indirect way of measuring the queen's age in the different zones. As the growth of the colony occurs by expansion and budding (Van Loon et al., 1990; Espadaler and Rey, 2001), additional differences, such as in the presence of cocoons of sexuals, and other brood stages, might be expected between the two zones and were therefore compared in this study.

We used the above mentioned information on queen densities and distributions to infer the colony queen number: it is enormous. We are aware of few earlier attempts to quantitatively assess the number of queens from other supercolonial ants: Newell and Barber (1913) mention 10–20 queens of the Argentine ant under large trees, and Horton (1918), in the same species, detected 13 queens/m^2 in a Californian fruit plantation. Ingram (2002a, b), by using genetic analyses, detected a variation in queen number per nest – from 0 up to >60 – and this was related to nest densities. Rosengren et al. (1993) counted >500 queens/nest in *Formica aquilonia* or >300 in *F. uralensis* and Higashi and Yamauchi (1979) estimated >10^6 to be the number of queens in a supercolony of *Formica yessensis*. The absolute queen number, in itself, may be of limited interest but considered jointly with the egg-laying rate – which varies according to queen age, colony size and temperature (Hölldobler and Wilson, 1990; Williams, 1990) – it can provide an estimate of the potential for colony growth and thus, for the potential future expansion of pest ants.

Material and methods

All counts, measurements and comments refer exclusively to the population from Seva (Barcelona, Spain; 41°48′N, 2°16′E; elevation 650 m; annual rainfall 775 mm). The oldest dates from which inhabitants remember these ants being a problem are from 1985. Such knowledge is lacking from other populations, both in Spain and elsewhere, and these sites may have different properties according to differences in ecological context or climatic conditions. The periphery of the colony was loosely defined as <20 m from the edge; usually the first 10 m allowed for sufficient sampling. The edge was detected through visual inspection of the soil, soil digging with a showel or by looking for cracks in concrete with soil debris, and always checking the identity of the ants.

Density of stones with workers

The number of stones with workers was assessed by counting the number of stones present in 45 randomly chosen squares distributed over the are of the whole population; 14 squares were in the periphery, and 31 in the center. Squares of 5 m × 5 m were delimited and every stone (size >5 cm) was counted and noted if worker ants were present. Squares are assumed to be independent samples.

Queen abundance

We compared the presence and abundance of queens at the center of the supercolony and at the periphery (Fig. 1). In ants, queens have a retinue of attending workers; therefore, before the appearance of sexuals, we did not expect to detect isolated queens without workers. We turned over stones no smaller than 5 cm in diameter (= length + width/2) and noted the presence or absence of queens, workers, eggs, larvae, and cocoons. When queens were present it was also noted how many there were. We continued until 100 stones with workers had been censused in each zone. Each stone was measured at its longest width, length and height, to control for a possible relationship between stone size and the presence of any of the stages noted above. Censuses were done in late April and early May 2002, before the appearance of the new generation of sexuals in June. In addition to the number of queens under stones, the density of queens elsewhere was assessed by soil sampling with a manually operated soil auger that extracted a volume of approx. 300 cm^3 (10 cm diameter, 15 cm deep). This procedure has already been used by Boomsma and Van Loon (1982) to estimate worker populations of underground ants. At 50 random points, distributed over the area of the whole population, we extracted four soil cores at the corners of a 1 m square. Soil was crumbled over a white plastic box (25 × 40 cm), and ants (queens, workers) were counted. This also allowed a gross estimation of the total number of workers in soil for the whole colony.

Egg-laying rates

Laying rates were obtained in the laboratory. In late May, and before the appearance of the sexuals, we collected queens from the central zone and from the periphery. Thus, queens were at least one year of age. Each queen was isolated with 5 workers in tube nests, fed an artificial diet and the eggs were counted one and three days later. Nests were maintained at three different temperatures (25 °C, 28 °C and 31 °C). Ten replicates were established per temperature and for each colony zone. Eggs were counted under a dissecting microscope. After the egg-laying test, live queens were individually weighted.

Statistics

The number of queens under stones and egg laying rates

The number of queens under stones in the two zones was strongly skewed and no transformation achieved or approximated normality. Thus, we used two non-parametric approaches: First, a simple Mann-Whitney test and second, a bootstrapping procedure (1000 random replicates) to estimate the mean number of queens. By doing this we got a distribution that gives a reasonable approximation of the limits of variation for the mean (Efron and Tibishirani, 1993). For both analyses, we used 'zone' as the independent variable and the raw number of queens as the dependent variable. An attempt to estimate the total number of queens in squares of 5 m × 5 m and confidence limits was also done with bootstrapping: a random sample with replacement and with the same sample size for each distribution (sample size = 200 for the number of stones with workers and with/out queens and sample size = 45 for the number of occupied stones in 25 m^2)

Figure 1. Extension of the *Lasius neglectus* colony in the population of Seva (NE Spain). Data up to May 2003. A more recent, very small population of the argentine ant (*Linepithema humile*) occurs just south of the colony

was done and the means for each sample multiplied so as to get a measurement of the number of queens in 25 m^2. This was repeated 1000 times. Egg laying rates of queens from different colony zones were compared with a repeated measures ANCOVA, with zone (center, periphery) and temperature (25 °C, 28 °C, 31 °C) as fixed factors, and queen fresh weight as a covariate.

Brood stages

To test if workers relocate the brood stages towards the periphery through local migrations, to provide a task force for the colony expansion, eventual differences in the presence of the different stages (eggs to pupae) in the two zones (center vs. periphery) were analysed using a Generalized Linear Model (GLM), with presence-absence as the response variable (binomial variate). Colony zone was used as a categorical factor in the model and stone volume as a continuous predictor. The model included a binomial error structure and a logit link function.

Binomial confidence intervals for the proportion of stones with the various developmental stages were calculated according to Rohlf and Sokal (1995; Table P). The five tests are clearly a family of related tests, addressing the common null hypothesis of there being no difference between the center and the periphery. This situation requires adjustment for multiple tests (Chandler, 1995; Rice, 1989). Routines for the bootstrapping were done using Simstat and Matlab; other statistical analyses were run with Statistica 5.5.

Results

Queen density

Roughly one third of the stones had workers under them (Table 1). When queens were found under stones, they were always attended by workers: no isolated queens were detected. Stone size, as expected, was correlated with queen number although volume explained only 6.25 % of the variance. The number of queens per stone at the periphery (mean ± s.d.: 1.18 ± 2.38; n = 100) was not different (Mann-Whitney U test, two tailed; U = 4982; Z = 0.05; p = 0.95) from that in the central zone (1.38 ± 2.87; n = 100) (Fig. 2); comparisons using bootstrapping did not show any difference between queen number either: the probability of obtaining the observed difference of 0.2 queens, or bigger, is > 0.59. Therefore, for the estimation of the total number of queens in squares of 5 m × 5 m and its variation, we used the global mean of 1.28 ± 2.63 queens per stone; ranging from 0 to 14 queens (n = 200 stones with workers). Queens in the soil were collected only in three of the 200 soil cores. Two cores had a single queen and in a third, we recovered two queens (one alive, and one dead which had been cut in half).

Estimation of queen and worker number

We obtained a cumulative estimate of the queen number by summing estimates from densities under stones and of queens in the soil. Both estimates are very different and are treated separately. By using data of queens under stones, the bootstrapping procedure produced a mean ± s.d. of 6.34 ± 1.79 queens under stones per area of 25 m^2. Confidence limits (± 95 %) of this mean value are 3.34 and 10.17 (Fig. 3). In the Argentine ant in urban settings nests are more abundant *under* pavements (Passera, pers. comm.) and we have often seen workers of *L. neglectus* coming out from cracks with soil debris in roads. So, we have not discounted the surface occupied by pavements and roads (4 ha) from our calculations. As the supercolony occupies at present an area of 14 ha, this number would lead to an estimate of 35504 ± 10024 queens for the total number of queens under stones. From the 200 soil cores extracted only three – from three dis-

Table 1. Stone density and occupancy by ants *Lasius neglectus* at Seva (NE Spain). (n = 45 randomly chosen squares distributed over the whole population; 14 squares were in the periphery and 31 in the center)

	Total stones	+ workers	– workers
Mean	16.64	4.97	11.66
s.d.	24.24	8.11	17.40
Median	7	2	7
Range	0–127	0–42	0–85
Total	749	224	525

Neither the number of stones found at the center and at the periphery (ANOVA: $F_{1,43} = 0.008$; $P = 0.92$) nor the number of stones with workers (ANOVA: $F_{1,43} = 1.3$; $P = 0.25$) were different.

tinct plots – showed positive for queens: two had a single queen and the third had two queens. We accept that 200 cores is probably too small a sample for an accurate estimate; the interesting point, however, is that these samples show that queens do not need the protection of a stone and may be found in the soil throughout the habitat, without any visible marker of their presence at the surface. Given the area of the extracting device (78.5 cm^2), extrapolation of the entire 14 ha of infested area, gives an astounding number of 356580 queens in the soil.

Workers were found in 35 of the 50 random points and in 84 of the 200 cores (four per point), indicating a rather uniform presence over the whole occupied area. This last sample unit had a mean ± s.d of 6.28 ± 20.0 workers/core (range: 0–173), giving a rough estimate of 1.12 ± 10^8 workers in the soil, for the entire colony (workers foraging in trees are not included).

Brood distribution

Stones with eggs, small (= worker + male) cocoons, big (= queen) cocoons and queens were not more frequent in either zone (Table 2). Across the colony, 78.5% of all sampled stones had some brood stages present and 44% had brood stages but no queen was detected. Larvae were found to be statistically more abundant at the center of the colony ($P = 0.04$). A Bonferroni correction made all differences non-significant.

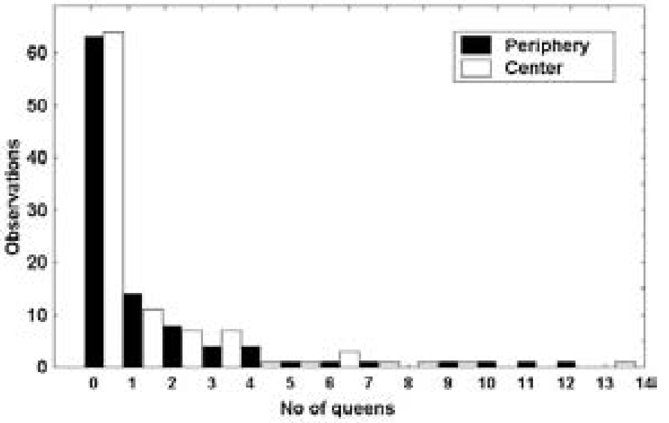

Figure 2. Frequency distribution of the number of queens of *Lasius neglectus* under stones with workers, at the central zone and the periphery of the colony. (n = 100 stones with workers per zone). Population of Seva (province of Barcelona, Spain). Mean ± s.d: 1.38 ± 2.87; n = 100; center. Mean ± s.d.: 1.18 ± 2.38; n = 100; periphery

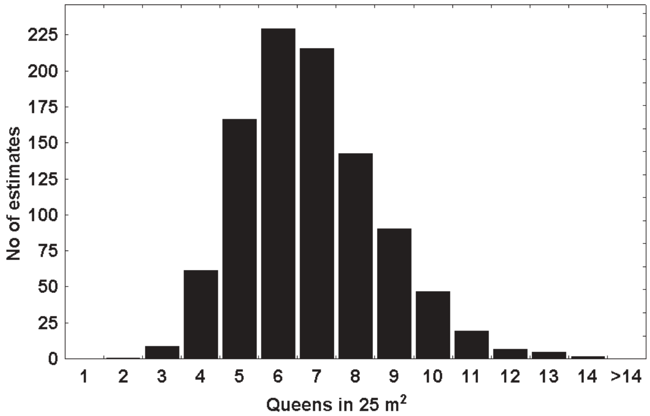

Figure 3. Distribution of bootstrappings (n = 1000) estimating the total number of queens in 25 m^2. Each entry is a replicate of a bootstrap of the mean number of queens per stone (based on 200 stones with workers) × the mean number of stones with workers (based on 45 squares of 25 m^2). Numbers along the X-axis indicate the upper class limits. Mean ± s.d.: 6.34 ± 1.79

Table 2. Distribution of the brood stages and queens. Figures indicate the number of stones at two colony zones (central part vs. periphery) (n = 100 stones/zone) with a given brood stage or queens (presence/absence). Binomial confidence limits calculated according to Rohlf and Sokal (1995; Table P). P indicates before Bonferroni corrections differences in a generalized linear model that included stone volume as continuous predictor (see text)

	Eggs	Larvae	Small pupae	Big pupae	Queens
Center	23	81	14	18	36
95% confidence limits	15–32	72–88	8–22	11–27	27–46
Periphery	23	64	10	21	37
95% confidence limits	15–32	54–73	5–17	14–30	28–47
P	0.81	0.04	0.22	0.60	0.36

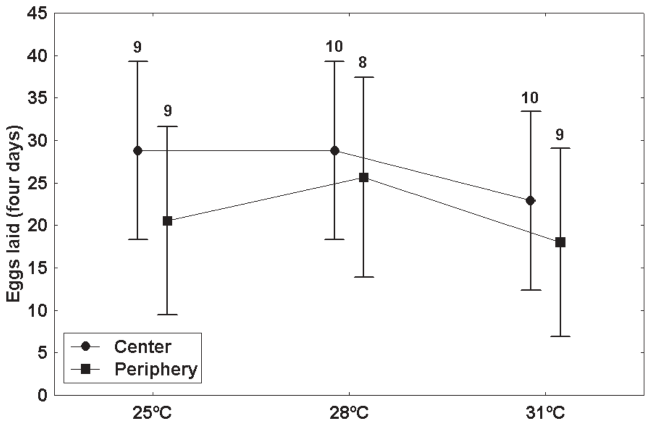

Figure 4. Egg laying rates of queens of *Lasius neglectus* from the center and the periphery of the colony set at three temperatures. Mean ± s.e. Figures on top indicate the number of replicates

Egg-laying rates and weight of queens

Four queens died during the observations and four did not lay any egg. For the remaining 52 queens neither zone ($F_{1,44}$ = 2.5; P = 0.11) nor temperature ($F_{2,44}$ = 0.10; P = 0.90) had any effect on the egg-laying rate during four days (Fig. 4). Queens laid a mean ± s.d. of 24.2 ± 16.3 eggs in four days. The fresh weight of queens from the center (mean ± s.d.; 8.21 ± 1.34 mg; n = 28) was not different (t-test; t = 0.15; P = 0.87) from the weight of peripheral queens (8.27 ± 1.41 mg; n = 24).

Discussion

The spatial pattern of the colony components (queens, workers, brood stages) has been rarely addressed in unicolonial ants. Recently, Ingram (2002 a, b) showed spatial flexibility in the social structure of the Argentine ant in several populations in Hawaii: queen number, nestmate relatedness and nest size change along a gradient of time and distance from the edge of the invasion. The ecological context, ephemeral nest sites, nest densities, competitive interactions or frequently disturbed habitats, could be responsible for that variation. That population, though, occupies a very large area (~330 ha) and was detected >30 years ago. In the colony of *Lasius neglectus* studied – occupying a much smaller area and detected >15 years ago – we did not find any asymmetry in queen distribution at the center or the periphery. The degree of polygyny, egg laying rates of queens from distinct zones and the presence/absence of brood stages (eggs to pupae) were also not detected to be different. Boomsma et al. (1990) detected some viscosity in the Budapest population of *L. neglectus*, corresponding to a significant worker relatedness of 0.14, although its significance depended on the scale of observation and density of sampling. Here we do not expect differences in brood density at the center or at the periphery unless some kind of differential transport of brood elements operates. Although it is possible that mated queens, old and young, undertake some limited horizontal displacements, the large colony size, over a big surface, allows for possible genetic structuring within the colony. One consequence of that eventual brood transfer would be to homogenize genetic relatedness. A genetic study at a microgeographical range of the Seva population is needed to address this question. The discrepancy with Ingram's studies could be explained by assuming that the whole area occupied by *L. neglectus* is already saturated immediately after expansion, that all nesting opportunities are now filled, and that only the opening of new habitats, following complete urbanization, allows colony expansion. Studies of other populations are needed to address plasticity in the social structure of *L. neglectus.*

The density of workers is not much different from what is known about other *Lasius* species (Table 3). Notwithstanding, since regions, biotopes and methods to estimate density are multifarious, direct comparisons are suspect. In particular, it has been shown that ant abundance increases with net primary productivity (Kaspari et al., 2000) and specific identity is also of major concern (Kaspari, 2001). This renders a useful comparison of these numbers problematic. It is worth noting that we found workers in 84 of the 200 soil cores, indicating a rather regular presence of workers over the whole surface occupied by the colony. Estimates of worker density in soil for other NE Spanish populations of *L. neglectus*, from different ecological conditions, are currently planned.

Based on relatedness deduced from allozyme variation, Boomsma et al. (1990; their Fig. 2) developed a model relating queen number and frequency of intranidal mating. They showed that 'nearly exclusive intranidal mating would require hundreds of coexisting nest queens'. Their figures

Table 3. Density of workers of several *Lasius* species (workers per m^2)

Ant species	Region	Density	Author
L. niger	Denmark	1590	Nielsen, 1974
L. niger	Schiermonikoog island	15–1339	Boomsma and Van Loon, 1982
L. psammophilus (as *L. alienus*)	Denmark	2100–5068	Nielsen and Jensen, 1975
L. alienus	Berkshire	1167–6398	Waloff and Blackith, 1962
L. flavus	England	420–1130	Odum and Pontin, 1961
L. flavus	Schiermonikoog island	3573–7448	Boomsma and Van Loon, 1982
L. flavus	Yorkshire	13	Pickles, 1940
L. neglectus	NE Spain	800	this work

even show a queen number of thousands when intranidal mating is absolute. Our data on queen numbers fit their prediction exactly. The colony studied is one large, single colony that occupies a sharply limited area (Fig. 1) and, within its limits it is difficult to determine individual nests. It is highly likely that the colony has grown during >15 years – inhabitants remember the ants from approximately the year 1985 on – without input gene flow. The closest other colony of this species (Taradell) is 9 km away as the crow flies and both are likely reproductively isolated, unless humans transport the ants, given the absence of nuptial flights in this species. Thus, we assume the growth of both supercolonies has been largely independent, although the possibility remains that both initiated from the same source. Molecular analyses should provide information on the actual relationships between these colonies. Gene flow remains an affair within the colony. Mapping of colony limits from 1999 to 2003 shows that expansion is an ongoing process and that it is strictly limited to the periphery. No buds have been detected further away in four years, except for an isolated transport of soil from within the colony that was immediately chemically treated and eliminated.

The invasion of new habitats is a complex process, with many factors involved (Walters and MacKay, 2003). In the population of *Lasius neglectus* studied it is highly likely that expansion is enhanced by the construction of premises in the urbanized lots. The process involves the following steps: i) clearing and elimination, usually by in situ burning of cut vegetation; a majority of trees are left untouched; ii) construction of buildings; iii) gardening introducing soil, planting of green and ornamental plants and bushes. Inhabitants provide sufficient summer irrigation to maintain vegetation – and the ants – in good health. If we consider that at the periphery, in the area that has not been invaded, 27 ant species have been detected (unpub. obs.), we hypothesize that these species have been displaced by urban development, leaving an enemy free space (Sax and Brown, 2000) for *L. neglectus*, which may then expand and colonize these new habitats. There, with little or no competition, ants can exploit local trees and their whole complement of aphids, as the studied population they do, from late April to late September, on a 24/7 schedule (unpub. obs.). An alternative hypothesis for the absence of differences between the center and the periphery in all measured variables could be that the resolution of the study is not small enough to detect any potential change in density at the edge, or that the edge, as defined here, was too large to detect any difference. A more detailed study of the edge might give important additional data. Also, the measuring of foraging distances from the edge might shed light on the mechanisms – if any – of competition between *L. neglectus* and native ants. If native ants suffer a lot from the interaction with *L. neglectus*, the ring created around the colony could be quickly inhabited by new bud nests.

The small number of soil cores that contained queens offers limited information and the estimate of the total queen number for the colony through soil cores is to be treated with caution. Notwithstanding, the order of magnitude for the total number of queens, is between 10^4 (estimates using sampling under stones) and 10^5 (estimates by soil cores), and this is consistent with the model derived from genetic studies on the Budapest colony (Boomsma et al., 1990; their Fig. 2) describing the relationship between the frequency of intranidal mating and the number of queens.

Acknowledgments

The authors thank Mauro Santos for help with the bootstrap analysis. We are deeply indebted to J. Boomsma, L. Passera and J. Pedersen for helpful comments, suggestions and language corrections on the first draft of this paper. Two anonymous referees patiently edited and helped to make the manuscript much more understandable. Their comments are heartily acknowledged. The SIMTRAD polished the final version. Josep Palmarola (Seva) and Josep Mª Vives (DARP, Generalitat de Catalunya) for logistic support. Funding was provided by DGESIC (REN 2000-0300-CO2/GLO).

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Article III

Effect of a *Lasius neglectus* (Hymenoptera, Formicidae) invasion on native ant communities in Northeast Spain.

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Submitted to Acta Oecologica

Abstract. Our study documents the negative effects of an invasion of a non-indigenous ant species, *Lasius neglectus,* on the local ant fauna in three Mediterranean suburban areas in Northeast Spain. Three sampling methods (baits, pitfall traps and beating trays) were used to sample abundance and diversity at the invaded and the non- invaded areas. The effect of the invasive ant *L. neglectus* was assessed at three levels: number of species, number of individuals and ant biomass. The diversity in native ant species in the invaded area by *L. neglectus* was significantly lower than the diversity in natives found in the non-invaded area. The number of ants collected from trees and pitfall traps (population A, Seva) was twenty-three times higher in the invaded than in the non-invaded area and consisted almost entirely of *L. neglectus* workers, and there were 3.8 times more ant species in the non-invaded area. Population B (Matadepera) presented a total of 3.5 times more abundance of ants in the invaded area and there were 2.8 more different species in the non-invaded area than in the invaded. Population C (Taradell) presented 53 times more ants' abundance also in the invaded area than in the non-invaded area and there was 2.5 times more ant species in the non-invaded than in the invaded area. Border areas between invaded and non-invaded areas presented transitional characteristics.

Keywords: biodiversity; tramp species; invasive ants; Spain; *Lasius neglectus*

Introduction

Biotic invaders are those not native species that establish a new range in which they proliferate, expand, and persist to the detriment of the natural environment (Mack *et al.*, 2000; McGlynn, 1999). Biotic invaders can in many cases inflict enormous environmental damage: animal invaders can cause extinction of vulnerable native species through predation, grazing, competition, and habitat alteration (Mack *et al.*, 2000). These alterations are collectively a threat to global biodiversity that is second in impact only to the direct destruction of habitat (Walker and Steffen, 1997). Invaders are collectively altering global ecosystem processes (Vitousek *et al.*, 1996; Holway et al, 2002), evolutionary processes (Hoffsmeister et al, 2005) and are by any criteria major agents of global change today (Lövei, 1997; Mack *et al.*, 2000).The importance of humans as dispersal agents of potential invaders via human transport and commerce has been widely reported (Lubin, 1984; Mack *et al.*, 2000; Wetterer *et al.*, 2006). When some of these species reach a vulnerable habitat, this potential becomes real and the invasion starts. The vacant niche hypothesis suggests that island communities and some other disturbed areas (fire, urbanisation, etc.) are relatively poor in view of numbers of native species and thus cannot provide “biological resistance” to non-indigenous species (Simberloff, 1986). Man-mediated disturbances of the habitat can produce this vacant niche effect highly susceptible to biological invasions, as many invasions are reflections of other changes rather than agents of change themselves (Vitousek *et al.*, 1996). Transferred ants that have widespread geographical distributions closely tied with urban areas and human activity (Passera, 1994), like *L. neglectus*, can become a real pest in the infested area (Espadaler et al. 2004).

On a population-level, invasions can have a severe impact on native species. Introduced invasive ants such as the Little red fire ant (*Wasmannia auropunctata*), the red fire ant (*Solenopsis invicta*), the Argentine ant (*Linepithema humile*) or the big-headed ant (*Pheidole megacephala*) devastate large fractions of native ant communities by aggression (references in Lubin, 1984; Porter et al., 1990; Cole et al., 1992; Williams, 1994; Human and Gordon, 1996 and 1999; Holway, 1999). However, not only direct competition can act as an exclusion mechanism of native ants. Other mechanisms of exclusion could be the exploitation of the same resources or the occupation of disturbed habitats before native species colonise. Some biological traits of the invasive species of ants seem to favour the expansion: intranidal mating, poligyny, and absence of intraespecific aggression (Passera, 1994; Espadaler & Rey, 2001) but no determinant biotic factors appear to stop it. The enemy release hypothesis (ERH) has also tried to explain the expansion of non-indigenous species but has been recently revised (Colautti et al., 2004) with highlighting contradictory evidence for ERH in community and biogeographical studies.

The aim of our study was to determine the effect of the current invasion of the polygynous invasive ant *Lasius neglectus* in North-east Spain upon the local native ant fauna in three study populations.

Materials and methods

Species and populations

Lasius neglectus was described from Budapest, Hungary (Van Loon *et al.* 1990). See http://www.creaf.uab.es/xeg/Lasius/ for the most recent update of its distribution. We have studied three populations located in the Northeast of Spain: A) Seva, Barcelona (41º48'N, 2º16'E). This huge colony occupies at present a continuous surface of >17 hectares in a suburban zone at 650 m a.s.l. The area is under a Mediterranean climate, with a wet spring and fall and a dry winter and summer. Mean annual temperature is 11.5 ºC and mean annual rainfall 775 mm. Houses with gardens are sparse and some contain exotic vegetation. Within the limits of the urbanization, the area occupied by *L. neglectus* is here defined as the invaded zone. The rest of the urbanisation does not contain *L. neglectus* and is defined as the non-invaded zone. B) Matadepera, Barcelona (41º37'N, 2º2'E). The ant colony is localized in a suburban zone, highly urbanised, at 570 m a.s.l. Mean annual temperature is 13.5 ºC and mean annual rainfall 775 mm. Vegetation consists of pine trees (*Pinus halepensis*), evergreen oaks (*Quercus ilex*), exotic bushes and trees within gardens. The area occupied by the colony has been estimated at 28 ha. C) Taradell (41º88'N, 2º30'E) Barcelona. The colony is located in the middle of an urbanised area at 650 m a.s.l. Mean annual temperature is 14ºC and mean annual rainfall is of 775 mm. This population occupies a smaller surface (6 ha) and the ants are more abundant along a seasonal stream.

Lasius neglectus has never been observed in natural habitats in Spain but only in disturbed areas from where the original vegetation has been cleared, substituted or heavily modified. Throughout the areas colonised, queens are found isolated or in small groups (up to 15 queens; Espadaler *et al*., 2004) under medium-sized stones. In four of the thirteen known Iberian populations polygyny has been ascertained. *Lasius neglectus* workers forage extensively on trees and on ground surface; thus, they can affect two distinct groups of ants: a) arboricolous species and b) soil nesting ants that can forage both on soil surface and trees or have a limited foraging to soil surface. The pest status of this ant was clear from the first reports (Boomsma *et al,* 1990; Espadaler, 1999; Tartally, 2000; Seifert, 2000). In Spain, affected inhabitants of private properties invaded by *Lasius neglectus* reported extensive presence inside homes, occupation of electrical conductions with damage to electrical outlets, phones and electric swimming pool engines. Outside, ant aphid-attendance, in enormous numbers has caused the death of some of the affected trees. As a consequence of public pressure, preliminary massive control essays have been conducted at the Seva population, with promising results (Rey & Espadaler, 2004).

Sampling methods

The dichotomy invaded vs. non-invaded was used initially to define the sampling space, and the criteria was by visual detection of *L. neglectus* ants on trees or under stones. However, on the first census we already detected the presence of abundant *L. neglectus* in a few of the traps, trees or baits initially allocated to the non-invaded area, depending on the population, and this observation was used to define a third status, a border area, in which *L. neglectus* and other species were present. The border was detected in populations A and B; in population C, no border was detected. This is a highly dynamic and narrow zone, at the periphery of the invaded area and in which no nesting of *L. neglectus* is implied.

At the population A, three different statuses were detected: an invaded area, a border area comprising the invasion front and a non-invaded area at least 100 m from the invasion front. Six censuses were performed, one each month during the activity season. The first census was made in April, at the beginning of the activity period of the colony of *Lasius neglectus* and the last census was in September 2000. Populations B and C were studied under the same scheme but censuses were conducted only for the three months of maximum activity: May, June and July, from year 2001. In population B a border area was detected.

For each census, three sampling methods were used to estimate the effect of *L. neglectus* on the diversity and abundance of ants present in those different statuses. Each sampling method was applied to different spatial points.

1) *Beating trays* were used to sample the ants nesting on trees or attending aphids. Because of the observed ant-aphid attendance by *L. neglectus*, we expected to test the effect of the pest over arboricolous ants or ants feeding on trees. Trees --evergreen oaks (*Quercus ilex*)-- were similar in size. In population A we marked five trees in the invaded area, five in the border and ten in the non-invaded area and each were checked during every census.

Population B had ten trees for the invaded area and nine for the non-invaded; population C had ten trees in both the invaded and non-invaded areas; no border area was detected for those populations with this sampling method. All trees were marked and checked at each census. A beating tray was held under the canopy, which was then struck two times in each of the four corners of the tree with a long stick. Ants were collected with a pooter and preserved in spirit. In the laboratory ants were counted and determined to species level.

2) *Baits* using canned tuna fish and sugared water on top of glass slides were placed in straight transects, with ten baits per transect. Baits were placed at three meters distance one from each other. Five transects (=fifty baits) were placed in each experimental area (invaded and non-invaded). Baits were left for an hour, and checked for the presence of epigaeic ants. The number of ants on top of the slide and their specific identity was recorded; the ants were kicked off at the same point. In population B a border area of coexistence with other species was detected, and two of the ten transects were within it. In populations A and C no border was detected using baits.

3) *Pitfall traps.* Plastic cylinders (21mm wide and 12 cm long) were buried flush with the soil surface with as little disturbance to the surrounding soil and litter as possible and to avoid the digging-in effect that can inflate the number of ants captured (Greenslade, 1973; Majer, 1978). The cylinders were left closed in the ground for fifteen days before the beginning of the experiment. Pitfall traps consisted of 15 mm wide (internal diameter) and 20 mm at the mouth, 12 ml plastic vials. At each sampling point we placed the pitfall traps in a pattern resembling the five on a dice, filling them up to 6 ml with soaped water to reduce water surface tension and avoid insect escape. Each trap was placed three meters distant from the others. Vials were inserted inside the cylinders so that the lip of the vial was flush with the surface. In population A we set three sampling points in the invaded zone, two in the border and five in the non-invaded zone. In population B four points were in the invaded area, two in

the border and four in the non-invaded area. In population C no border area was detected, so five points were placed in the invaded area and five in the non-invaded area. Traps were left for 48 hours in the field and collected afterwards. Samples were transferred immediately to other vials filled with alcohol to preserve them prior to identification. All ants were counted and determined to species level. Because of the huge number of ants collected in some of the vials from the invaded zone, numbers of ants were estimated by counting a sub-sample of two hundred ants. Then, both the sample and sub-sample were dried at 60º for 24 h until constant weight and the number of ants of the original sample was inferred using the dry weight obtained.

Ant biomass

Specific ant dry biomass was obtained through the relation between specific head length (mm) and dry biomass (mg) that was shown by Kaspari & Weiser (1999) to be very high in the subfamilies Myrmicinae ($r^2 = 0.93$) and Dolichoderinae ($r^2 = 0.76$) and by Espadaler & Gómez (2001) for the ants belonging in the subfamily Formicinae ($r^2 = 0.96$). Head lengths were obtained from a data base for Iberian ants (Gómez & Espadaler, 2001). For Myrmicinae species, ant dry mass = $(5.1475 \times 10^{-1})\ HL^{3.361}$; in Dolichoderinae ants, dry mass = $(3.870 \times 10^{-1})\ HL^{2.621}$; in Formicinae ants, dry mass = $(4.101 \times 10^{-1})\ HL^{2.681}$.

Statistical analysis

Data from the three populations were analysed separately. A repeated-measures analysis of variance was used to compare three response variables: abundance of ants, number of ant species and ant biomass. As variances of the data set for abundance of ants were heterogeneous we applied a ($\log_{10}$ x+1) transformation that resulted in homogeneity of variances and normalised residuals (visual inspection). Data concerning the number of species found was distributed normally and directly analysed. Data on total ant biomass was $\log_{10} x+1$ transformed in order to normalise data. Data in text are presented as non-transformed means ± s.d.

Data from beating trays and pitfall traps. Units analysed were as per tree or per sampling point. The between-subjects factors were status (invaded, border, non-invaded in A; invaded, non-invaded in B and C), and the within-subjects factor was census (3 for populations B and C, 6 for population A). In population B we didn't analyze border data for traps because no border trees were found. To control for different efficiencies of sampling methods (pitfall traps, beating trays) they were nested in status and not explored further.

Data from baits. Units analysed were as per transect. Those data were analysed with a single way ANOVA with status having only two levels (invaded, non-invaded) in population A and C and three (invaded, non-invaded and border) in population B, and census (3 or 6 as noted above) as a repeated measures factor.

Biodiversity measurement

As a descriptive measure of total ant species diversity within population, status and common sampling method we used the Shannon Diversity Index (Magurran, 1988). In order to avoid the inflating recruitment effect, a square root transformation was applied over the original data in ant abundance. We used only data from common sampling methods for different status within populations. In the A population only data from traps and trays were used because results of common sampling methods for all three status (invaded, border and non-invaded) were available. In population B data from baits and traps were used as common sampling methods for all three status (invaded, border and non-invaded) and all data were used for population C and only two status (Invaded, non-invaded).

Results

Pitfall traps and *beating trays*

1) Ant abundance: In all of the populations studied the presence of *Lasius neglectus* dramatically reduced the probability to find native ants in trees and soil surface situated in the invaded area (Table I, II and III, Baits, Trays and Traps). Native epigaeic ants and arboricolous ants failed to coexist with *L. neglectus* in the invaded areas. In population A two queens belonging to two hypogaeic ant species were found in the invaded area as well as some strays. In this population the status (MANOVA, $F_{2,\ 24}$ =78.89; P<0.001), census (MANOVA, $F_{5,\ 120}$ = 0.18; P<0.001) and their interaction (MANOVA, $F_{10,\ 120}$ = 2.80; P=0.003) were significant factors. Population B and C followed the same pattern for the status factor (MANOVA, population B: $F_{1,23}$=18.22; P=0.0003, population C: $F_{1,26}$=81.153; P<0.001) and census (MANOVA, population B: $F_{2,\ 46}$ = 21.42; P<0.001; population C: MANOVA, $F_{2,\ 52}$ = 3.60; P=0.03). The interaction of both factors was not significant. The maximum monthly mean number of ants collected with the pitfall traps in the invaded zone of the population A was of 22298 ± 8761 per sampling point in the census of June; for the non invaded area of the same population numbers are much smaller: a maximum of 1110 ± 2449 ants (census of August). In the same population, the maximum monthly mean number of ants collected with pitfall traps in the border zone was of 511 ± 627 and most of them (98.3%) were *L. neglectus* workers. The number of ants collected from trees and pitfall traps for all censuses in population A was twenty-three times higher in the invaded than in the non-invaded area and consisted almost entirely of *L. neglectus* workers. Population B presented a total of 3.5 times more abundance of ants in the invaded areas and population C had 53 times more ants' abundance also in the invaded areas than in the non-invaded areas (see Tables I-III). Two queens, one of *Lasius mixtus* and one of *Lasius myops* were trapped in the invaded

area; we ignore if they represent hipogaeic ant species that can coexist with *L. neglectus* or if they reached the traps on the wing.

2) Ant species: In population A the number of species found was different between the three different status described above (MANOVA, $F_{2,24}$=7.00; P=0.004). However the border area contained a number of species that did not differ statistically from the invaded area (Scheffé test; P=0.093) or from the uninvaded area (Scheffé test; P=0.463). So, differences in number of species were due to invaded and non-invaded areas. The census factor was significant (MANOVA, $F_{5,120}$=14.40; P<0.001) and the interaction status-census was also significant ((MANOVA, $F_{10,120}$=7.60; P<0.001).

The number of species per different months (MANOVA, $F_{10,120}$ =7.60; P<0,001) followed a similar pattern than the number of ants collected, where summer is always the most diverse in terms of ant species and the abundance of ants. In populations B and C the main effect was also due to status. In population B the number of ant species found was significantly different in areas infested or non-infested (MANOVA, $F_{2,23}$=39.03; P<0.001). Census time was also significant (MANOVA, $F_{2,46}$=5.59; P=0.006). The interaction status-census was not significant. Population C also had differences in the two status level studied (MANOVA, $F_{1,26}$=31.78; P<0.001). Census-time was not significant in this population neither was the interaction status-census. In short in population A there were 3.8 times more ant species in the non-invaded area (see Table I). In population B there were 2.8 more different species in the non-invaded area than in the invaded. In population C there was 2.5 times more species richness in the non-invaded than in the invaded area (see Table 2 and 3).

3) Ant biomass: Data on ant biomass for the three populations was also studied. The total ant biomass per sampling point in the invaded area of population A was statistically different (MANOVA, $F_{2,24}$=48.9; P<0.001) and greater than in the border and non invaded areas. Differences were due to the huge numbers of *L.neglectus* workers collected in the

invaded area during the activity period. No differences were found in population B (ANOVA, $F_{1,23}$=0,92; P=2.001) with border areas not included, but significant statistical differences were detected in population C (ANOVA, $F_{1,26}$=40.9; P<0.001), where the number of ants was also much higher in the invaded area (Table IV).

Baits

1) Ant abundance: Results from baits also indicate a major influence of the presence of *L. neglectus*. In population A, both factors (status and census) were statistically significant for abundance of ant workers. The invaded area in the population of Seva contained a higher number of ant workers than non-invaded sites (ANOVA, $F_{1,8}$=7,84; P<0,0232). Differences in the abundance of ants for the different census were also found (ANOVA, $F_{5,40}$=8,92; P<0,001). The number of ants recorded in the invaded area with this sampling method was lower than with beating trays or pitfall traps, with recorded values ranging from a maximum of 313 ± 155 ants per census (May) to a minimum of 37 ± 30 ants in October. The maximum number of ants obtained from the non-invaded zone was 246 ± 133 in July. Therefore maximum mean values of ant worker abundance did not differ so much between the invaded and non-invaded areas. However, exclusively *L. neglectus* workers were found in the invaded zone and only two epigaeic ant workers (*Formica rufibarbis*) were recorded in areas invaded and these, given their low number, were probably strays. Direct aggression for the food provided in baits has never been observed but transients passing through the middle of the colony have been observed to be directly attacked by *L. neglectus* workers in large numbers, causing the escape of the foreign ant. Neither in population B nor C were there differences in the number of ants in baits found for the different studied areas.

2) Ant species: In population A the same effects were obtained for the richness of ant species, that differed significantly between invaded and non-invaded areas (ANOVA, $F_{1,8}$=30,75; P<0,001). Because we used the number of different species of ants found in each

transect for each one of the six censuses performed, and not the cumulative number of different ant species found, differences between invaded and non-invaded areas were not so evident but still statistically significant. Bait sampling gave us the maximum number of ant species diversity from the three sampling methods used in this study.

In population B and c significant statistical differences were found for the number of species found in baits for both factors: status and census. Population B and C respectively contained different numbers of species for the different areas studied (ANOVA, $F_{2,7}$=10.476; P=0.007 and ANOVA, $F_{1,8}$=17.956; P=0.002) and also for each census performed (ANOVA, $F_{2,14}$=6.382; P=0.01 and ANOVA, $F_{2,16}$=5.032; P=0.02).

Thirty ant species have been found in the whole A area studied (Table I, twenty-nine are native and only one is a non indigenous species (NIS): *L.neglectus*). Probably none of these species could establish inside the invaded area and only transients could be found, but for a few workers of hypogaeic species (*Solenopsis* sp.). The same occurs in B, where the population of native ants seems to be less abundant (a total of 18 native different species found and two NIS: *L. neglectus* and *L. humile*; see Table II) than in populations A or C. In population C, the total number of different ant species found was of 27 (Table II), and the only NIS was *L. neglectus*.

Shannon diversity index

Our aim was to compare within populations the effects of *L. neglectus* invasion on indigenous ant fauna. Comparing the different invasion status within the same population we gain a better idea of biodiversity loss. Invaded areas show a smaller Shannon index (Table 5) than non-invaded areas. In population A the border had an intermediate value, whereas in population B the border was of similar magnitude than the non-invaded area. No comparisons between the three localities studied have been carried out due to different abiotic conditions (mainly altitude, max. and min. temperatures and water availability).

Discussion

Here we present the first quantitative evidence of a *Lasius neglectus* invasion on native ant biota. Local ant species diversity, number and biomass are considerably affected. Two of the three populations studied presented the same distribution pattern for the *Lasius neglectus* pest: An invaded area where no coexistence between native epigaeic ants and *L. neglectus* was found; a border area where *L. neglectus* was not exclusive and coexistence with other species was common, and a non-invaded area where only native ant species were found. In population C the *L. neglectus* colony was only present within the urbanised area and no border area was detected probably due to the preserved natural surroundings around the colony.

Similar numbers in species richness were found for all populations in trays and trees being always the non-invaded areas the richest in diversity of species. In population A there were 3.8 times more ant species in the non-invaded area (see Table I). In population B there were 2.8 more different species in the non-invaded area than in the invaded. In population C there was 2.5 times more species richness in the non-invaded than in the invaded area (see Tables II and III).

The invaded areas had, invariably, a colony core where the number of *L. neglectus* was huge. Some transient ants, when crossing the centre of the colonies, were attacked by *L. neglectus* workers. No aggression was found between *L. neglectus* workers from the same super-colony even when distances were considerable far (*unpub. obs*.). Inter-nest aggression from different populations is currently being tested but it seems that as in other invasive ants (Giraud et al., 2002), is very low or completely inexistent. It remains to be seen if other populations of *L. neglectus* detected behave in the same manner or if there are two or more super-colonies existing. *L. neglectus* is highly aggressive against other *Lasius* ant species coexisting at the edges of the supercolony (Cremer et al., 2006). However, there is a possible coexistence with hypogaeic ants, not checked yet, as they require a specific sampling procedure. In the invaded areas the number of ant species found was always very low, usually only one: *L. neglectus* but the amount of ants recorded was very high and all pertained to the same invasive species.

The coexistence area was called the border area. As explained in the sampling methods section, this was an *a posteriori* definition as, previously to the allocation of the traps and baits, we had not considered this situation. The number of ant species was higher than in the invaded areas but never as diverse as the non-invaded areas. The same occurred with the number of ants recorded: usually quite high but never reaching the levels of the invaded areas. These numbers were usually due to the presence of *L. neglectus* ants however, no nesting of *L. neglectus* was required. Therefore, these border areas act as a temporal diffuse edge with a possible buffer effect. In other invasive ants such as Argentine ants *(Linepithema humile)* this border area was also described as ephemeral mixed communities of coexistence between Argentine ants and other native ant species (Human and Gordon, 1996). After some time, many of these communities are reduced to a single species: the Argentine ant. This displacement was due to direct aggressive interactions and reduced foraging success by native ants. *L. neglectus* shows very low levels of intraspecific aggression within the same supercolony (aggression tests; *unpub. obs.*) However it shows high interspecific aggression (Cremer et al., 2006). The supercolony contains such a high numbers of ants that it likely prevents any intrusive workers and, specially, of founding queens from other ant species. So, similar mechanisms appear to be acting with *L. neglectus* expansion within the border areas turning into invaded areas and moving along the non-invaded areas although this needs documentation (see in http://www.creaf.uab.es/xeg/Lasius/ expansion process of population A). No biotic mechanisms are likely to stop the expansion of the ant in disturbed sites at the unset of colony expansion. However a high number of native ants and a natural habitat surrounding the edges, may maintain the border area for longer periods of time, as it has been described for argentine ants (Holway, 1998a).

The maximum level of biodiversity of ants was found in the non-invaded areas. Some of them were quite natural with native vegetation and little human disturbance. Most of these areas were cleared of bushes and fires were performed in order to “clean” the area. Not all three populations contained the same number of native species due to the diverse geographical areas (Table I, II and III).

The total number of ant workers collected was always lower in the non-invaded than in the invaded areas, as well as the total ant biomass. It was always caused by the huge number of *L.neglectus* workers and not due to size (*L. neglectus* is relatively small in comparison with some much larger ant species).

The expansion mechanisms of the pest are still unknown and not directly tested but urbanisation processes and habitat fragmentation (Suarez et al., 1998) seem to favour the expansion of this pest ant. The vacant niche hypothesis (Simberloff, 1986) can also explain the rapid expansion of this pest in perturbed areas. In the three studied populations the urbanisation process of the area has provoked, in our opinion, this vacant niche effect that has been used by *Lasius neglectus* to expand and displace the native ant fauna of the area initiated from an invasion focal point. The surrounding non-urbanized areas remain with semi-natural vegetation and have not been as of yet invaded.

At the edge of invasion fronts, direct interactions and reduced foraging success from native ants due to huge numbers of *L. neglectus* workers can cause the emigration of other native ant species present as it occurs with Argentine ants (Holway, 1999). Its large colony size, due to their reproductive strategy, implying intranidal mating and poliginy (Espadaler and Rey, 2001) may be the most important competitive factor as it has been observed for other invasive ants such as *Solenopsis invicta* (Morrison, 2000) or *Linepithema humile* (Holway, 1998b; Aron, 2001; Suarez et al., 1998).

The biological characteristics of *L. neglectus* favour the expansion of the ant. Intranidal mating increases mating success and reduces the predation risk associated to mating flights. Although daily egg lying rates of >1 year old queens are not high (6 eggs/day), the huge number of queens in the colony (>35000; possibly many more; Espadaler *et al.* 2004) implies the production of >200000 eggs per day and thousands of workers are produced during each reproductive period. Two cohorts of workers are produced each year during the activity period, which means thousands of ants per nest and millions per super colony. Also the possibility of independent colony founding in the laboratory although not proved in the field, may avoid the complete eradication of the pest. This independent colony founding could be another strategy of survival in extreme conditions.

It remains to be seen if other non-ant arthropod communities (Rey and Espadaler, in prep.) and local ecological processes as pollination (Blancafort and Gómez, 2005) or seed dispersal (Gómez and Oliveras, 2003) are affected by the presence of *Lasius neglectus* colonies. The only biotic limitation would be the absence of aphids but because they are widely distributed and abundant it never represented a constraint in any of the studied areas. If insecticide aphid treatments are performed in infested areas, ants can be affected by food deprivation (Rey and Espadaler, 2004). No apparent direct predators have been seen, only indirect observations (soil scratches and bird droppings) of bird predation have been detected (*unpub. obs*.).

Abiotic factors could be a limitation for *L. neglectus*. A limiting factor would be the humidity level or water supply. The success of other invasive species such as *L. humile* was correlated strongly and positively with water availability and soil moisture (Holway, 1998a; Holway and Suarez, 2006) and only abiotic factors seemed to determine the rate of invasion of argentine ants. So access to water would be the most important abiotic factor. All three studied populations have got temporal streams and gardens with a continuous water supply by watering regimes.

However the presence of *L. neglectus* does not always imply an invasive problem. *L. neglectus* has been detected in many European locations coexisting with other ant species and not yet determined to be an invasive ant. The main question is: Why do certain colonies of *L.neglectus* become a pest? Are these populations in different stages of establishment or invasiveness? (Sakai et al. 2001). Here we have detected variation in the degree of effects upon local ant fauna: in population A and C are obvious, but less apparent in population B. Further studies are required to detect direct biotic or abiotic constraints that avoid or limit the expansion to an invasive level, affecting biodiversity as in our study populations. There is lack of biogeographical studies on native *L. neglectus* populations so nothing can be said about the enemy release hypothesis (see Colautti et al., 2004). Data concerning reproductive biology in their native habitats are badly needed and no comparison with introduced populations can be made. Other communities of arthropods may be affected by the presence of *L. neglectus* ant colonies (*in prep.*) and this further demonstrates major biotic effects on the invaded areas.

In conclusion, in terms of the ecological impact of the garden ant, *Lasius negletus* we detect a dramatic biodiversity loss of native ant species where *L. neglectus* is present, acting as an invasive ant. Further studies are required to characterize all the generalized steps (Sakai et al. 2001) concerning the evolution of *Lasius neglectus* as an invasive ant.

Acknowledgments

To J. M. Vives and M. Rojo for logistic support; this work is supported by a DGESIC (REN2000-0300-C03-01) and MCYT (CGL2004-05240-C02) grant. To Dr. Simon Mackenzie for suggestions and language correction.

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Table I. Species of ants captured with three sampling methods (baits, pitfall traps, beating trays) in Seva, population A. Each table entry is the cumulative numerical abundance of each species for the three sampling methods in each zone and for the six censuses. (Q): queen. After specific name, in parentheses, dry mass in mg.

Species/**Status**	**Invaded**			**Border**		**Non invaded**		
Sampling method	Baits	Traps	Trays	Traps	Trays	Baits	Traps	Trays
Aphaenogaster gibbosa (0.95)	-	-	-	-	-	-	139	-
Camponotus aethiops (3.27)	-	-	-	-	-	-	-	1
Camponotus cruentatus (3.91)	-	-	-	-	-	18	2	7
Camponotus pilicornis (4.76)	-	-	-	-	-	-	-	11
Camponotus truncatus (0.43)	-	-	-	-	8	-	-	6
Crematogaster scutellaris (0.53)	-	-	-	-	-	-	-	5
Formica gagates (0.88)	1	-	-	2	18	66	312	487
Formica gerardi (0.93)	-	-	-	-	-	4	-	-
Formica rufibarbis (1.24)	1	1	-	-	1	14	-	-
Lasius cinereus (0.32)	-	-	-	-	-	-	6	133
Lasius grandis (0.45)	-	-	-	-	1	256	-	-
Lasius mixtus (Q*)*	-	1	-	-	-	-	-	-
Lasius myops (Q)	-	1	-	-	-	-	1	-
Lasius neglectus (0.22)	5132	157975	5790	1790	2578	-	-	-
Temnothorax unifasciatus (0.13)	-	-	-	-	-	4	-	-
Temnothorax kraussei (0.15)	-	-	-	-	1	-	-	2
Temnothorax niger (0.09)	-	-	-	-	-	2	-	-
Temnothorax nylanderi (0.15)	-	-	-	-	-	-	7	-
Temnothorax parvulus (0.10)	-	-	-	3	-	-	-	-
Temnothorax rabaudi (0.15)	-	-	-	-	1	-	-	-
Temnothorax racovitzae (0.09)	-	-	-	-	-	1	-	-
Messor barbarus (5.65)	-	-	-	-	-	1	-	-
Messor capitatus (3.95)	-	-	-	-	-	5	1	-
Messor structor (2.82)	-	-	-	-	-	2	-	-
Myrmica spinosior (0,96)	-	-	-	-	-	299	-	-
Pheidole pallidula (0.45)	-	-	-	-	-	297	5566	55
Plagiolepis pygmaea (0.03)	-	1	1	20	7	103	42	4
Proformica nasuta (0.48)	-	-	-	-	-	-	2	-
Solenopsis sp. (0.07)	-	-	-	1	-	-	-	-
Tapinoma ambiguum (0.21)	-	1	-	4	-	20	27	-
Tapinoma nigerrimum (0.34)	-	-	-	-	-	602	1	109
Tetramorium impurum (0.29)	-	-	-	-	-	438	53	-
Total individuals	5134	157980	5791	1820	2615	2132	6159	820
Total species	3	6	2	6	8	17	13	11

Table II. Species of ants captured with three sampling methods (baits, pitfall traps, beating trays) in Matadepera, population B. Each table entry is the cumulative numerical abundance of each species for the three sampling methods in each zone and for the three censuses. After specific name, in parentheses, dry mass in mg.

Species/**Status**		**Invaded**		**Border**		**Non**	**invaded**	
Sampling method	Baits	Traps	Trays	Baits	Traps	Baits	Traps	Trays
Aphaenogaster gibbosa (0.95)	-	-	-	-	-	213	10	-
Camponotus cruentatus (3.91)	2	-	-	22	-	42	-	35
Camponotus pilicornis (4.76)	-	-	-	-	2	-	-	6
Camponotus truncatus (0.43)	-	-	2	-	-	-	-	-
Crematogaster scutellaris (0.53)	-	-	-	41	12	102	15	8
Formica gagates (0.88)	-	-	-	9	11	-	-	5
Formica gerardi (0.93)	-	-	3	2	5	-	19	23
Formica rufibarbis (1.24)	-	-	-	2	-	-	-	-
Formica subrufa (1.22)	-	-	-		-	2	-	-
Lasius grandis (0.45)	-	-	-	-	-	-	-	14
Lasius neglectus (0.22)	4603	10148	2161	166	1884	-	1	-
Temnothorax parvulus (0.10)	-	-	-	-	1	-	15	-
Temnothorax recedens (0.12)	-	-	-	-	-	-	2	-
Linepithema humile (0.11)	-	-	-	-	-	10	-	-
Myrmica spinosior (0.96)	-	-	-	56	145	12	415	-
Pheidole pallidula (0.45)	-	17	-	19	11	2544	2788	1
Plagiolepis pygmaea (0.03)	214	9	-	95	20	61	105	5
Solenopsis fugax (0.07)	-	-	-	-	1	-	2	-
Tapinoma ambiguum (0.21)	-	-	-	47	-	-	-	-
Tapinoma nigerrimum (0.34)	-	-	-	-	-	48	-	-
Total individuals	4819	10174	2166	459	2092	3034	3372	97
Total species	3	3	3	10	10	9	10	8

Table III. Species of ants captured with three sampling methods (baits, pitfall traps, beating trays) in Taradell, population C. Each table entry is the cumulative numerical abundance of each species for the three sampling methods in each zone and for the three censuses. After specific name, in parentheses, dry mass in mg.

Species/**Status**	**Invaded**			**Non invaded**		
Sampling method	Baits	Traps	Trays	Baits	Traps	Trays
Aphaenogaster gibbosa (0.95)	-	-	-	-	7	-
Camponotus aethiops (3.27)	-	-	1	5	2	5
Camponotus cruentatus (3.91)	-	-	-	10	-	5
Camponotus piceus (0.56)	-	-	-	2	-	7
Camponotus pilicornis (4.76)	-	-	1	-	-	-
Camponotus truncatus (0.43)	-	-	11	-	-	3
Formica gagates (0.88)	-	-	10	82	31	65
Formica gerardi (0.93)	-	2	-	23	40	15
Formica pratensis (2.12)	-	-	-	-	-	65
Formica rufibarbis (1.24)	-	-	-	108	6	21
Lasius cinereus (0.32)	-	-	-	311	-	-
Lasius emarginatus (0.41)	-	-	-	2	-	43
Lasius grandis (0.45)	-	-	-	-	302	25
Lasius neglectus (0.22)	4515	58853	8064	-	-	-
Temnothorax nylanderi (0.15)	-	-	2	-	2	-
Temnothorax parvulus (0.10)	-	-	-	-	3	-
Temnothorax rabaudi (0.15)	-	-	-	-	-	1
Messor capitatus (3.95)	-	-	-	23	2	-
Myrmica spinosiori (0,96)	-	16	-	56	268	-
Myrmica specioides (0.59)	-	-	-	73	41	-
Mymecina graminicola (0.22)	-	-	-	-	2	-
Pheidole pallidula (0.45)	-	20	1	366	87	2
Plagiolepis pygmaea (0.03)	11	-	-	104	21	25
Solenopsis fugax. cfr. (0.07)	-	-	-	-	1	-
Tapinoma ambiguum (0.21)	-	-	-	235	14	-
Tapinoma nigerrimum (0.34)	-	-	-	149	83	3
Tetramorium impurum cfr. (0.29)	-	-	-	1140	56	-
Total individuals	4526	58891	8090	2689	968	285
Total species	2	4	6	16	18	14

Table IV. Total ant biomass (mean dry mass ± s.d. (mg)) per month, per sampling point and status.

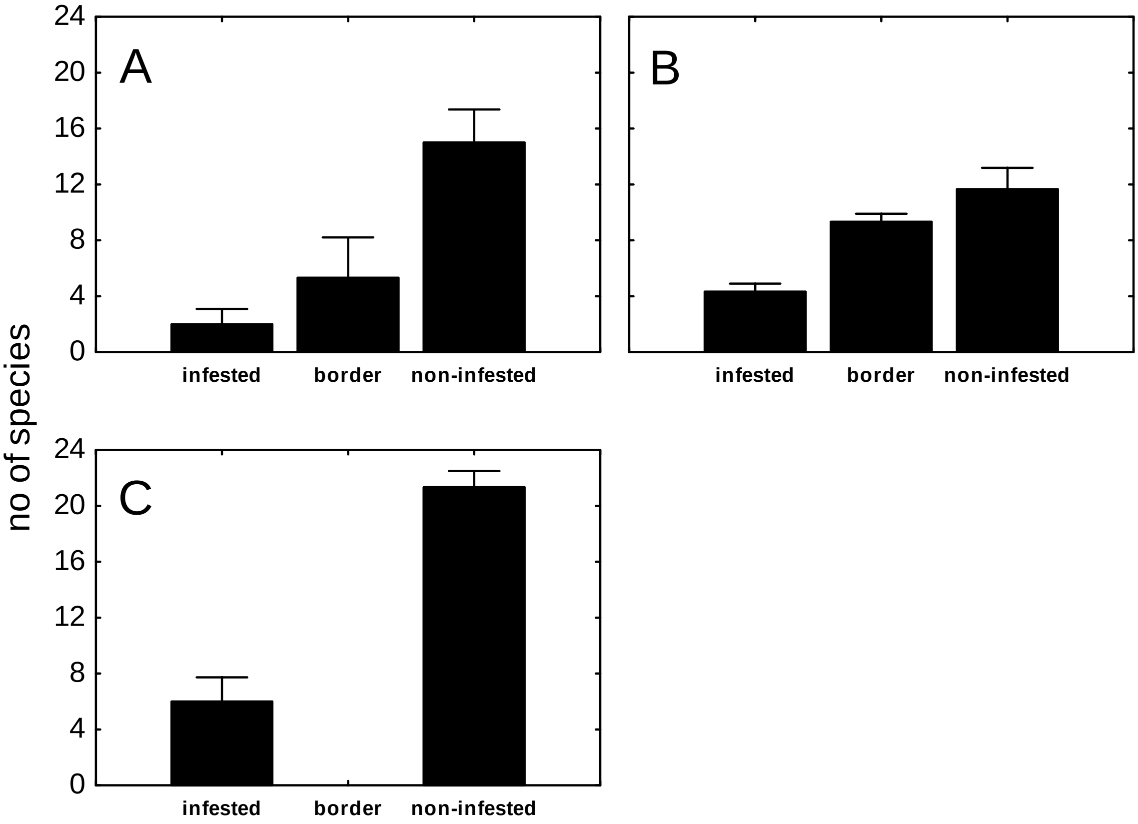
ANT BIOMASS(mg)/ AREA STUDIED	**A**	**B**	**C**
Invaded	3820±1811	186±216	879±768
Border	67±76	98±127	----
Non-invaded	206±607	143±333	40±55

Ants were collected with pitfall traps (n=5) at each sampling point. Data for three censuses (May, June, July) of the three populations studied.

Table V. Shannon diversity index results for all data collected for the three different localities studied with common sampling methods within populations.

POPULATION STUDIED/STATUS	**Invaded**	**Border**	**Non-invaded**
A. Muntanyà (Traps & Trays)	0,091	1,062	1,984
B. Matadepera (Traps & Baits)	0,518	1,941	1,870
C. Taradell (Traps,Baits&Trays)	0,443	-------	2,864

Fig 1. Differences in the total number of ant species (mean±s.d.) found between invaded areas, border and non-invaded zones in the tree populations studied. (Population A: Seva; B: Matadepera; C: Taradell) for the total number of census and sampling methods.



Article IV

Invasion of the garden ant, *Lasius neglectus* (Hymenoptera, Formicidae), in North-East Spain: impact on non- ant arthropod communities

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Running head: effect of *Lasius neglectus* on arthropods.

Submitted to Biological Invasions

Abstract

Our study documents the effects of the invasive garden ant (*Lasius neglectus*) on non-ant arthropod communities over three Mediterranean populations located at the North-East region of Spain (Population A: Seva; B: Matadepera and C: Taradell). All areas have been invaded by colonies of *L. neglectus* with different degrees of invasiveness. Two sampling methods were used: beating trays for arboreal arthropods and pitfall traps for soil surface arthropods. We compared arthropod abundance and order richness from invaded and non-invaded adjacent areas. Ant effects are variable depending on arthropod identity and the population studied. Results indicate: a) an enhancement of canopy arthropods, these being due to Homoptera, the unique order positively favored by the ant invasion; b) an impoverishment of the number of soil arthropods in invaded populations; c) an impoverishment in the number of arthropod orders both in canopies and surface in invaded areas. Insects, such as Psocoptera, showed significant low levels of abundance in invaded areas. Predators such as Araneida and other Hymenoptera were also significantly less abundant in invaded areas. Other orders negatively affected by the presence of the ant were Collembola and Diptera probably due to direct predation of the eggs, juvenile or adult forms by *L. neglectus*.

Key Words: ant abundance, arthropods, biological invasion, biodiversity, invasive ants, *Lasius neglectus,* polygyne ants, species richness, Spain, tramp species.

Introduction

The effects of invasive ant colonies on native ant communities have been widely studied (Williams 1994; Human and Gordon 1996; Holway 1998; Suarez et al. 1998; Morrison 2002; Walters 2006; Rey and Espadaler, submitted) and most of them observed striking declines in the abundance and species richness of these native communities. *Lasius neglectus* ants have been described as a tramp species, sometimes invasive. It displaces other coexisting native ant species and dramatically reduces ant biodiversity (Boomsma et al. 1990; Tartally 2000; Espadaler 1999; Espadaler and Rey 2001; Rey and Espadaler, submitted).

However, the effects of ant invasions on non-ant arthropod communities remain poorly studied and published results are variable. Human and Gordon (1997) did a review of past studies, comparing their study with four others on the effects of invasive ants on invertebrate communities. They found a positive correlation between invasive aphid-tending ants and Homoptera. Most of the studies resulted in negative effects over the native arthropod fauna (Haines and Haines 1978; Lubin 1984; Porter and Savignano 1990; Human and Gordon 1997). Recent studies show somewhat variable results: on one hand some studies find no effects on arthropod abundance and richness (Heterick 1997; Holway 1998; Berghoff et al. 2003; Gerlach 2004) whereas on the other hand significant negative effects are reported (James et al. 1999; Hoffman et al. 1999; Morrison 2002; Walters 2006) and some of the effects are considered a serious potential threat to the native invertebrate fauna.

Lasius neglectus is an aphid-tending ant, highly dependent on aphid honeydew although in some instances, mainly in spring, individuals are seen carrying small prey (Collembola, Psocoptera). Nests are active from April to late October (depending on the year climatic conditions) with foraging ants active throughout the entire day (24 hours) (unpubl. data).

Preliminary quantitative measures indicate that *L. neglectus* ants can extract up to 2.98 Kg honeydew per tree per year on Holm oak (*Quercus ilex*) (Paris 2005) and as much as 950 cc honeydew per month on poplar trees (*Populus nigra*) (http://www.creaf.uab.es/xeg/Lasius/). So, we would expect as well a remarkable influence on invertebrate population composition and abundance in invaded areas. From our data we confirm a substantial decline of native ant species within the limits of the *L. neglectus* supercolonies (Rey and Espadaler, submitted).

Here we report upon the composition and abundance of non-ant arthropod communities in three supercolonies of *L. neglectus* compared with adjacent non-invaded areas. From previous studies we expected to find different numbers of total non-ant arthropod abundances and richness in both areas (invaded vs. non-invaded).

Methods

Populations Studied

We conducted our study at three different locations in the Northeast of Spain, all of them with an invasive population of *Lasius neglectus*. One location was el Muntanyà, in Seva (41º48’N, 2º16’E; Population A), an urbanized area surrounded by natural Mediterranean vegetation, mainly oak woodland and some pine trees (*Pinus halepensis*). This area has been heavily invaded by *Lasius neglectus*. The colony occupies at present a continuous surface of >17 hectares and has been the most intensively studied population. The second location was Matadepera (41º37’N, 2º2’E; Population B), also an urbanized area close to heavily populated urban areas but with semi-natural Mediterranean vegetation surrounding it. The third location was in Taradell (41º88’N, 2º30’E; Population C). This was also an urbanized area surrounded by some agricultural land, natural oak woods and riparian vegetation. All three populations are located between 500 and 650m a.s.l, with main annual temperatures ranging from 11.5ºC to 14ºC and an exact mean annual rainfall of 775mm.

Non-ant arthropod communities' surveys

To sample soil arthropod communities we set up an unbaited pitfall trapping survey. To sample canopy arthropods we used beating trays. Those surveys were done both at invaded and non-invaded areas for the populations studied. We specified different degrees of invasion at a spatial level. At population A, three different statuses were detected: an invaded area, a border area comprising the invasion front and a non-invaded area at least 100 m from the invasion front with no *L. neglectus* present. Three censuses were performed, one each month during the peak activity season (May-July). Populations B and C were studied under the same scheme. A border area was detected in population B but only using trays. No border was found in C. The border is a highly dynamic and narrow zone, at the periphery of the invaded zone, as documented by Human and Gordon (1996). In this periphery, no nesting of *L. neglectus* is implied. In the statistical analyses the border was not considered.

Soil arthropods sampling

Pitfall traps were 12 ml plastic vials and 20 mm at the opening. Pitfall traps were buried flush with the soil surface with as little disturbance to the surrounding soil and litter as possible and to avoid the digging-in effect that can inflate the number of ants captured (Greenslade 1973, Majer 1978). At each sampling point we placed five pitfall traps in a pattern resembling the five on a dice, filling them up to 6 ml with soaped water to reduce water surface tension and avoid insect escape. Each trap was placed three meters distant from the others except for the pitfall trap placed in the center of the square. In population A we set up three sampling points in the invaded zone, two in the border and five in the non-invaded zone. In population B four points were in the invaded area, two in the border and four in the non-invaded area. In population C no border area was detected, so five points were placed in the invaded area and five in the non-invaded area. Traps were left for 48 hours in the field and collected afterwards. Samples were transferred immediately to other vials filled with alcohol to preserve them prior to identification. At the laboratory non-ant arthropods were counted and identified to order level.

Canopy arthropod sampling

Beating trays were used to sample the arthropods living or feeding on trees. Because of the observed ant-aphid attendance by *L. neglectus*, we expected to test the effect of the pest on coexisting arthropods, aphids and their predators. In population A we marked five trees in the invaded area, five in the border and ten in the non-invaded area. Population B had ten trees for the invaded area and nine for the non-invaded. Population C had ten trees in both the invaded and non-invaded areas; no border area was detected for that population. Trees --Holm oaks (*Quercus ilex*)-- were similar in size (visual inspection). A beating tray was held under the canopy, which was then struck two times in each of the four corners of the tree with a long stick. Invertebrates were collected with an entomological aspirator and preserved in jars filled with alcohol. At the laboratory non-ant arthropods were counted and identified to order level.

Statistical analysis

Factors analyzed were populations (A, B and C), status (invaded, non-invaded) and sampling method (pitfalls and trays). The response variable was the number of arthropod orders and their abundance (number of individuals) per sampling point for the three censuses (as repeated measures). As variances of the data set for abundance of arthropods were heterogeneous we applied a ($\log_{10}$ x+1) transformation that resulted in homogeneity of variances and normalized residuals (visual inspection). Figures and tables, though, show non-transformed data. Data concerning the number of orders was normally distributed and directly analyzed. As we did not have a complete dataset due to the sampling design of the three status levels (border absent in C and partial in B), only data from invaded and non-invaded areas were analyzed.

Results

Abundance of arthropods

Significant statistical differences in number of arthropods collected was found for invaded and non-invaded areas (all populations; ANOVA, $F_{1,68}$ =9.52; P=0.002) with the non-invaded areas containing a higher number of ground dwelling arthropods than the invaded areas (see figure 1a: ground-dwelling arthropods) and the arboreal arthropods being more abundant in the invaded areas (see figure 1a: canopy arthropods). Statistical differences were also found for different populations studied ($F_{2, 68}$ =14.12; P<0.001; Figure 2a: population A, B and C). Population A was the most affected by the ant invasion and other arthropod numbers were very low or almost inexistent in invaded areas. We also found significant statistical differences for different habitats studied (soil or canopies): $F_{1, 68}$ =31.29; P<0.001 (see figure 1a), with canopies being always richer in number of collected arthropods in the invaded area. Populations don’t behave differently regarding to status, as the interaction is not significant.

Arthropod orders

Similar results to those obtained for abundance were found. All populations had significant statistical differences for the number of orders of arthropods found in invaded and non-invaded areas (ANOVA, $F_{1, 68}$ =27.60; P<0.001; see fig. 1b). Populations always had more orders in non-invaded areas. Significant statistical differences were also found for the three different populations studied (A, B and C): $F_{2, 68}$ =17.81; P<0.001 (see figure 2b).

Ground-dwelling arthropod orders where less abundant than canopy arthropod orders and statistically different ($F_{1, 68}$ =46.85; P<0.001).

Overall arthropod communities

Abundance of individuals was higher in the invaded areas in population A and C for arboreal arthropods mainly due to Homoptera, an insect group that seems to be favored in the presence of *L. neglectus* ants (Pop. A and C respectively: T-test, df=4, P=0.002 and P=0.0063; see Table 1). In tree canopies, Araneida, Hymenoptera and Psocoptera were negatively affected by the presence of *L. neglectus* ants, with significantly lower values in invaded areas (T-test, df=4, Pop. A: Araneida P=0.016; Hymenoptera P=0.005; Pop. C: Psocoptera P=0.0001). For soil arthropods only Diptera in population A was negatively affected by the presence of *L. neglectus* (T-test, df=4, P=0.022). No significant statistical differences were found for other orders but invaded areas contained lower numbers of total individuals and orders (see Table 2).

The maximum number of different arthropod orders collected was of 8.2±2 for canopy arthropods for the month of May in C population (Table 3). Significant statistical differences were found for the number of orders collected in both habitats. We always found major number of orders in canopies, but for one month (June) in one population (C) (see table 3), than in soil surface for all three populations and for different months sampled. The number of orders had the maximum in May for all three populations and statuses (Table 4).

Discussion

L. neglectus probably reached new areas throughout human commerce (see Van Loon et al. 1990), invading disturbed human habitats. Acting as an opportunistic ant, it almost completely excludes other native ants coexisting in the area (Van Loon et al. 1990; Seifert

2000) and produces major changes on the abundance and richness of native ants (Rey and Espadaler, submitted). The dramatic biodiversity loss of native ants in invaded areas is the most important consequence observed until now.

Here we studied the impact on arthropod communities coexisting with three *L. neglectus* supercolonies. There were differences in number of orders found and abundances between arboreal and ground-dwelling arthropods. Abundances were significantly different for invaded and non-invaded areas, with non-invaded areas containing higher numbers of ground-dwelling arthropods. On tree canopies the number of arthropods in the invaded areas was higher than in the non-invaded areas (Figure 1a). Aphids were mainly responsible for increased abundance of arthropod individuals in trees for invaded areas (see Table 1). As *L. neglectus* is an aphid attending ant it was already expected that this order would be favored by the huge amount of ants on trees. Some studies with other invasive ants (*Pheidole megacephala* and *Linepithema humile)* reach similar conclusions (Gonzalez et al. 1999; Wilson 2005; Holway 2002), with different effects on the plants they feed upon (reviewed in Lach, 2003). However, Paris (2005) did not find statistical significant differences in abundances for a very common and obligate myrmecophile homopteran (*Lachnus roboris*) attended by *L. neglectus* as compared with those attended by a native *Lasius* ant (*L. grandis*). In the present study this situation was observed only in population B (see table 1) suggesting that different levels of infestation may affect results. However, negative effects were observed on canopy arthropods. Araneida, Hymenoptera and Psocoptera were usually the most affected orders (see Table 2). The huge amount of *L. neglectus* ants present may affect Hymenoptera and Araneida as they could be direct competitors for prey. Indirect competitive exclusion may be also a mechanism to explain these results. Sanders and Christian (2007) found strong intraguild interactions between ants and spiders, where higher densities of ants affected the abundance and biomass of web-building spiders. Similar results for spiders have been found with other invasive ants such as *Linephitema humile*, *Wasmannia auropunctata* or *Anoplolepis gracilipes* (reported in Human and Gordon, 1997). *L. neglectus* workers can also act as predators on other arthropods and in some instances individuals have been seen carrying small prey (Collembola and Psocoptera; (Paris 2005; pers. obs.). Certain types of organisms with reduced mobility may be more vulnerable than others to direct predation (Human and Gordon 1997; Floren et al. 2002). Psocoptera feed on vegetative plant parts and/or plant exudates with a foraging microhabitat overlap with *L. neglectus* and are a potential occasionally prey. In fact, data from Paris (2005) indicate that even though *L. neglectus* is not a highly predacious ant (total percentages of predation over arthropods from one year data range from a maximum of 11.54% in May to a minimum of 0.60% in September), Psocoptera represent between 26 to 31 % of their prey. Diptera had always fewer individuals in invaded areas and predation over adults has been detected (pers. obs.). Lepidoptera larvae are potential vulnerable prey because of their reduced mobility and this could explain their lower presence in invaded habitats from studied populations.

For ground-dwelling arthropods only Diptera contained significantly less individuals in population A, however in B and C populations the tendency was the same: fewer individuals in invaded areas. This could be explained by predation of Diptera eggs, juveniles and larvae by *L. neglectus* ants (Human and Gordon 1997). All populations showed a decline in Collembola and predation by the invasive ants (pers. obs.) could be an important reason for their lower numbers in invaded areas. This negative effect on Collembola was also found by Cole et al. (1992), Human and Gordon (1997) and Heterick (1997). Alternatively, Collembola have been shown to be particularly vulnerable to disturbance (Springett 1976; Deharveng 1996).

Interference with the foraging activity of other arthropod orders can explain the lower numbers of invertebrates recorded in invaded areas, where in population A, were very low.

Most of them probably moved into non-invaded areas as suggested by Human and Gordon (1997) to avoid direct or indirect competition, although this needs proper documentation in the here studied populations. Non affected orders could survive thanks to exoskeleton protection (Coleoptera), chemical defenses, high mobility or simply not microhabitat overlaps (Cole et al. 1992, Porter and Savignano 1990, Human and Gordon, 1997). Furthermore an extrinsic factor, the level of human-mediated disturbance of the area, might affect the non-ant arthropod communities. In this hypothesis, the effect of the invasive ant would not be a direct cause of the low levels of other arthropods but a consequence of disturbance as well (Floren and Linsenmair, 2001).

Long-term studies of impacts on arthropod communities invaded by non-indigenous ants indicate that, initially, the abundance and species richness of arthropods decreased precipitously but with time, populations returned to pre-invasion levels and the invasive ant was not as abundant being only a negative factor during, and shortly after, the initial phase of an invasion (Morrison 2002). Our study populations are probably at different stages of invasion, where population A shows the most intense effect. At this location we found a major decline of native ants (Rey and Espadaler, submitted) and of non-ant arthropod communities.

The estimated time of invasion in population A is at least 22 years (in 1985 it was first detected as an indoors nuisance). However, we have no data about the time of invasion in the other two populations studied. It is possible that these negative effects can vary with time but only further research or long-term studies could confirm this.

Acknowledgements

To J. M. Vives and M. Rojo for logistic support; this work is funded by a DGESIC (REN2000-0300-C03-01) and MCYT (CGL2004-05240-C02) grant. To Dr. Simon

Mackenzie for suggestions and language correction. To Victor Bernal for helping in arthropod samples identification.

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Figure 1. Differences in non-ant arthropod abundances and richness for invaded and non-invaded areas for the two habitats studied. Data from all populations (n=3). Symbols indicate means (means±sd/sampling point): Population A (pitfall traps: n=3 invaded areas, n=5 non-invaded areas / beating trays: n=10 invaded areas, n=9 non-invaded areas). Population B (pitfall traps: n=4 each status / beating trays: n=10 invaded, n=9 non-invaded areas). Population C (pitfall traps: n=5 each status / beating trays: n=10 each status); whiskers are 95% confidence interval.

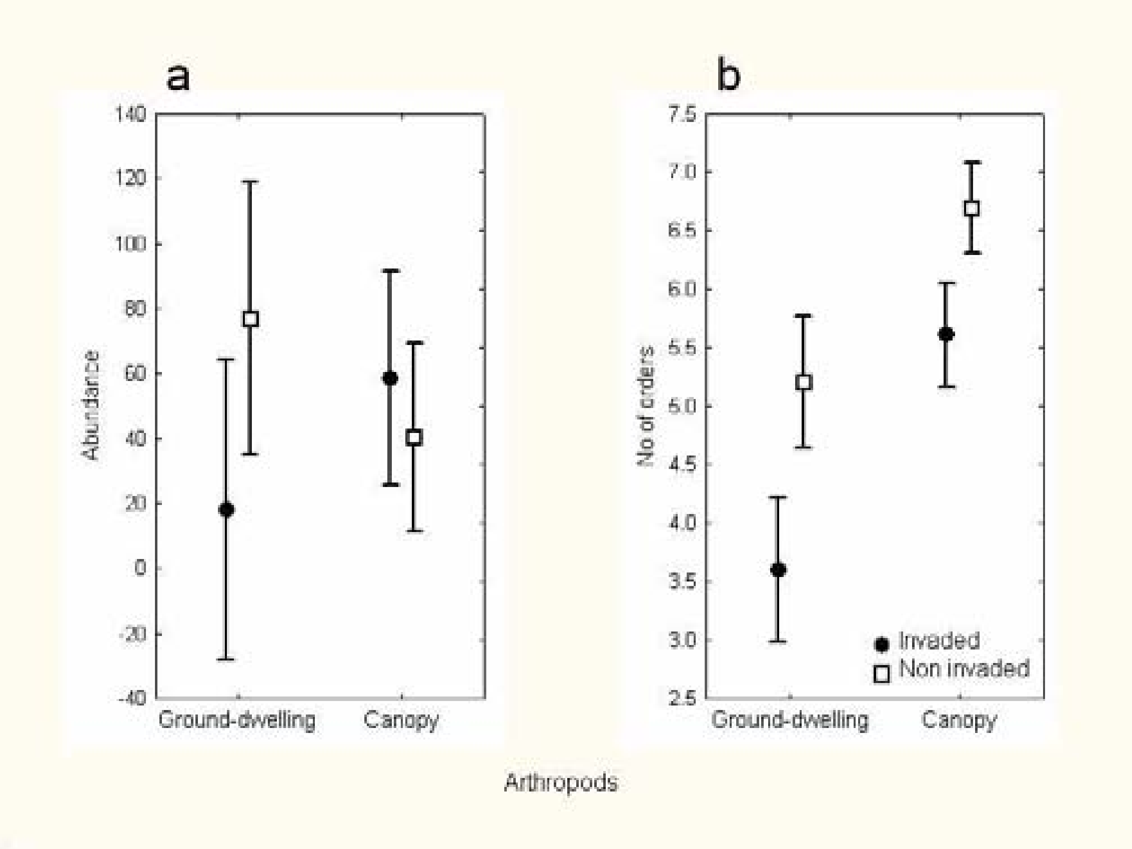

Figure 2. Differences in abundance and richness of non-ant arthropods for invaded and non-invaded areas from all three populations studied, two habitats and three censuses performed (May, June and July). Symbols indicate means (means±sd/sampling point): Population A (pitfall traps: n=3 invaded areas, n=5 non-invaded areas / beating trays: n=10 invaded areas, n=9 non-invaded areas). Population B (pitfall traps: n=4 each status / beating trays: n=10 invaded, n=9 non-invaded areas). Population C (pitfall traps: n=5 each status / beating trays: n=10 each status); whiskers are 95% confidence interval.

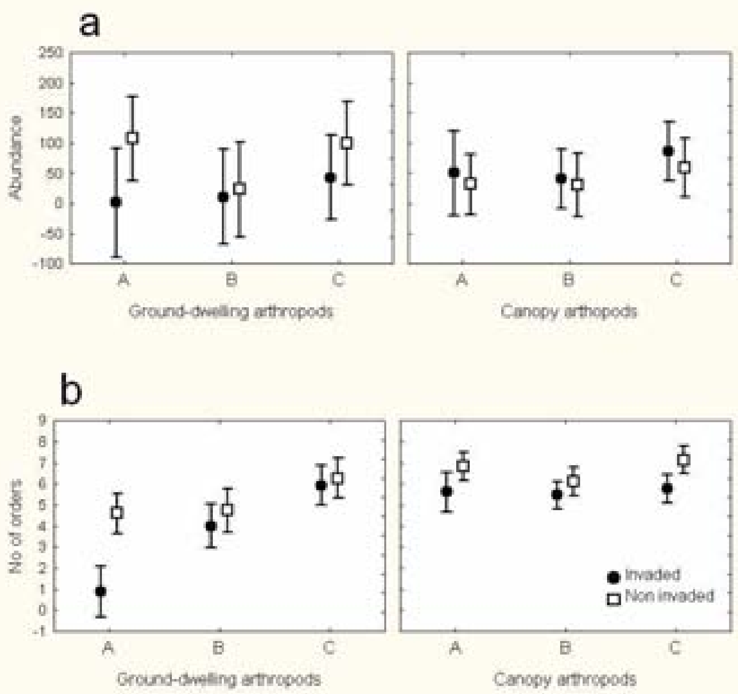

Table 1. Number of non-ant arthropods individuals on canopies per populations and status for major taxonomic groups of arthropods (means±sd / sampling point; n=3 months). Means are calculated from the cumulative number of individuals collected each month with beating trays for sampling point (trees).

Taxa/Population	A		B		C	
Status,Sampling points	Invaded n=10	Non-invaded n=9	Invaded n=10	Non-invaded n=9	Invaded n=10	Non-invaded n=10
Acarina	12.7±18.6	3.7±4	1.6±2.8	1±1.7	3.6±3.7	21.6±26.3
Araneida	**16.3±9.3**	**57.7±15.6***	51±17	65±41	74.6±15.3	95.3±19.5
Coleoptera	77.3±88.9	56±37.5	38.3±38.7	54.3±56.3	97.33±96.2	47±41.7
Diptera	0.7±1.2	5.7±4.7	2±1.7	3.3±2.8	4.6±2.5	5.3±7.5
Heteroptera	15.7±12	24±22.6	35.3±17	22.6±12.7	73.3±61.2	28.3±15.3
Homoptera	**84.3±4.7**	**29.7±12.7***	202±298.1	31.3±12.7	**558±153.9**	**82.3±32.7***
Hymenoptera	**2±1**	**10.7±2.5***	6.6±4.5	10.6±9.8	8.3±4.7	8.3±3.5
Lepidoptera	1.7±2.9	10.7±9	2.3±4	31±50.2	3.3±4.9	3.6±6.3
Psocoptera	35.7±11.6	59.3±38.8	35.6±54.8	13.6±14.3	**30.6±7.5**	**278.6±29.9***
Thysanoptera	0	0	21.6±18	33.6±38	4.33±6.6	3±1

Asterisks indicate significant differences in abundances between invaded and non-invaded areas (T-test; df=4, P<0.05)

Table 2. Number of ground-dwelling arthropods per populations and status for major taxonomic groups of arthropods (means±sd / sampling point; n=3 months). Means are from the cumulative number of orders collected each month with pitfall traps for sampling point.

Taxa/Population	A		B		C	
Status,Sampling points.	Invaded n=3	Non-invaded n=5	Invaded n=4	Non-invaded n=4	Invaded n=5	Non-invaded n=5
Acarina	0	25.3±23.9	6.3±5.6	4.6±2.8	72.3±102	18.3±6.0
Araneida	1±1.7	8.6±5.5	5±4	4.6±4.7	8.3±4.7	9.6±6.4
Collembola	0	482±705.7	18.3±21.4	37.3±34.0	91.3±58.1	419.3±494.1
Coleoptera	1.3±2.3	2±1.73	1±1	1.3±1.1	7.3±2.8	9.6±4.0
Diptera	**0**	**4.3±2.08***	0.6±0.5	12±9.1	3.6±1.5	7.3±4.0
Heteroptera	0.6±0.5	3±3	1±1	0.6±1.1	3±3.4	0.3±0.5
Homoptera	0	1.3±1.1	3±2.6	1±1.7	15±12.1	3.6±2.0
Hymenoptera	0.3±0.5	1.3±0.5	0.6±0.5	5±8.6	2.3±1.5	1.6±1.5
Opilionae	0.3±0.5	2.3±3.2	2.6±3.0	5.3±7.5	3.3±2.5	6.6±6.0
Orthoptera	0	2.3±2.5	0.6±1.1	2.3±2.5	0	0
Psocoptera	0	3±3.6	0	0	0.3±0.5	0

Asterisks indicate significant differences in abundances between invaded and non-invaded areas (T-test; df=4, P<0.05)

Table 3. Mean number of arthropod orders captured (±s.d.) at

the two habitats studied (ground and canopy) for the three populations studied and the three months sampled using all sampling methods: pitfall traps and beating trays.

Month/**Population**	**A**		**B**		**C**	
Habitats	Ground	Canopy	Ground	Canopy	Ground	Canopy
May	5±2.6	6.9±1.8	4.6±1.7	7.4±1.4	6.4±2.5	8.2±2
June	2.8±2.2	6.8±1.3	4.5±1.7	5.4±1.4	6.8±2.2	5.5±1.5
July	2.5±2	5.4±1.5	4±1.9	4.4±1.3	5.1±1.7	5.6±1.8

Table 4. Mean number of arthropod orders captured (±s.d.) for the two status studied (invaded, non-invaded) for the three populations studied and the three months sampled with both methods (pitfall traps and beating trays).

Month/**Population**	**A**		**B**		**C**	
Status	Invaded	Non-invaded	Invaded	Non-invaded	Invaded	Non-invaded
May	5.1±2.4	7.4±1.54	6.2±2	6.9±1.8	6.8±2.3	8.4±2
June	4.8±3	6.2±1.7	4.7±1.1	5.5±1.8	5.6±1.5	6.3±2.1
July	4.2±2.2	4.6±2.1	4±1.5	4.6±1.4	5±1.4	5.8±2.1

Article V

Published in 2005!

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

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J. Agric. Urban Entomol. 21(2): 99–112 (April 2004)

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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[1] Accepted for publication 2 December 2004.

[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.

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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).

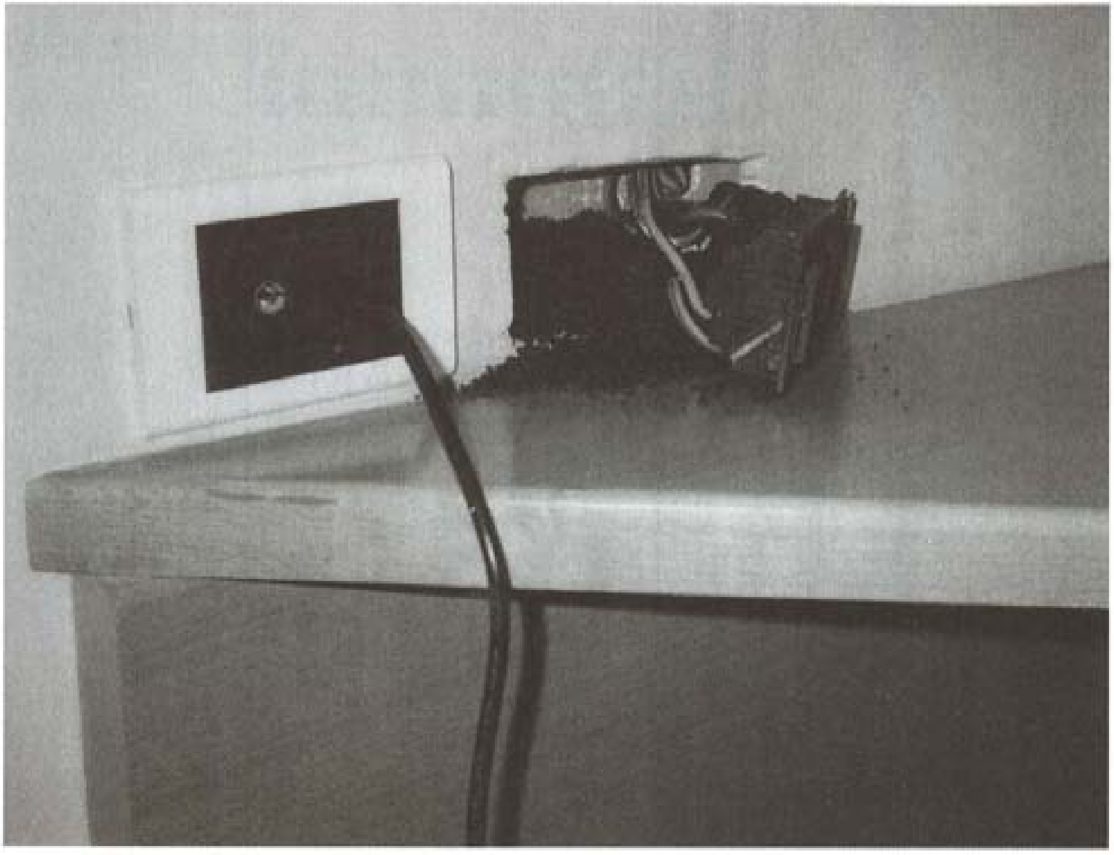

Fig. 1. Dead *Lasius neglectus* ants inside an electrical outlet. Ants were killed by applications with available commercial spray (brand unknown) a few days before. Spraying was routinely done every 2 weeks, with similar results as those shown here. (Photo courtesy of Montserrat Jorba, Matadepera, Spain).

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;

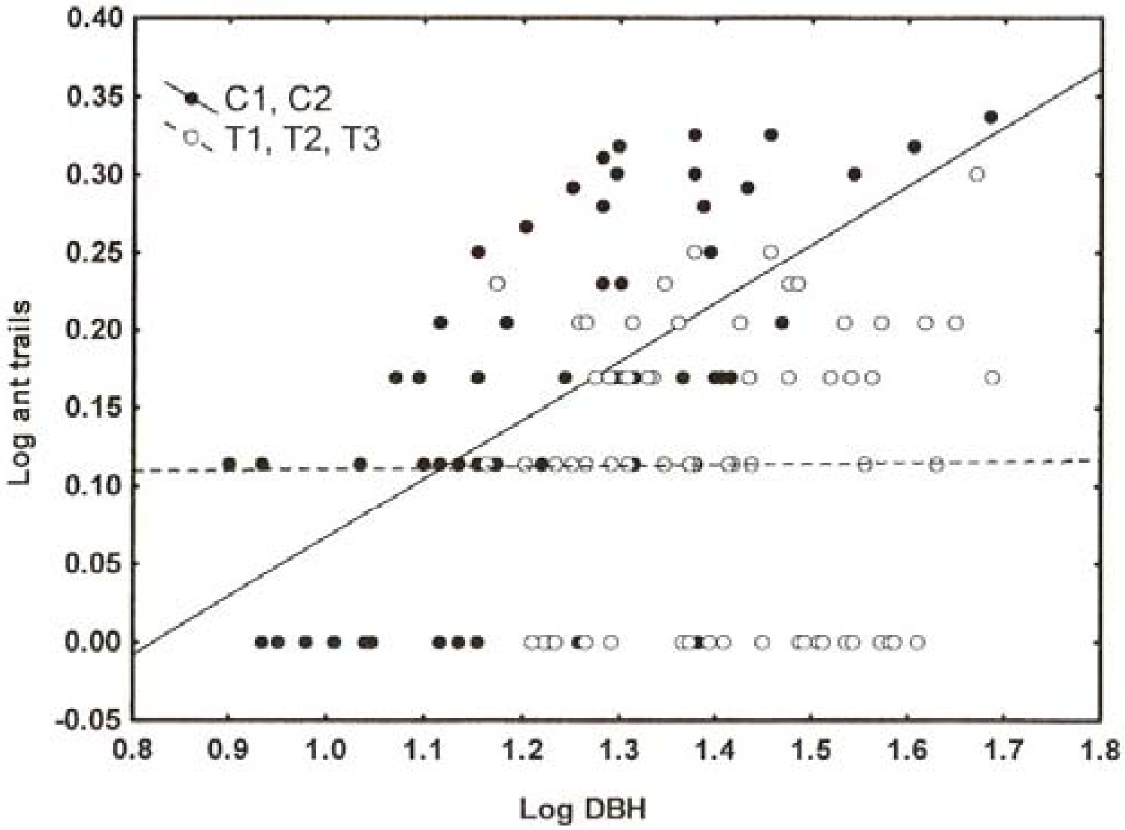

Fig. 2. Relationship between the number of *Lasius neglectus* ant trails found in each tree trunk and the diameter of the tree trunk (*Quercus ilex*) in the control areas (C1, C2; $R^2 = 0.38$; $P < 0.001$) and the treated areas (T1, T2, T3; $R^2 = 0.01$; $P = 0.28$) in 2002. In the treated area, the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

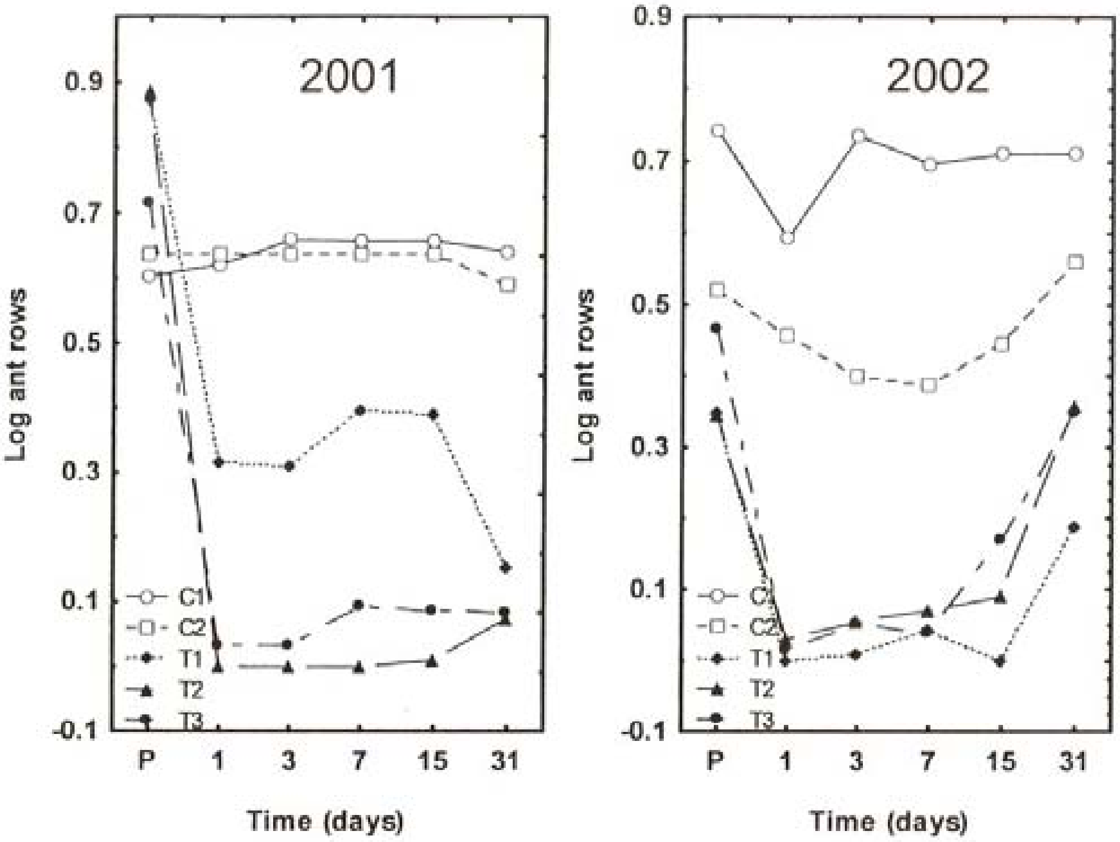

Fig. 3. Mean number of ant trails (log10 transformed) on tree trunks before the treatment (P) and after 1, 3, 7, 15, and 31 days. C1 and C2: control areas; T1, T2, T3: treated areas. Data from 2001 and 2002. Heavy rains in 2002 diminished the effect of trunk sprays.

$P < 0.001$. 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

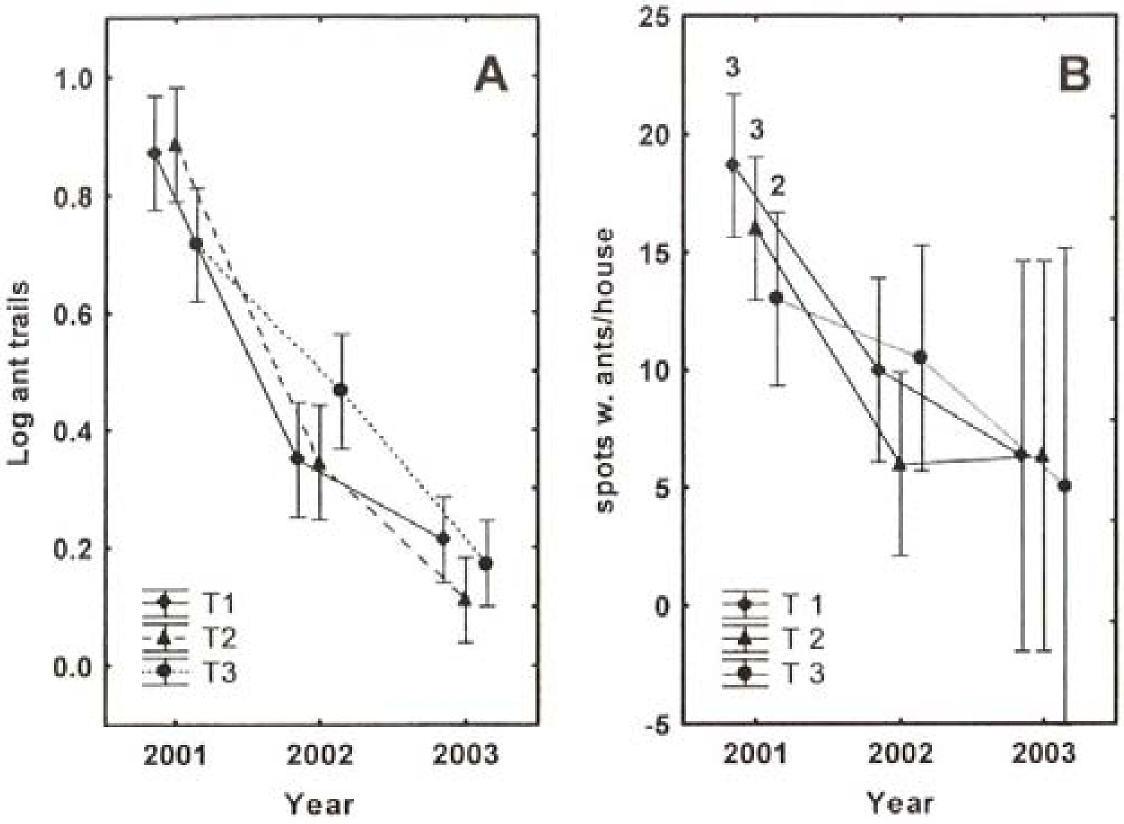

Fig. 4. Mean (±95% c.i.) number of ant trails (log10 transformed) on tree trunks (A) and number of perimeter spots with ants per house (B; digits over figures indicate number of houses) in the 3 treated zones (T1, T2, T3; n = 30 trees per zone) before any treatment (2001), immediately before the treatment 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).

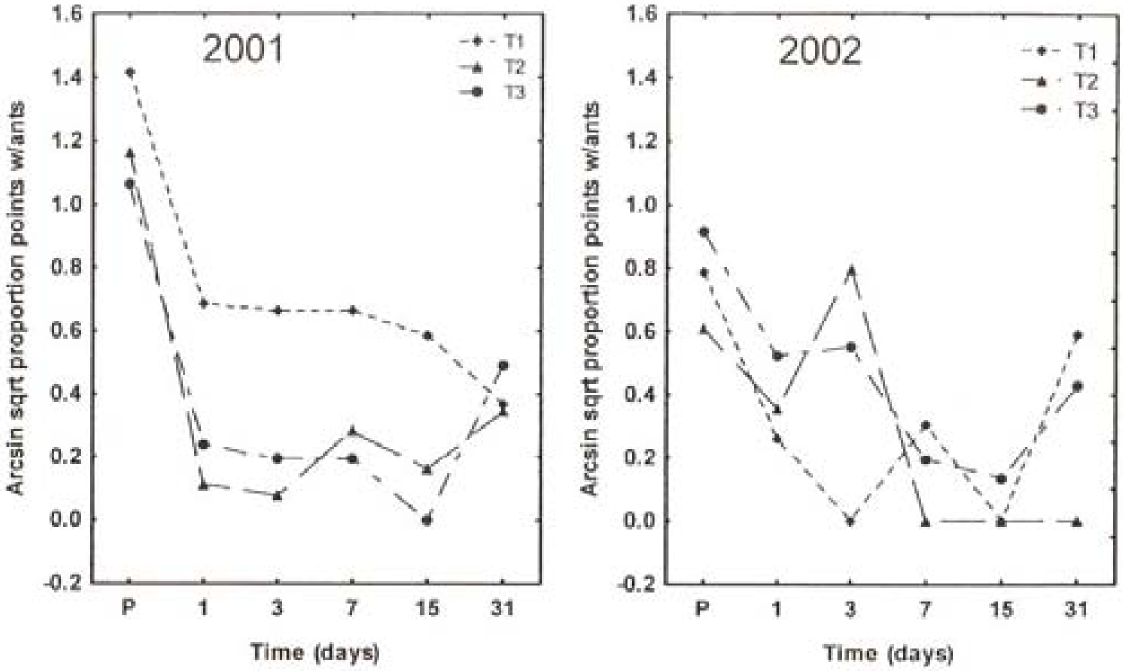

Fig. 5. Mean number of perimeter spots (arcsin transformed) with ants per house before the treatment (P) and after 1, 3, 7, 15, and 31 days. T1, T2, T3: treated areas. Data from 2001 and 2002.

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.

Acknowledgments

Two anonymous referees made useful critical comments and improved the readability of the manuscript. Their help is sincerely acknowledged. We thank Josep Palmarola (Seva) and Josep Mª Vives (DARP, Generalitat de Catalunya) for logistic support. Llorenç Badiella and Oliver Valero (Servei d'Anàlisi Estadística) helped with statistical analysis. Partial funding was provided by grants DGESIC (REN 2000-0300-CO2/GLO), MCYT (CGL2004-05240-C02-01), and the Abertis Foundation.

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