



Universitat de Girona

**ESTUDI D'ASPECTES BIOLÒGICS DE LA
FORMIGA ARGENTINA (LINEPITHEMA
HUMILE, MAYR) ENCARAT AL CONTROL DE
LA PLAGA EN ECOSISTEMES NATURALS**

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(*Linepithema humile*, Mayr) encarat al control de la
plaga en ecosistemes naturals**

per

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(*Linepithema humile*, Mayr) encarat al control de la
plaga en ecosistemes naturals**

Tesi doctoral

Memòria presentada per Sílvia Abril i Meléndez
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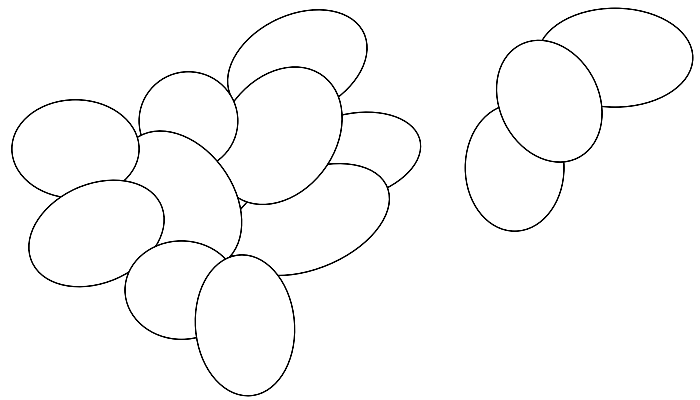
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INTRODUCCIÓ GENERAL I OBJECTIUS

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Introducció

1. Les Invasions biològiques

Les invasions biològiques són el resultat d'un complex procés que s'inicia amb la importació d'organismes cap a nous indrets, sovint allunyats del seu hàbitat natiu, on els seus descendents proliferen, es dispersen i persisteixen (Elton 1958, citat a Mack et al. 2000). No és un fenomen ni recent ni exclusiu de l'ésser humà, tanmateix en els darrers 200 anys la freqüència, abast geogràfic i nombre d'espècies involucrades s'ha disparat exponencialment com a conseqüència directa del creixement del comerç i transport humà (Mack et al. 2000). Per sort, s'estima que només una de cada mil espècies importades a nous hàbitats esdevenen invasores (Williamson & Fitter 1996).

Les conseqüències de les invasions biològiques solen ser ecològicament i econòmicament devastadores (Pimentel et al. 2000). S'estima que només als Estats Units les pèrdues econòmiques associades tant als efectes de les espècies invasores sobre l'agricultura, silvicultura i altres segments de l'economia com al seu control, assoleixen la impactant xifra de 137 bilions de dòlars anuals (Pimentel et al. 2000). A nivell ecològic, l'efecte de les invasions biològiques pot arribar a ser molt divers. En algunes ocasions, les espècies invasores poden arribar a beneficiar a algunes espècies natives, ja sigui perquè els serveix d'aliment o perquè estableixen amb elles relacions comensalistes (Crooks 2002), però en general, els efectes de les invasions biològiques solen ser negatius per a l'ecosistema: introducció de malalties, depredació o pasturatge de les espècies natives, extinció d'espècies natives per contaminació genètica, etc. Amb tot, l'efecte més greu d'una invasió biològica es troba a nivell d'ecosistema, ja que pot arribar a provocar la disrupció d'un ecosistema sencer, sovint a causa del reemplaçament de les espècies natives per les exòtiques, generant una alteració en la composició i estructura de les comunitats animals i vegetals (Vitousek 1990). Aquesta alteració es sol traduir en una dramàtica pèrdua de diversitat i homogeneïtzació de la biota a les àrees envaïdes (McKinney & Lockwood 1999; Olden et al. 2004). Per aquest motiu,



les invasions biològiques constitueixen una de les causes principals de la disminució de la biodiversitat global (Mack et al. 2000).

Les espècies invasores engloben un elevat i divers nombre de tàxons diferents. D'entre tots ells, el grup dels artròpodes, a més de ser un grup amb un potencial molt elevat per dispersar-se a través de grans distàncies (Simberloff 1989; Lawton 1995), també és el que posseeix més facilitat per a introduir-se accidentalment a nous territoris gràcies a l'ajuda de l'ésser humà (Vitousek et al. 1996; Ward et al. 2006). Això es fa pal·lès en el fet que dins el llistat de les pitjors espècies invasores del món (www.iddg.org/database/welcome) hi ha diverses espècies de formigues, les quals provoquen importants efectes negatius en ecosistemes d'arreu del món. Una d'aquestes formigues invasores és la formiga argentina (*Linepithema humile* Mayr), l'espècie estudi d'aquesta tesi.

2. La formiga argentina

La formiga argentina (*Linepithema humile*) està catalogada dins el llistat de les 100 pitjors espècies invasores del món segons l'ISSG (Invasive Species Specialist Group, www.issg.org/database/welcome). És una formiga petita -les obreres medeixen de l'ordre dels 2-3 mm de llargada, els mascles uns 3 mm i les reines fèrtils uns 5 mm (Newell & Barber 1913) -monomòrfica, de color marró i caràcter nerviós (Figura 1).



Figura 1 Obrera de formiga argentina (*Linepithema humile*). A l'esquerra transportant una larva de mascle alimentada amb menjar tenyit amb Rhodamina A. Fotos: David Estany.

2.1. Origen i distribució

La formiga argentina és originària de l'Amèrica del Sud (Tsutsui et al. 2001) des d'on ha aconseguit dispersar-se amb l'ajuda de l'ésser humà, envaint ecosistemes mediterranis i subtropicals de tots el continents- excepte l'Antàrtida- així com nombroses illes oceàniques (Passera 1994; Suarez et al. 1998, 2001; Roura-Pascual et al. 2004) (Figura 2). A la Península Ibèrica la primera cita de la seva presència va ser a Portugal l'any 1907 (Martins 1907), mentre que a Espanya va ser citada per primer cop a València l'any 1923 (Font de Mora 1923; García Mercet 1923). La seva preferència per zones humides i de clima temperat (Holway et al. 2002a) condiciona la seva distribució a la Península Ibèrica on la podem trobar bàsicament al llarg del litoral Mediterrani i Atlàntic (Espadaler & Gómez 2003) (Figura 3). A la resta d'indrets de l'interior i litoral cantàbric la seva presència esporàdica sembla més associada a zones urbanes, on els microclimes presents a l'interior dels edificis generen unes condicions de temperatura i humitat adequades per a la seva supervivència (Suarez et al 2001; Espadaler & Gómez 2003).

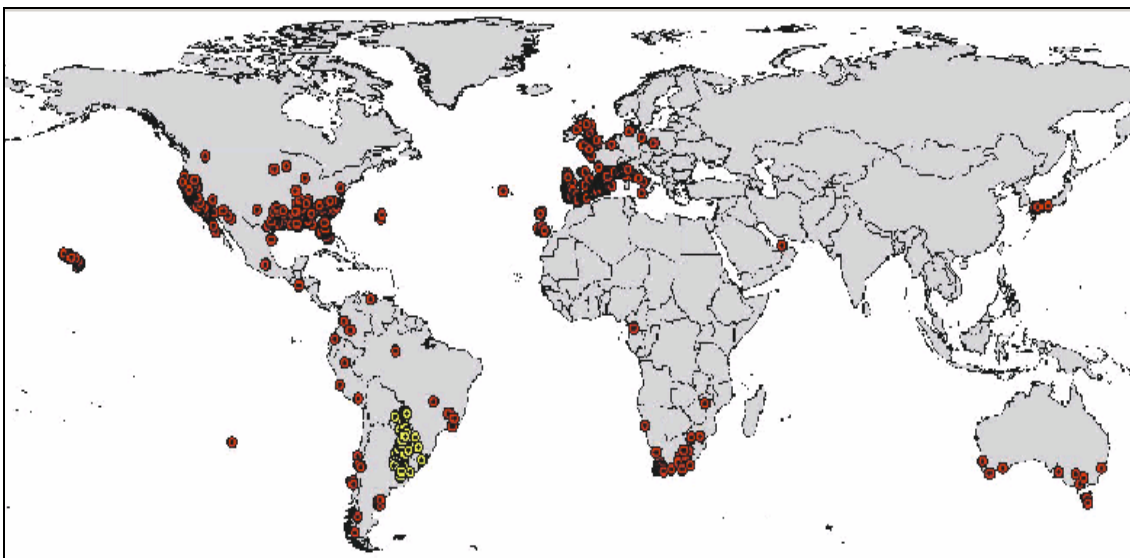


Figura 2 Distribució mundial de la formiga argentina. Els cercles grocs fan referència a les poblacions natives i els vermells a les poblacions introduïdes. Font: Roura-Pascual et al. 2004.

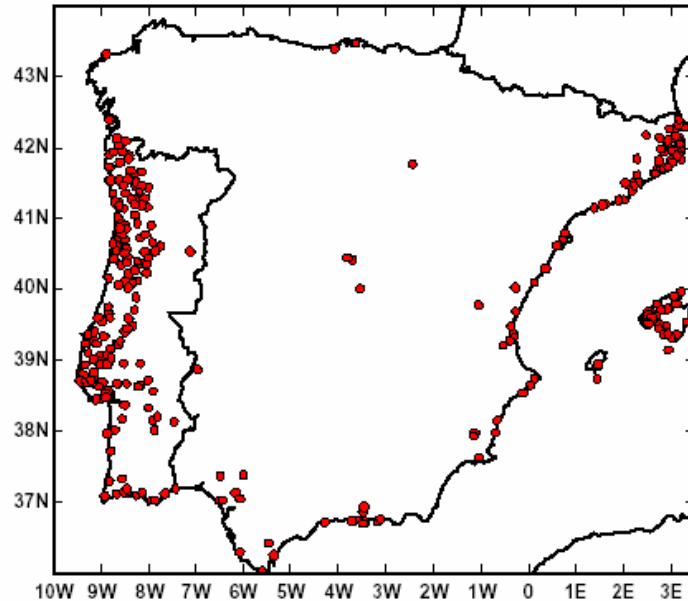


Figura 3 Distribució de la formiga argentina a la Península Ibèrica. Font: Espadaler & Gómez 2003).

2.2. Fases de la posta i cicle reproductiu de la formiga argentina

Com la resta d'espècies de formigues, la formiga argentina pot generar tres tipus de descendència: mascles, obreres i reines, mentre que la posta està formada per ous, larves i nimfes (Figura 4).

Els ous de formiga argentina són el·líptics, anacarats i lluents i la membrana que els envolta és extremadament prima i delicada. Al niu, tant natural com artificial, és freqüent trobar-los agrupats en massa (obs.pers.) gràcies a la capa mucilaginosa que cobreix la seva superfície (Newell & Barber 1913). A les colònies de formiga argentina, l'únic individu capacitat per a pondre ous és la reina, les obreres són completament estèrils (Holway et al. 2002b).

Dins la fase larvària, es poden distingir diferents graus de desenvolupament. En aquest sentit, Benois (1973) distingia entre larves petites, mitjanes i grosses. Les larves, a excepció d'alguns moviments de contracció, són immòbils (Newell & Barber 1913). Són l'únic estadi de la posta que requereix ser alimentat per a poder créixer i desenvolupar-se (Markin 1970a) i només en els darrers estadis de desenvolupament, quan la larva ja és

distingible a ull nu, es poden diferenciar les castes que s'estan gestant gràcies a la seva mida (obs. pers.).

Les nimfes són nues, és a dir, la larva de formiga argentina no s'envolta d'un capoll de seda per a poder dur a terme el seu desenvolupament com en altres espècies de formigues, sinó que en tot moment es poden distingir les diferents parts de la seva anatomia. Recent transformada a partir de l'estadi larval, la nimfa és completament blanca i es va enfosquint a mesura que es va apropant a l'eclosió. Els ulls també canvien de coloració: de transparents passen a ser vermells i quan es troba a les fases finals de maduració es tornen negres (Silva Dias 1955; obs. pers.). A més, les obreres recent nascudes, caracteritzades per la seva coloració més clara, gairebé transparent, s'engloben dins d'un estadi anomenat en anglès estadi "callow" (Newell & Barber 1913). El cos de l'obrera "callow" s'enfosqueix ràpidament i passades 24-72 hores de l'eclosió de la nimfa, l'obrera recent nascuda esdevé irreconeixible respecte la resta d'obreres de la colònia (obs.pers.)

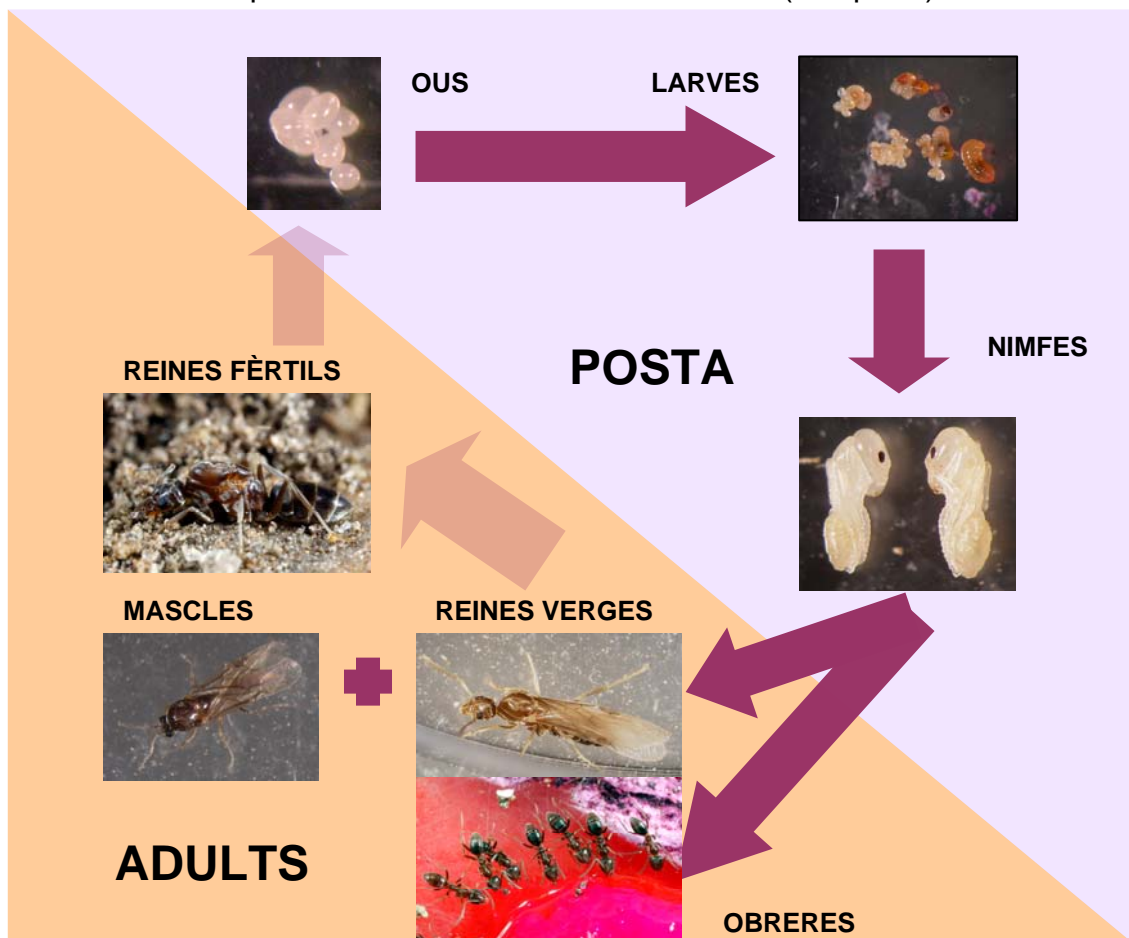


Figura 4 Cicle biològic de la formiga argentina. El triangle superior engloba les diferents fases de la posta, mentre que el triangle inferior engloba totes les formes adultes.

Pel què fa a la seva fenologia reproductiva, sembla que les reines de formiga argentina només ponen ous durant els mesos de primavera, estiu i tardor. A l'hivern entren en un període de repòs fisiològic, motivat per les baixes temperatures, i que és essencial per la supervivència de les reines. Al laboratori, si les reines es mantenen actives artificialment durant aquesta etapa, ponen alguns ous al principi, però acaben morint després d'un període de subsistència sense pondre (Benois 1973).

D'altra banda, la quantitat d'ous generats durant els períodes de posta varia estacionalment (Benois 1973) (Figura 5). Així doncs, a la primavera (finals març-maig) les reines entren dins un període de màxima posta d'ous. Al mes de maig es genera un màxim de larves provinents de la posta de primavera i aquestes, alhora, generen un màxim de nimfes al mes següent. Aquestes nimfes generen mascles i obreres de manera explosiva als mesos de juny i juliol respectivament, malgrat que les obreres provinents de la posta primaveral continuen apareixent fins al mes d'octubre i els mascles fins a finals de juliol (Figura 5). A l'estiu, la posta d'ous per part de les reines disminueix dràsticament (Benois 1973). Aquest fet pot ser causat per la matança d'aproximadament el 90% de reines fèrtils al niu per part de les obreres al mes de maig (Keller et al. 1989), i al fet que les reines fèrtils no apareixen fins al juliol-agost (Markin 1970b). A la tardor, però, hi torna a haver un segon període de màxima posta, més lleu que el primaveral, però molt més fort que el període de posta estival (Benois 1973). A partir dels ous postos a la tardor (setembre-octubre) es generen les larves que entraran en estat hivernant durant els mesos de desembre a febrer. Aquestes larves reprenen el desenvolupament a partir del mes de març, juntament amb l'ascens de la temperatura ambiental, i es converteixen en nimfes a partir de mitjans de maig (Benois 1973). El fet que les primeres larves de reina s'observin al mes d'abril indica que les reines es generen a partir dels ous postos a la tardor l'any anterior (Benois 1973). Dels ous postos a la tardor també es generen obreres, que naixeran a l'estiu de l'any següent (Benois 1973). Les reines verges eclosionen uns 15 dies després de l'aparició dels mascles i l'acoblament es fa a l'interior del niu fins al mes d'agost (Benois 1973).

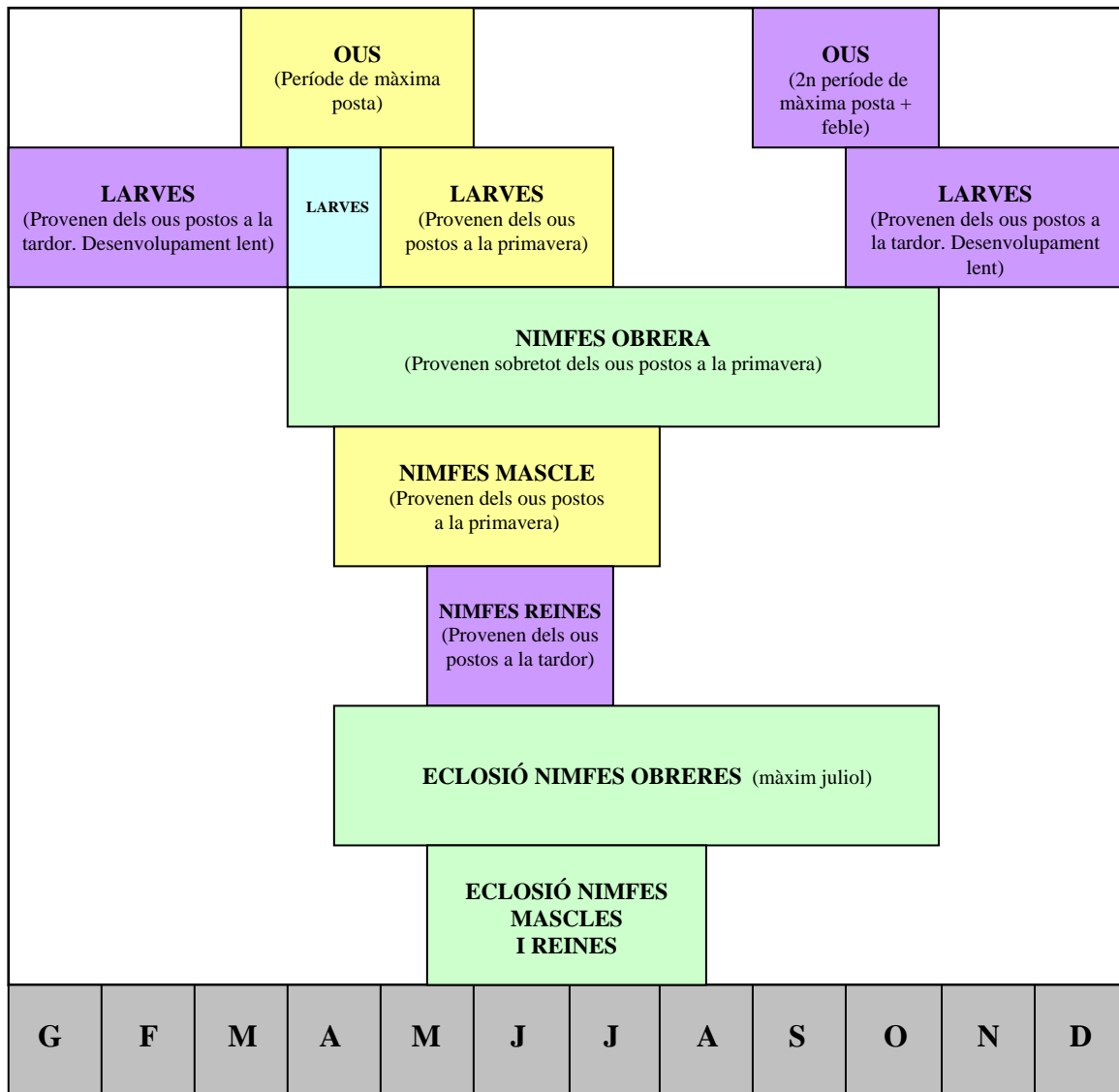


Figura 5 Fenologia reproductiva de la formiga argentina basat en l'estudi realitzat per Benois (1973) a la regió d'Antibes (SE França). Els requadres grocs representen la posta provinent dels ous postos a la primavera i els requadres de color morat representen la posta provinent dels ous postos a la tardor. Els requadres verds engloben tant la posta provinent dels ous postos a la primavera com la provinent dels ous postos a la tardor, i el requadre blau representa el solapament entre el període de larves que provenen de la posta d'ous de tardor i les larves que provenen de la posta d'ous de primavera.

2.3. Característiques que determinen el seu caràcter invasor

La formiga argentina, així com les formigues invasores en general, posseeix unes característiques biològiques clau per al seu èxit com a espècie invasora a

les àrees que colonitza. Una d'aquestes característiques és la unicolonialitat, és a dir, l'absència d'un comportament agressiu entre individus de diferents nius (Passera 1994; Holway et al. 2002b). Aquesta manca de "frontera comportamental" entre els diferents nius permet a l'espècie assolir elevadíssimes abundàncies d'individus i, en conseqüència dominar hàbitats sencers mitjançant la formació del que es coneix com a "supercolònia". En alguns casos, com a la zona oest dels Estats Units o a Europa, aquestes supercolònies arriben a assolir milers de kilòmetres d'extensió (Tsutsui et al. 2000; Giraud et al. 2002), en les quals els individus que en formen part es reconeixen com a membres de la mateixa colònia i no s'agredeixen malgrat trobar-se físicament allunyats per una distància tan exageradament gran. L'existència d'aquestes supercolònies és una evidència inequívoca de l'amenaça potencial d'aquesta espècie a les àrees envaïdes. Altres característiques clau que potencien la capacitat invasora de la formiga argentina són el seu règim omnívor (Markin 1970c), la presència de més d'una reina al niu (poligínia) (Passera 1994; Holway et al. 2002b), fet que li permet mantenir una aportació constant i elevada d'individus a la colònia, i també la manca de vol nupcial (la còpula dels sexuals es realitza a l'interior del niu) (Passera 1994) que li permet assegurar la màxima supervivència de les reines i, per tant, la màxima productivitat de la colònia. Aquestes característiques, juntament amb la manca de competidors naturals, depredadors i paràsits a les àrees introduïdes (Holway et al. 2002b), el fet que petits propàguls de l'espècie són capaços de fundar una nova colònia (Hee et al. 2000) i la tolerància de l'espècie a un ampli rang de condicions ambientals (Holway et al. 2002a), són les principals causes de la dominància d'aquesta espècie respecte a les espècies de formigues natives. Ara bé, potser la característica més important en referència al seu potencial invasor a escala global és la seva predilecció per viure en zones estretament relacionades amb l'ésser humà, com cultius, zones urbanes o abocadors (Passera 1994). Aquesta "relació" amb l'ésser humà la proveeix del transport necessari per a dispersar-se arreu del món a través de la dispersió per salts o "jump-dispersal" (Suarez et al. 2001). Aquesta dispersió es dona principalment a través d'hàbitats humanitzats i alterats (Suarez et al. 1998). No obstant això, cada vegada hi ha més evidències de la seva capacitat

per envair hàbitats naturals (Cole et al. 1992; Human & Gordon 1996; Holway 1998; Carpintero et al. 2005; Gómez et al. 2003).

2.4. Efectes ecològics de la invasió

En el cas concret de la invasió de la formiga argentina en ecosistemes naturals, l'efecte més evident i, en conseqüència, més àmpliament estudiat arreu del món és el desplaçament de gairebé totes les espècies de formigues natives presents a les àrees afectades (Donnelly & Giliomee 1985; Ward 1987; Majer 1994; Cammell et al. 1996; Human & Gordon 1996, 1997, 1999; Way et al. 1997; Suarez et al. 1998; Holway 1999; Gómez et al. 2003). Sembla ser que aquest desplaçament ve motivat per la superioritat numèrica de la formiga argentina respecte la resta d'espècies de formigues natives, la qual li permetria trobar el recurs i monopolitzar-lo molt més ràpidament (Human & Gordon 1996; Oliveras et al. 2005) i, per tant, desplaçar a les espècies natives a través de la competència per explotació i interferència (Hölldobler & Wilson 1990). A més d'afectar la mirmecofauna local, la formiga argentina també està implicada en la disminució de la diversitat i abundància d'altres invertebrats, depredant sobre ous, larves i algunes formes adultes d'artròpodes a més de constituir-se com a fort competidor d'alguns d'ells (Cole et al. 1992; Human & Gordon 1997; Bolger et al. 2000; Huxel 2000). Així, l'efecte de la formiga argentina sobre els ecosistemes naturals és devastador, no només perquè provoca una elevada reducció de la biodiversitat de la zona, sinó també perquè, en desplaçar formigues i artròpodes clau de l'ecosistema, també pot arribar a afectar processos ecològics fonamentals com ara la pol·linització (Visser et al. 1996) o la dispersió de llavors (Bond & Slingsby 1984; Gómez & Oliveras 2003). Aquest efecte sobre espècies que es troben als nivells més bàsics de la piràmide tròfica pot generar, de retruc, efectes en espècies de nivells tròfics superiors. En aquest sentit, la reducció de les poblacions de vertebrats insectívors com la musaranya grisa, *Notiosorex crawfordi* (Coues) (Laakkonen et al. 2001) o el llangardaix cornut, *Phrynosoma coronatum blainvillei* Gray (Suarez et al. 2000) a zones del sud de Califòrnia afectades per la invasió, sembla ser causada per la disminució de les poblacions d'artròpodes. Pel que fa a la Península Ibèrica, s'ha vist que la presència de la formiga argentina sembla comportar una

disminució de la condició física dels polls de mallerenga blava, *Parus caeruleus* L., la qual cosa podria repercutir en la supervivència dels polls un cop emancipats (Roca 2004).

2.5. Prevenició i control de la invasió

Malgrat els continus esforços per controlar o eradicar aquesta espècie de les zones on esdevé una plaga, avui dia encara resta per descobrir un mètode realment eficaç que controli o elimini la formiga argentina de manera permanent dels hàbitats afectats per la invasió. En vivendes i zones urbanitzades, l'estratègia a seguir per controlar la invasió és la de limitar l'accés a l'aigua, aliments i zones per niar així com també mantenir les plantes lliures de pugons i altres hemípters productors de melassa que oferirien una font d'aliment permanent a la formiga (Soeprono & Rust 2004a). En cultius, fins ara la manera més eficaç de controlar la plaga és utilitzant barreres químiques o físiques que impedeixin a la formiga l'accés als pugons de les plantes (Shorey et al. 1992; Soeprono & Rust 2004). Altres mètodes de control utilitzats per a combatre la plaga es basen en l'ús d'insecticides aplicats en forma d'aerosols o insecticides granulats (Van Schagen et al. 1994; Gordon et al. 2001; Soeprono & Rust 2004b; Soeprono & Rust 2004c; Costa et al. 2005). Aquest tipus de mètodes, però, presenten el gran inconvenient que només mata als individus per contacte directe, i elimina bàsicament obreres farratjadores però no afecta ni a les reines ni a les larves (Soeprono & Rust 2004a), que es mantenen protegides a l'interior del niu conservant el potencial regenerador de la colònia. Un altre gran inconvenient, sobretot quan es tracta d'aplicar aquest tipus de mètodes de control en ecosistemes naturals envaïts, és que aquest tipus de tractaments solen ser insecticides d'ampli espectre que no només eliminen les obreres de formiga argentina sinó també altres insectes, la desaparició dels quals pot arribar a generar altres plagues (Smith et al. 1995). Un altre mètode de control químic és l'ús d'esquers tòxics d'efecte retardat (Baker et al. 1985; Krushelnycky & Reimer 1998a,b; Silverman & Roulston 2001; Klotz et al. 2004; Krushelnycky et al. 2004). Aquests esquers tòxics permeten la introducció del verí a través de la trofolaxia a tots els individus de la colònia inclosos les reines i les larves, i evita l'eliminació massiva d'altres espècies d'insectes com en el

cas dels insecticides d'ampli espectre. Aquest darrer mètode és el que ha proporcionat resultats més satisfactoris a l'hora de frenar l'expansió de la formiga argentina en ecosistemes naturals (Krushelnycky et al. 2004). Tanmateix, i malgrat la lenta regeneració de l'espècie a les zones tractades, la suspensió del tractament acaba provocant que s'acabin assolint els nivells d'invasió inicials (Krushelnycky et al. 2004). En aquest sentit, malgrat els múltiples esforços per frenar l'expansió de la plaga en ecosistemes naturals, encara no s'ha trobat cap mètode que permeti frenar la invasió de manera permanent i molt menys la eradicació de la plaga a les zones envaïdes.

Curiosament, el què més crida l'atenció de tots els mètodes de control proposats a nivell mundial per frenar l'expansió i/o erradicar aquesta espècie invasora, és la gran manca d'informació referent a la seva biologia, que d'altra banda podria arribar a ésser clau per tal d'elaborar un mètode eficaç de control. Dit d'una altra manera, abans de combatre una espècie, potser el més lògic seria conèixer-la en profunditat per tal de saber quins són els seus punts febles. Però en el cas de la formiga argentina, malgrat els extensos estudis elaborats arreu del món sobre els múltiples impactes, econòmics, domèstics i ecològics i sobre possibles maneres de controlar la plaga, encara queda per estudiar bona part de la seva biologia com a espècie, de la qual gairebé tot el que se'n sap és a través d'uns pocs estudis realitzats a principis del segle passat (Newell & Barber 1913; Silva Dias 1955; Benois 1973). No ens ha d'estranyar doncs, el fet que en el passat s'hagin dut a terme dràstiques campanyes d'eradicació d'aquesta espècie mitjançant fumigacions massives amb productes tòxics com el DDT (Diclor-difenil-tricloroetà) (Van Schagen et al. 1994), que al marge de la seva efectivitat controlant la plaga, presentaven greus afeccions a la fauna de la zona tractada. D'altra banda, des del punt de vista ecològic, també crida l'atenció el fet que, malgrat els efectes negatius d'aquesta espècie invasora sobre ecosistemes naturals, fins ara s'han dut o s'estan duent molt pocs esforços per a poder controlar la plaga en aquestes zones (Soeprono & Rust 2004a). Per aquest motiu, l'objectiu bàsic d'aquesta tesi és el d'omplir els buits d'informació referents a la biologia de la formiga argentina que poden arribar a ser cabdals per al disseny de metodologies encarades al seu control en ecosistemes naturals.

Objectius

Tenint en compte que fins ara el mètode de control que sembla que obté els millors resultats és el tractament mitjançant esquers tòxics d'efecte retardat (Krushelnycky et al. 2004), i que dins els factors que poden afectar l'eficàcia d'aquests esquers s'inclouen l'estat fisiològic i reproductiu de la colònia (Krushelnycky & Reimer 1998a; Rust et al. 2000) o l'atractivitat de l'esquer per a les obreres farratjadores (Soeprono & Rust 2004a), el **capítol 1** d'aquesta tesi té com a objectiu analitzar l'espectre dietari de la formiga argentina en ecosistemes naturals tant a nivell qualitatiu com quantitatiu, així com la seva activitat de cerca d'aliment tant diària com anual. Aquesta informació és essencial per avaluar quin és el millor moment per tal d'aplicar mesures de control mitjançant esquers tòxics en ecosistemes naturals en termes de la fisiologia reproductiva de la colònia. També ens permetria determinar quin tipus nutricional és més atractiu per les obreres segons la casta que es vulgui afectar i l'època de l'any en què es faci el tractament.

Però què passa si la zona a tractar és una zona d'interès natural dins la qual no és permès l'ús d'agents químics per a combatre la plaga? En aquests casos es presenta la necessitat de desenvolupar altres metodologies de control de la plaga que no presentin cap tipus d'intervenció química per tal de gestionar la invasió en aquestes zones. Així, tenint en compte que les reines són les que s'encarreguen principalment de la regeneració de la colònia, una possible manera de frenar l'expansió de la invasió sense l'ús d'agents químics podria ser l'eliminació mecànica d'aquesta casta a les àrees afectades. Tanmateix, abans de generar i testar nous mètodes de control basats en l'eliminació massiva de reines és necessari un estudi que analitzi les fluctuacions de la densitat d'individus d'aquesta casta al llarg de l'any, per tal de saber quin és el millor moment per actuar. Aquest és l'objectiu del **capítol 2** d'aquesta tesi.

Queda palès que combatre la invasió és una tasca molt difícil. L'extensa distribució de l'espècie, juntament amb les dificultats titàniques per a poder frenar i/o eradicar les poblacions introduïdes un cop establertes, fan de la prevenció la millor estratègia per a controlar la plaga (Holway et al. 2002b). La determinació de les àrees més susceptibles de ser envaïdes per l'espècie pot ser una eina molt valuosa per a prevenir la seva aparició a noves àrees. Per tal

de determinar quines són aquestes àrees, es fa molt necessari un millor coneixement dels factors biòtics i abiòtics que determinen la distribució geogràfica de la formiga argentina. L'obtenció d'aquesta informació permetria la creació de bons models de predicció de la distribució potencial de l'espècie tant a escala global com regional, els quals contribuirien enormement al disseny de programes de gestió de la plaga per a prevenir la seva disseminació. Així, els models de predicció poden resultar eines indispensables per a gestionar i prevenir l'expansió de la plaga en ecosistemes naturals, però la bona calibració dels mateixos per tal que ofereixin prediccions fiables sobre el rang potencial de distribució de l'espècie es basa en les dades utilitzades per a generar el model. És per aquest motiu que es fa indispensable l'estudi dels diferents factors abiòtics i biòtics que condicionen el fet que l'espècie s'estableixi en una zona i no en una altra. Fins ara, múltiples estudis d'arreu del món han avaluat la influència dels factors abiòtics sobre la invasió de la formiga argentina (Holway 1995; Way et al. 1997; Holway 1998; Human et al. 1998; Paiva et al. 1998; Suarez et al. 2001; Menke & Holway 2006), els quals juntament amb altres dades sobre la seva distribució geogràfica han permès la creació de models de predicció de la potencial distribució de la formiga argentina tant a escala local com a escala global (Suarez et al. 2001; Hartley et al. 2006; Roura-Pascual 2006; Roura-Pascual et al. 2004, 2006). Aquests models, però, es basen únicament en els factors abiòtics com a principals elements que determinen la distribució geogràfica de l'espècie. Tanmateix, s'ha de tenir en compte que l'establiment d'una espècie invasora en una determinada àrea és un fenomen complex que molt probablement requereix altres elements-clau com a responsables de la seva expansió o limitació geogràfica a les zones introduïdes, com ara les relacions biòtiques amb la resta d'éssers vius (predació, mutualismes, parasitisme, competència, etc.) o els requeriments fisiològics de l'espècie per tal que la colònia sobrevisqui, creixi i es dispersi (Helmuth et al. 2005). La principal raó de la manca d'integració d'aquests factors a l'hora de crear models de predicció és probablement l'escassetat de dades disponibles referents a aquests tipus de processos biològics. Fins ara només existien un parell de treballs en els quals s'estimés un model de predicció de la distribució potencial de la formiga argentina en base a les seves necessitats fisiològiques per establir-se (Hartley & Lester 2003; Krushelnycky et

al. 2005) els quals es van realitzar amb dades obtingudes a principis i gairebé mitjans del segle passat (Newell & Barber; Benois 1973) sense rèpliques ni control de les condicions experimentals. En un intent d'omplir aquest buit, els **capítols 3 i 4** tenen per objectiu obtenir nova informació sobre els efectes de la temperatura en el desenvolupament i supervivència de les diferents fases juvenils de la formiga argentina així com en la posta de les reines. Aquest coneixement és essencial per a, posteriorment, generar models de predicció del rang potencial d'establiment de la invasió en base a la fisiologia de l'espècie, fet aquest que ha estat abordat en el darrer capítol d'aquest treball. Així, el **capítol 5** té com a objectiu utilitzar les dades obtingudes en els dos capítols anteriors per a crear dos models de predicció del rang potencial d'establiment de la plaga a Catalunya en base a les seves necessitats fisiològiques. Aquesta informació és essencial per a omplir la manca gairebé absoluta en l'ús de dades fisiològiques en models predictius i dona peu a la seva millora tant a escala global com a escala local.

Àrea d'estudi

El treball de camp realitzat als dos primers capítols de la tesi es va dur a terme a la Serra Llonga, una cadena de petits puigs de menys de 400 metres d'alçada situada a l'extrem sud-est del massís de les Gavarres, a tocar del nucli de Castell d'Aro (Baix Empordà, Girona) a uns 4 km de la línia de costa Mediterrània (Figura 6).

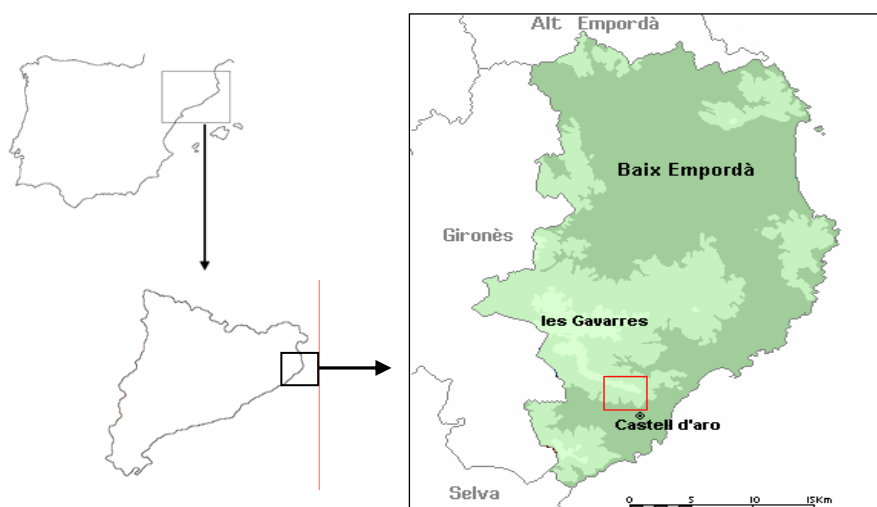


Figura 6 Localització de l'àrea d'estudi.

El clima de la zona és típicament mediterrani, amb una temperatura mitjana anual entre 14 i 15 °C, amb pic màxim els mesos de juliol i agost (temperatura mitjana mensual de 22-23 °C) i mínim al gener (8-9 °C). Les precipitacions són moderades, d'entre 750 i 800 mm de precipitació mitjana anual, amb màxims a la tardor i a la primavera (mes més plujós l'octubre amb 130-140 mm) i mínims a l'estiu (mes més sec el juliol amb 20-30 mm) (Font: Atles Climàtic de Catalunya: <http://magno.uab.es/atles-climatic/>).

En aquesta àrea hi podem trobar zones envaïdes per la formiga argentina i zones no envaïdes. La proximitat entre elles fa que presentin unes característiques físiques, ambientals i ecològiques molt similars.

La naturalesa silícica del sòl a la nostra zona, així com en la major part del massís, fa que sigui la sureda (*Quercetum ilicis galloprovinciale suberetosum*) la comunitat dominant. Aquesta la trobem representada amb diferents graus de desenvolupament, des de zones àmpliament dominades per la surera (*Quercus suber* L.), passant per zones amb un recobriment arbori menys atapeït format per sureres més o menys esparses i de vegades algun pi pinyer (*Pinus pinea* L.) en combinació amb una brolla silícicola més o menys densa en l'estrat arbustiu, i fins a zones on la comunitat vegetal esdevé gairebé totalment arbustiva i formada per diferents subassociacions de la brolla comuna d'estepes i bruc boal (*Cisto-Sarothamnetum catalaunicum*). Aquesta alternança dels diferents estadis de desenvolupament de la sureda mostra els diferents graus de pertorbació que ha sofert la zona, tant per activitats humanes com a causa del foc.

Cria experimental de colònies de la formiga argentina

El treball de laboratori realitzat als capítols 3 i 4 de la tesi requereix el manteniment en captivitat de colònies de formiga argentina. Les colònies es varen mantenir en nius artificials, els quals van ser dissenyats amb l'objectiu que permetessin:

- a. La cria d'obreres i sexuats a partir de la posta d'ous de les reines.
- b. La observació de les diferents fases de la posta per tal de poder estudiar el seu temps de desenvolupament.

- c. La manipulació de reines i obreres d'una manera pràctica i còmoda.
- d. La neteja de manera ràpida i eficient.
- e. La incubació en estufes o incubadors.
- f. El manteniment de la colònia sense risc de fugues.

Els nius dissenyats eren una variant dels creats per Passera et al. (1988), i estaven formats per una caixa de plàstic rectangular (180x115x35mm) connectada a una segona caixa de plàstic més petita (75x50x25mm) mitjançant un tub per l'interior del qual hi passava una metxa de cotó d'uns 10 mm de diàmetre. Aquesta metxa de cotó estava en contacte permanent amb un cotó amarat d'aigua a la caixa més petita, i transmetia per capil·laritat la humitat a l'escaiola que hi havia a la caixa de plàstic rectangular on hi havia instal·lada la colònia de formigues, oferint al niu d'aquesta manera una humitat que oscil·lava entre el 70 i el 80% (Figura 7). Per evitar fugues, es van recobrir les parts internes amb Fluon.

La neteja dels nius es feia diàriament, i consistia en retirar les obreres mortes i el menjar del dia anterior. Amb això s'evitava l'aparició d'àcars paràsits, ja que en proves prèvies als experiments d'aquesta tesi es va observar que la manca d'higiene podia arribar a generar l'aparició d'aquest tipus de paràsits a l'interior dels nius artificials (Figura 8).

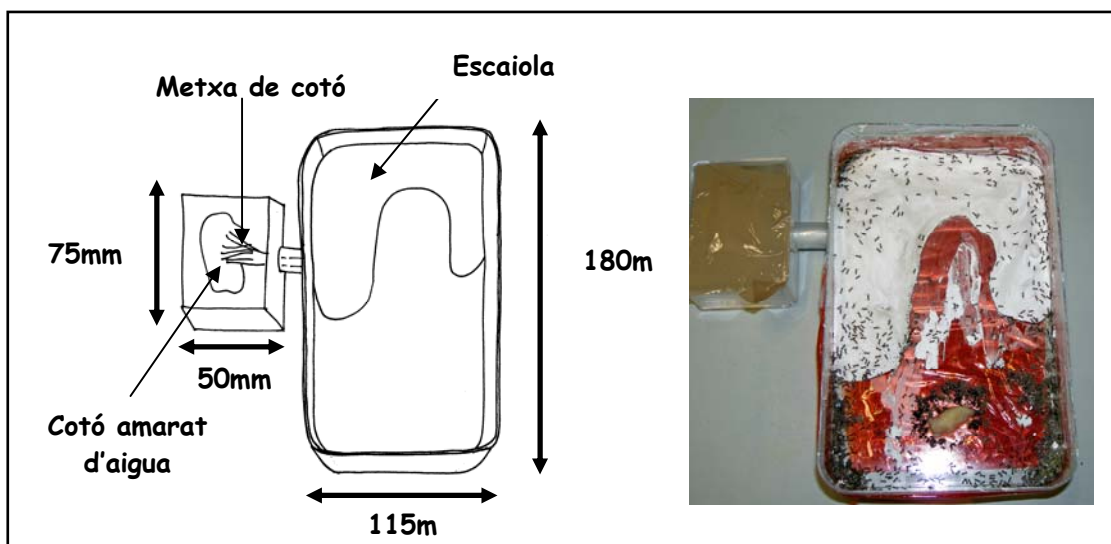


Figura 7 Esquema (a l'esquerra) i fotografia (a la dreta) dels nius artificials dissenyats per criar colònies de formiga argentina als capítols 3 i 4 d'aquesta tesi.



Figura 8 A dalt exemples de formigues argentines infestades per àcars: una obrera (a la dreta) i una reina (a l'esquerra). Les fotografies de baix mostren el grau d'infestació dels nius.

Juntament amb la neteja, diàriament es proporcionava nou menjar als nius. La dieta que es va subministrar va ser una variant de la dieta descrita per Keller et al. (1989). La variació consistia en proporcionar jalea reial enlloc de carn picada i de mel enlloc de sucre. Aquests canvis es varen fer tenint en compte la predilecció de les obreres d'aquesta espècie pels aliments líquids (Markin 1970c). Aquesta dieta va resultar ser força adequada per mantenir colònies de formiga argentina perquè permetia la producció de mascles, reines i obreres a partir d'ous postos per les reines de la colònia i una fecunditat força elevada en les reines, similar a la observada per Keller (1988) en reines incubades a la mateixa temperatura i utilitzant la dieta anteriorment referenciada. Concretament, l'aliment subministrat estava format pels següents ingredients: ½ litre d'aigua, 62 ml. mel, 5 gr. agar-agar, 60 ml. jalea reial, 4 ous, 100 gr. larves de l'escarabat de la farina (*Tenebrio molitor*), 1 gr. vitamines (Multivitamines BiCentury®).

Les larves de l'escarabat de la farina primer es picaven amb un quart de litre d'aigua i el líquid resultant es passava pel col·lador per descartar l'exoesquelet quitinós. Aquest líquid es coïa durant uns 5 minuts i quan estava tebi es mesclava amb els ous, la mel, la jalea reial i les vitamines. La resta d'aigua es barrejava amb l'agar i es portava a ebullició. Finalment es barrejava tot, s'abocava en plaques de petri i es deixava refredar fins que adquiria consistència gelatinosa. Les plaques amb el menjar es conservaven al congelador.

Es varen dissenyar uns tubs-niu per aïllar reines juntament amb algunes obreres per tal de fer els testos d'oviposició (capítols 3 i 4). A més, també es varen utilitzar per a observar el temps d'eclosió dels ous a diferents temperatures. Els tubs-niu consistien en tubs de 70 mm de llargada i 10 mm de diàmetre que estaven tapats per un primer tap, el qual era travessat per una metxa de cotó. Aquest tap estava recobert d'una placa d'escaiola a la part que donava a l'interior del tub i presentava una petita cavitat a l'altra banda la qual es va utilitzar com a reservori d'aigua. Finalment, un segon tap tapava el primer per tal que l'aigua que contenia no es vessés. Aquests tubs d'aïllament eren especialment útils per a realitzar testos d'oviposició perquè no ocupaven massa espai i perquè, en ésser transparents, permetien un còmode recompte i seguiment dels ous sota la lupa binocular (Figura 9).

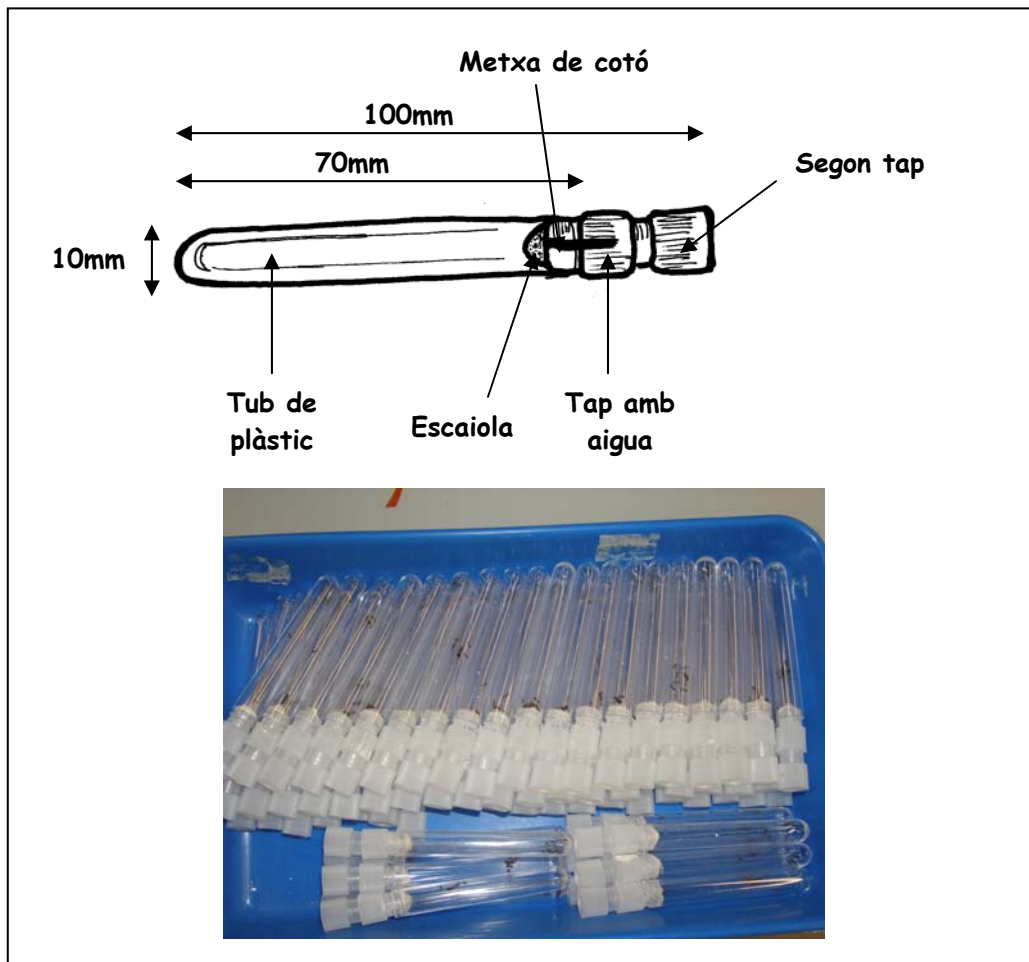


Figura 9 Esquema (a dalt) i fotografia (a baix) dels tubs d'aïllament utilitzats per realitzar testos d'oviposició a les reines (capítol 3) i per estudiar el temps d'eclosió dels ous (capítol 4).



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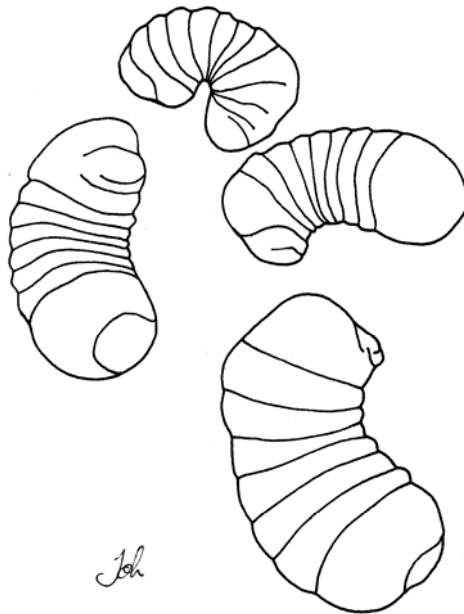
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(...) *La percepción del discurrir del tiempo es muy diferente entre los humanos y las hormigas. Para los humanos, el tiempo es absoluto. La periodicidad y la duración de los segundos se mantendrán iguales pase lo que pase. Entre las hormigas, por el contrario, el tiempo es relativo. Cuando hace calor, los segundos son muy cortos. Cuando hace frío, se alteran y prolongan hasta el infinito, hasta la pérdida de la consciencia de la hibernación. Esa elasticidad del tiempo les da una percepción de la velocidad de los hechos muy diferente de la nuestra. Para definir un movimiento, los insectos no utilizan sólo el espacio y la duración; añaden una tercera dimensión: la temperatura.*

Bernard Werber, *Las Hormigas*.



CHAPTER 1

Foraging activity and dietary spectrum of the Argentine ant (Hymenoptera: Formicidae) in invaded natural areas of the northeast Iberian Peninsula

Abril, S., Oliveras, J. and Gómez, C. *Environmental Entomology* (2007) 36(5): 1166-1173

Foraging activity and dietary spectrum of the Argentine ant (Hymenoptera: Formicidae) in invaded natural areas of the northeast Iberian Peninsula

Abstract

We analyze the foraging activity and the dietary spectrum of the Argentine ant (*Linepithema humile* Mayr) and select native ants on cork oaks from Mediterranean open cork oak (*Quercus suber*) secondary forests. The study area included invaded and non-invaded zones in close proximity. The Argentine ant's daily foraging activity was correlated to the abiotic factors studied, whereas the seasonal foraging activity is related not only to the variations in the average air temperature, but also to the trophic needs of the colony. Argentine ant workers focused their attention on protein foods during the queens' oviposition periods and during the larvae development phase, and on carbohydrate foods, such as honeydew, when males and workers were hatching. There were no significant differences over the entire year in the quantity of liquid food collected by the Argentine ant workers in comparison with the native ants studied. The solid diet of the Argentine ant on cork oaks is composed of insects, most of which are aphids. Our results have clear applications for control methods based on toxic baits in the invaded natural ecosystems of the Iberian Peninsula.

Introduction

The Argentine ant (*Linepithema humile* Mayr) is a widespread invader (see <http://www.issg.org/database>). Native to South America, it has been introduced worldwide due to human commercial activities (Hölldobler & Wilson 1990; Suarez et al. 2001). Its distribution range includes areas with Mediterranean-type climates throughout the world (Passera 1994; Suarez et al. 1998). In the Iberian Peninsula it is spread out extensively over the entire coastal band (Espadaler & Gómez 2003). Although the Argentine ant is associated with

human altered habitats (Suarez et al. 1998), there is increasing evidence of its ability to colonize natural areas with low anthropogenic disturbance (Cole et al. 1992; Human & Gordon 1996; Holway 1998a; Suarez et al. 2001; Gómez et al. 2003). It is widely reported that in these areas the Argentine ant affects the native fauna leading to changes in some essential ecological processes such as seed-dispersal (Bond & Slingsby 1984; Gómez & Oliveras 2003) and pollination (Visser et al. 1996; Blancafort & Gómez 2005) therefore having a negative impact on native biodiversity. The Argentine ant also produces negative economic effects by invading crops and plantations due to its mutualistic interactions with hemipterans which can affect the growth and production of the host plant (Buckley 1987; Ness & Bronstein 2004).

Attempts at eradicating established populations of Argentine ants have had little success. Current control methods focus on using toxic baits (Baker et al. 1985; Krushelnycky & Reimer 1998a, b; Silverman & Roulston 2001; Klotz et al. 2004). However, the success of this method depends on various factors including the attractiveness of the food for the foraging ants. Therefore, studies on the seasonal food preferences of the Argentine ant and its foraging activity periods are essential for effective use of toxic baits. To date, the most complete study on foraging and feeding behaviour of *L. humile* was done by Markin (1970a) in a California citrus grove, but in an invaded natural area only few studies present some data about the food preferences of this species (Human et al. 1998; Zee & Holway 2006).

The purpose of this paper is to examine the foraging activity times and the dietary spectrum of the Argentine ant in cork oaks from a natural ecosystem. This is a preliminary and indispensable step towards providing valuable information that can be used for developing control methods based on using baits in Mediterranean invaded natural ecosystems.

Material and methods

Study area and plant species. This study was carried out in open cork oak secondary forests dominated by *Quercus suber*, *Quercus ilex*, *Erica arborea*, *Cistus monspeliensis*, *Cistus salvifolius* and *Arbutus unedo* on the southern edge of Gavarres Massif, near the village of Castell d'Aro (NE Iberian



Peninsula) (41° 49' N 3° 00' E). The study areas are 4 km from the Mediterranean coast. The climate of this region is Mediterranean sub-humid, with 750-800 mm annual rainfall. In these areas there are invaded and non-invaded zones in close proximity (approximately 1.5 km. far apart) with similar environmental characteristics. Cork oak (*Quercus suber* L.) was chosen to study the foraging activity and dietary spectrum of the Argentine ant because it is the main tree species in the study areas and also because it allows us to study both the liquid and solid diet of this ant.

The exploitation of liquid sources in *Q. suber* throughout the year was studied to analyze how liquid food sources are used by the Argentine ant. In addition, we also studied the native ants which collect liquid sources from *Q. suber* to compare their behaviour with that of the Argentine ant.

The Argentine ant's daily foraging activity on cork oaks. We selected at random five cork oaks from the invaded zones and five more from the non-invaded ones. They were at least 10 meters far apart. In all the cases, the ants were nesting in the trees themselves (near the roots). Foraging activity was determined by counting the number of ants moving up and down the tree trunk for five minutes every hour during the sampling day.

To determine if a daily pattern existed in the Argentine ant's foraging activity sampling was carried out each hour over a 24 h period each month from May to September 2004 (warm period), and from December 2004 to January 2005 (cold period). The air temperature and the relative humidity for each sampling hour were also registered.

The correlation between the daily foraging activity and the abiotic factors studied was evaluated for each sampling day to assess whether the daily foraging activity and these factors were connected. Correlations were measured with the Pearson's correlation coefficient.

Differences between the diurnal foraging activity (from 9:00 to 20:00) and the nocturnal foraging activity (from 21:00 to 8:00) of all the sampling days corresponding to the warm period were compared using a one factor repeated measures ANOVA.

Seasonal foraging activity on cork oaks. We estimated the seasonal foraging pattern of the Argentine ant from 9:00 to 21:00, one day per month during the year 2004 and every 15 days during the years 2005/2006. The air



temperature and the relative humidity for each sampling hour were also registered.

The seasonal foraging pattern in cork oaks was also measured for the native ant community in order to evaluate possible differences between this community and the Argentine ant. In this case, sampling was carried out from 9:00 to 21:00 one day per month during a two year period (2004-2006).

The correlation between the seasonal foraging activity and the average air temperature and humidity registered during the sampling hours was measured.

Liquid food collection from cork oaks. The daily amount of liquid food collected by the ants was measured by capturing 20 ants going up the tree (without liquid) and 20 ants going down the tree (with the gaster full of liquid) every two hours during the sampling day. The captured ants were frozen to avoid losing liquid until they were weighed with a precision balance ($\pm 10^{-4}$ g) in the lab. The average difference between the weight of the ants with liquid and the weight of the ants without it was taken as an estimator of the amount of liquid food collected by twenty workers. Then was estimated the average amount of liquid food transported by a single worker. This value multiplied by the estimated foraging activity of the whole sampling day was used to estimate the daily amount of liquid food collected by the ants.

Solid prey captured by the Argentine ant on cork oaks. Every two hours for five minutes the ants that returned to the nest with prey visibly between their mandibles were counted as well as the total number of ants that entered the nest during this period. We calculated the proportion of ants that returned to the nest with solid prey between their mandibles in relation to the total number of ants which came back to the nest. Samples were taken from 9:00 to 21:00 on the same sampling days as for the Argentine ant's seasonal foraging activity observations. The solid prey captured by the Argentine ants was also collected and taxonomically classified in the laboratory.

All statistical analyses were run under the SPSS statistical package for Windows version 12.0.1 (Spss Inc., Chicago, IL, USA).

Results

The Argentine ant's daily foraging activity on cork oaks. The Argentine ant's daily foraging activity on cork oaks was continuous throughout the day and night during the warmer months (May to September) (Figure 1). During this period, nocturnal foraging activity was significantly higher than diurnal foraging activity ($F_{1,299} = 114.351, P < 0.001$).

The diurnal foraging activity pattern was negatively correlated with the air temperature and positively correlated with humidity for all five months sampled (Pearson's correlation coefficient: (temperature) $r > -0.573, P < 0.001$; (humidity) $r > 0.593, P < 0.001$).

During the cold months (December to February) foraging activity was mainly reduced to daily hours and correlated highly with the environmental temperature (Pearson's correlation coefficient: $r > 0.623, P < 0.001$). The foraging activity on the tree trunk stopped completely below five degrees centigrade (Figure 1). However, there was no association between environmental humidity and the daily foraging activity during these months (Pearson's correlation coefficient: $r < -0.299, P > 0.1$).

Seasonal foraging activity on cork oaks. The foraging activity of the Argentine ant on cork oaks was continuous throughout the year (Figure 2). However, in the colder months the foraging activity was much lower in comparison with the rest of the year. In May the foraging activity seems to rise until June and July, which are the months with maximum foraging activity, followed by a sudden decrease in August. There was a new increment in September, and after this period foraging activity gradually went down in association with the environmental temperature. We found a positive correlation between the temperature and the seasonal foraging activity on cork oaks (Pearson's correlation coefficient: $r = 0.729; P < 0.001$) (Figure 2). Whereas there was no association between the seasonal activity and the average humidity registered during the sampling hours (Pearson's correlation coefficient: $r = -0.177; P > 0.1$).

In the non-invaded trees, the only species present were the native ants *Crematogaster scutellaris* (Olivier) and *Lasius cinereus* Seifert.

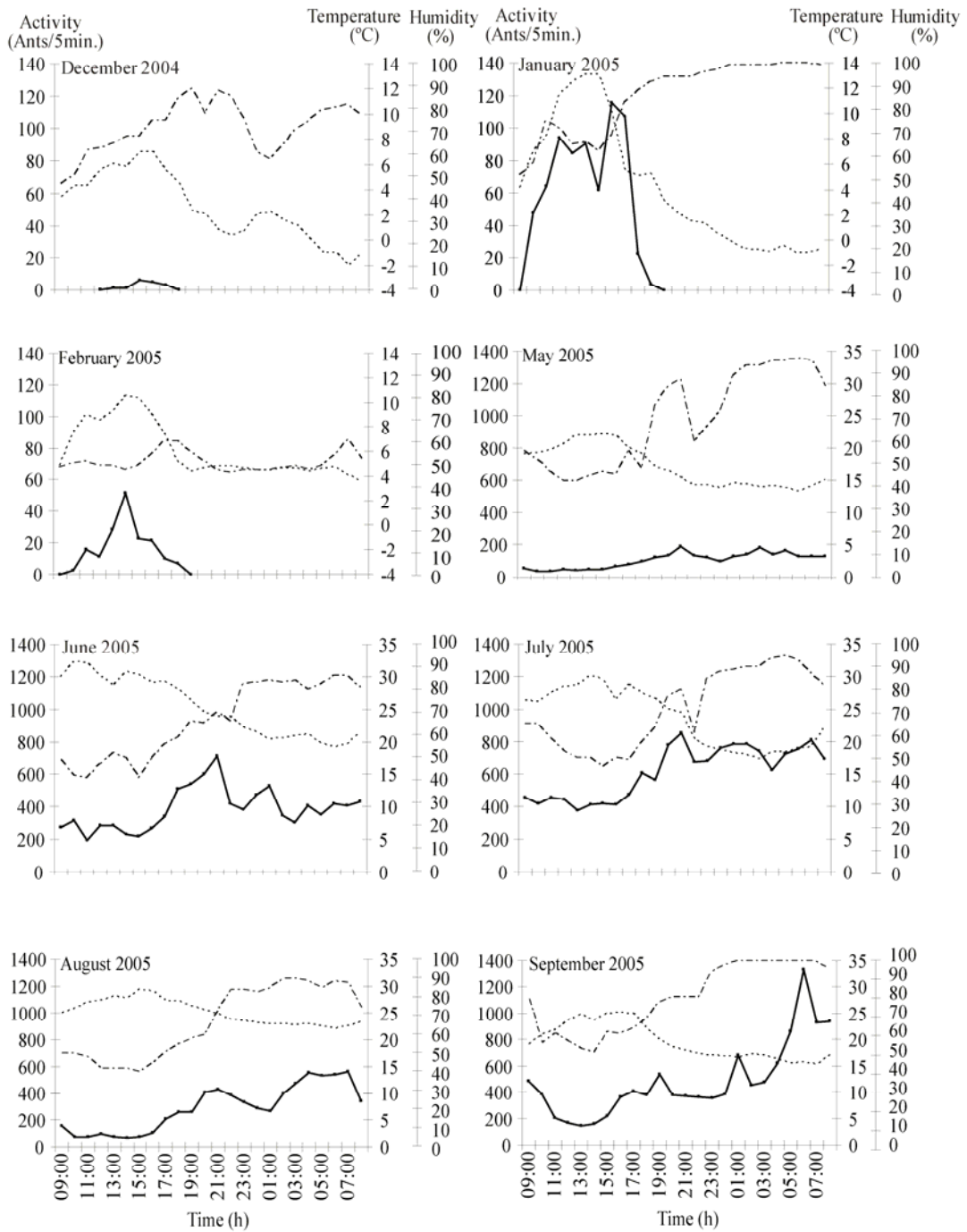


Figure 1 Average daily foraging activity (number of ants during five minutes) of the Argentine ant on five cork oaks during the warm and cold period. The solid line represents the average foraging activity of workers, the dotted line (.....) represents the air temperature registered each hour of sample and the dashed line (----) represents the relative humidity recorded each hour of sample.

The native ant's seasonal foraging activity on cork oaks was always lower than the seasonal foraging activity of the Argentine ants (Figure 2 and 3).

In addition, the native ants studied had a hibernation period from late November until the beginning of springtime (March), whereas the invaders didn't have a hibernation period.

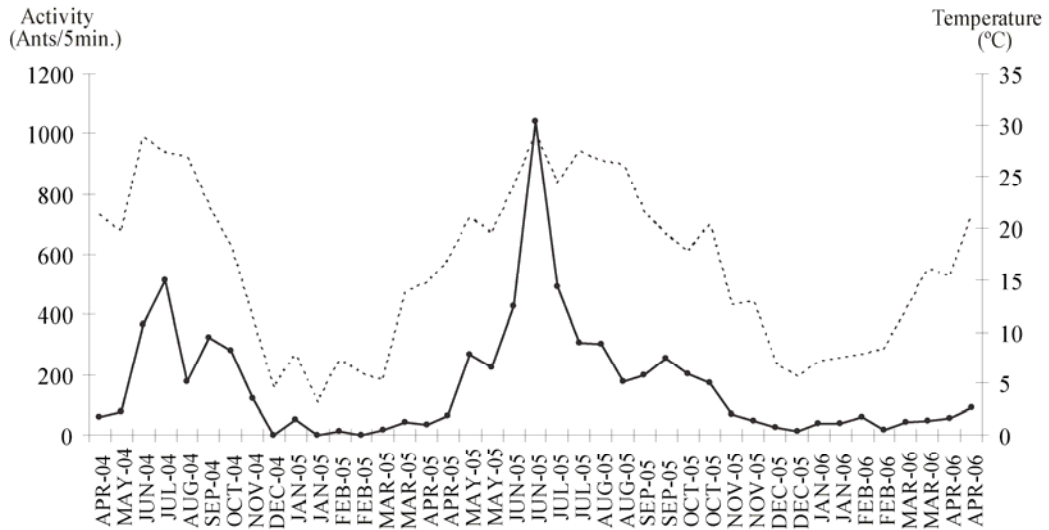


Figure 2 Seasonal foraging activity on cork oaks by the Argentine ant. The solid line represents the average foraging activity of workers (number of ants during five minutes) during the sampling day on five cork oaks. The dashed line represents the average daily temperature during the sampling day.

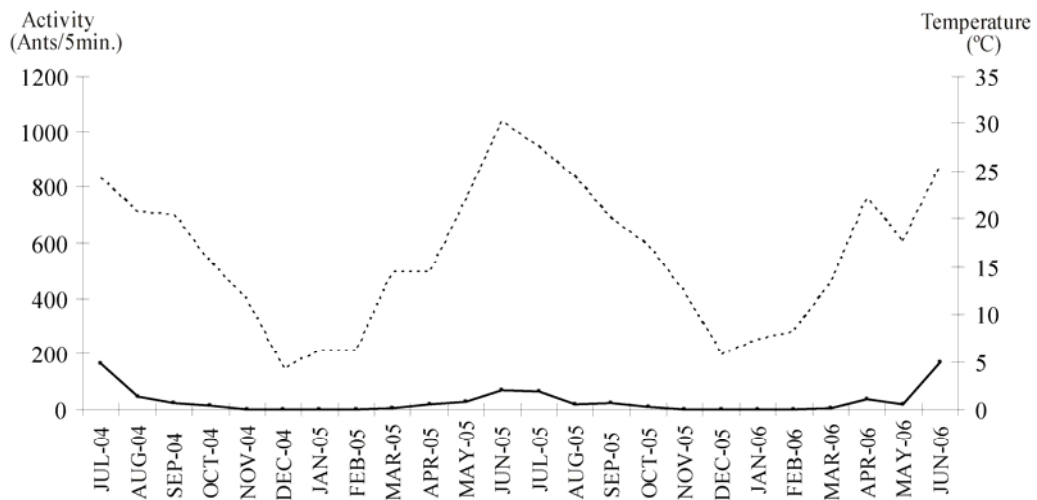


Figure 3 Seasonal foraging activity on cork oaks by the native ants studied: *Crematogaster scutellaris* and *Lasius cinereus*. The solid line represents the average foraging activity of workers (number of ants during five minutes) during the sampling day on five cork oaks. The dashed line represents the average daily temperature during the sampling day.

Liquid food collection from cork oaks. The daily amount of liquid food collected by the Argentine ants during the winter months was quite low in relation to the rest of the year (Figure 4). After this period, the liquid collection increased until June and July, which were the months of maximum liquid extraction. From those months on, the daily amount of liquid collected decreased gradually until December, but with a little peak in October.

Argentine ants collected more liquid food than the native ants during the hibernation period of the latter (November to March), and also during the month previous to this period (October). On the other hand, the native ants collected, in general, more liquid food than the Argentine ants during their periods of maximum activity (May to August). Nevertheless, these differences were little noticeable for the most part of the year (Figure 4).

The seasonal foraging activity pattern of the Argentine ant was closely related to the seasonal fluctuations of the estimated amount of liquid food collected in 24 h.

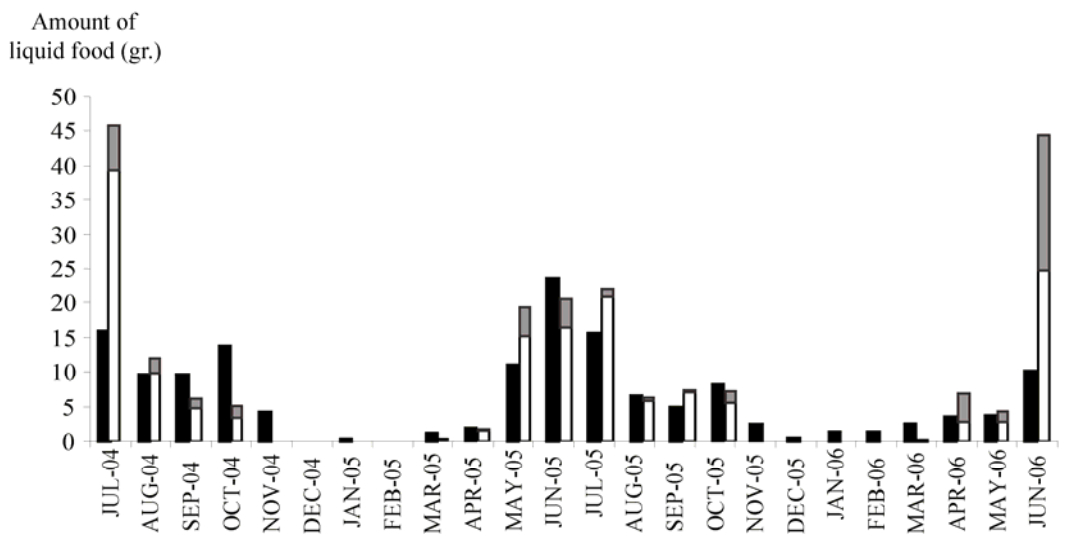


Figure 4 Estimated amount of liquid food collected in 24hrs by the Argentine ant (black bars) and by the native ants studied (white and grey bars): *Crematogaster scutellaris* (white bars) and *Lasius cinereus* (grey bars) on five cork oaks.

Solid prey captured by the Argentine ant on cork oaks. The Argentine ants (Figure 5) foraged for solid prey on cork oaks throughout the year except on the coldest days when foraging was null. Despite this almost continual effort foraging of solid prey was quite low since, even in the periods of maximum solid prey collection, less than six percent of workers that returned to the nest carried solid prey between their mandibles.

Spring (March to May) was the season of maximum foraging of solid preys followed by a gradual downward slope until July, which was the month of minimum solid prey foraging. There was another period of active foraging in autumn (September to December) with a peak in October.

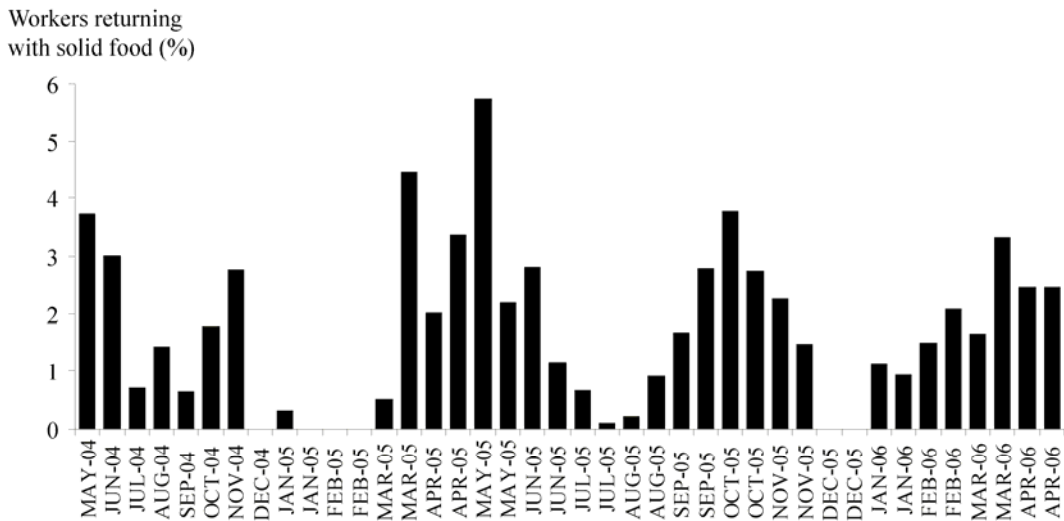


Figure 5 Proportion of Argentine ant workers returning to the nest with solid food collected from cork oaks.

Argentine ant workers collected a diversity of prey from the cork oaks (Table 1). The vast majority of prey was arthropods, both adult and immature forms. Within the immature forms, all the prey was insects whereas within the adult forms there were some arachnids. There were also a small proportion of vegetal remains (less than 2% of the total sample). The main order collected was the order Sternorrhyncha. Within this group almost all the samples were aphids. Some samples showed an advanced dehydration level and others were still alive when they were collected.

Table 1 Number and percentage of solid prey taken to the nest by the workers of the Argentine ant.

TYPE OF PREY	N	%	TYPE OF PREY	N	%
Adult stage	439	41.26	Immature stage	552	51.88
Sternorrhyncha	245	23.03	Sternorrhyncha	306	28.76
Thysanoptera	46	4.32	Lepidoptera	114	10.71
Psocoptera	45	4.23	Coleoptera	50	5.43
Hymenoptera	37	3.48	Heteroptera	28	4.70
Diptera	30	2.82	Unidentified nymph	19	1.78
Acarida	13	1.23	Thysanoptera	16	1.50
Coleoptera	10	0.940	Orthoptera	8	0.752
Collembola	8	0.752	Dermaptera	4	0.376
Araneida	4	0.376	Diplura	2	0.188
Orthoptera	1	0.094	Dictyoptera	2	0.188
			Hymenoptera	2	0.188
Unidentified	63	5.92	Heteroptera nymph	1	0.094
Plant remains	10	0.940			

Discussion

The Argentine ant shows seasonal variation in daily foraging activity patterns on cork oaks. During spring and summer the foraging activity is continuous all around the clock. This continual activity by *L. humile* has also been observed in California by Human et al. (1998). The native ant species studied, *Crematogaster scutellaris* and *Lasius cinereus*, also have a continual foraging activity pattern on cork oaks like other ant species' such as *Camponotus cruentatus* (Alsina et al. 1988), *Iridomyrmex purpureus viridiaeneus* (Greenaway 1981) and *Camponotus detritus* (Curtis 1985). Some authors have proposed to explain this behaviour by the fact that aphids produce honeydew continuously throughout the day and the workers tend to collect it at the same rhythm.

In the days of continual food gathering, the Argentine ant foraging activity on cork oaks is higher during the night. This behaviour is related to the abiotic factors studied (humidity and air temperature). It seems that the daily foraging activity of the Argentine ant on cork oaks during spring and summer days is limited by lower humidity and higher temperatures. Changes in these two factors can lead to changes in the daily activity pattern of this species. For this reason we believe that the continual foraging activity observed during the spring

and summer days can be explained by the abiotic conditions that occur throughout these months, which allow food gathering over a 24 hour period.

As in other ant species (Sanders 1972; Bernstein 1974; Alsina et al. 1988) the winter foraging activity on cork oaks became exclusively diurnal and related to the temperature but not to the relative humidity.

Below 5 °C the Argentine ant stops its foraging activity on cork oaks completely. Our results are in agreement with Markin's (1970a) results. However, the native ant species studied have a hibernation period from November to March, whereas the Argentine ants do not have a hibernation period and they are always busy as long as the air temperature allows them. This complete lack of a hibernation period for *L. humile* was also reported by Benois (1973) in the Antibes region (French Riviera) and Holway (1998b) in northern California.

The seasonal foraging activity pattern of the Argentine ant on cork oaks is closely related to the seasonal fluctuations in the amount of liquid collected by the workers. This may result from the fact that the main food source collected by this species is liquid food (Markin 1970a).

The Argentine ant gathers liquid the whole year round. This behaviour could be related to a lack of stored food caused by a great availability of liquid food throughout the year in the trees, basically honeydew secreted by Hemipterans.

Despite this continual liquid extraction, the average amount of liquid sources introduced into the nest by the Argentine ants during the winter months was almost insignificant. This may result from the low foraging activity on the trees observed during this period, which is most probably caused by the low temperatures. In spring the daily amount of liquid collected appeared to increase. It seems that this increment is not only related to the rise in the air temperature but also may be related to the beginning of the reproductive cycle that leads to the workers increasing their own activity to carry out several tasks, such as taking care of the brood or feeding the queens. Moreover, egg-laying is strongly influenced not only by abiotic conditions like temperature or humidity but also by the amount of food ingested (Benois 1973). The liquid food collected from cork oaks, most of it likely honeydew, may provide the workers with the necessary energy to carry out their work (Carroll & Janzen 1973). Therefore, it

is during these strong work periods when the foraging activity on cork oaks, and consequently the collection of liquid sources, appears to increase. In June and July, the collection of liquid is at its maximum. During these months a large number of males and workers hatch into the nest producing a strong demographic increase (Markin 1970b; Benois 1973). Since the main part of the liquid carried by the workers may be likely sugary liquids from Hemipterans exudates and the main consumers of this type of food in Argentine ant nests are males and workers (Markin 1970c), this sharp increment in liquid extraction may be related to the workers' need to meet the trophic requirements of these castes.

The second peak of liquid extraction in autumn seems to be related to the second reproductive period of this species described by Benois (1973). This new period of intense work would again cause an increment in the amount of liquid introduced into the nest. After this period, the amount of liquid food collected decreases with the fall in the temperature until the following spring, when the cycle begins again. Of course, it could be other possible non-excluding explanations to the seasonal differences in liquid sources collection by the foraging workers of the Argentine ant, such as the seasonal availability of this source on the trees.

Argentine ant workers extract more liquid from cork oaks than the native ant species during the winter months and during the adjacent months because of the hibernation period of the latter. However, the native ants collect much more liquid than the invaders during their months of maximum activity from May to July, even though their foraging activity on the trees is lower. These differences are due to the larger body size of the native species which allows them to gather larger quantities of liquid than the tiny Argentine ants. We observe that, in general, the Argentine ant workers collect throughout the year a similar amount of liquid food from cork oaks in relation to the native ants studied. Therefore, if we take into account that the direct physicochemical damage produced by the hemipterans can cause significant negative damage to the host plant (Buckley 1987), the possible damage caused by the ant-hemipteran mutualism to the host plant wouldn't get better or worse after the invasion. Nevertheless, further research is necessary to assess if the annual or monthly liquid extraction by Argentine ants and native ants has a significant



negative effect on the growth and reproduction of the host plant and also if there are differences in the amount of plants affected.

We observed two peaks of solid prey foraging during the year on cork oaks. The first occurred from March to June with a peak in May and the second from September to November with a peak in October. We must consider insects as a large source of proteins. The main consumers of proteins in the nest are larvae and queens because this nutrient is essential for the optimal growth and development of larvae and for the production of eggs by queens (Markin 1970c; Rust et al. 2000). Therefore, the high prey capture values found in spring with the peak in May correspond with the maximum oviposition rate period of the queens and with the maximum larvae densities in the nest (Benois 1973). The other period of intense foraging effort is in autumn, from September to November, coinciding with the second but weaker oviposition period of the year and with a second maximum in larvae in the nest in October (Benois 1973). Thus, one possible explanation to the seasonal variation of solid prey foraging by the Argentine ant workers is that it varies seasonally depending on the colony's demand for protein in relation to its colony growth phase.

These results are in agreement with the results obtained by Rust et al. (2000) in a California citrus grove. Edwards (1951) also came to the same conclusion observing the foraging ecology of the ant species *Lasius niger*. The workers of this species focus their attention on protein foods during their period of colony growth when there is a high density of developing larvae in the nest, whereas during the pupation period of the larvae they prefer to search for carbohydrate food to feed the adults.

The proportion of workers which enter the nest with solid prey in their mandibles is quite low, less than six percent. This value is similar to the values obtained for other ant species (Gotwald 1968; Sanders 1972; Lévieux 1975; Curtis 1985; Alsina et al. 1988); all of that had a low proportion of workers introducing solid prey into the nest with values between 3% and 5.9%. As most workers that enter the nest from cork oaks are carrying liquid food, the explanation for these low values could be due to the fact that the main food source of Argentine ants may be liquid food. Similarly, Markin (1970a) found that approximately 99% of the material carried into the nest by the Argentine ant workers in a Californian citrus grove is liquid food.

Small insects, both adults and immature forms, are the main group of prey collected by the Argentine ant workers. The captured prey is mainly dehydrated, although we also observed a small percentage of living prey being transported to the nest. This fact suggests that, in reference to the solid diet, *L. humile* is basically a scavenger ant, but can also occasionally play the role of hunter of small specific insects or tiny apterous forms such as small larvae or aphids. Small insects are also the main source of solid prey of other ant species (Lévieux 1975; Retana et al. 1986). Markin (1970a) also found that a large amount of the solid prey captured by the Argentine ants on citrus trees were hemipterans. He supposed that they were dead when the ants collected them, whereas in this study we found some living aphids being transported to the nest by the *L. humile* workers.

Since more than 50% of the prey collected from cork oaks by the Argentine ant workers are aphids, it is probable that these are not only protected by the workers for their honeydew source, but are also used as a source of protein. It seems that this behaviour is not singular of the Argentine ant. In that respect there are other ant species which also collect hemipterans as a protein source and at the same time establish mutualistic interactions with them in order to obtain carbohydrates from their honeydew. This is the case of *Oecophylla longinoda* (Way 1954), *Lasius flavus* (Edwards 1951), *Lasius niger* (Pontin 1958) and *Formica rufa* (Skinner & Whittaker 1981). It has been reported that *Formica rufa* preys only on the non-myrmecophilous species of hemipteran *Drepanosiphum platanooides* (Pontin 1958) and establishes a mutualism with the myrmecophilous hemipteran species *Periphyllus testudinaceus* (Skinner & Whittaker 1981). By doing this, they ensure a protein intake without harming their sugary sources. This may be a good explanation for the case of the Argentine ant in our study area, although further research on this point is necessary.



Conclusions

The Argentine ant's daily and seasonal foraging activity in a Mediterranean open cork oak secondary forest is strongly related to the abiotic conditions, especially to air temperature. Thus, during the coldest months the foraging activity of this species is limited to the warmest periods of the day, and during the spring and the summer months their foraging activity occurs mainly during the night. Moreover, the Argentine ant workers change their food preferences depending on the phase of the reproductive cycle, collecting more protein food in spring (March to late May) and autumn (September to November) to feed the queens and the developing larvae, and more carbohydrate food during the rest of the year, especially during June and July, in order to feed the newborn males and workers of the colony. These results are essential for improving the current knowledge on the Argentine ant's foraging behaviour in invaded natural areas in order to initiate control methods based on using baits.

Acknowledgements

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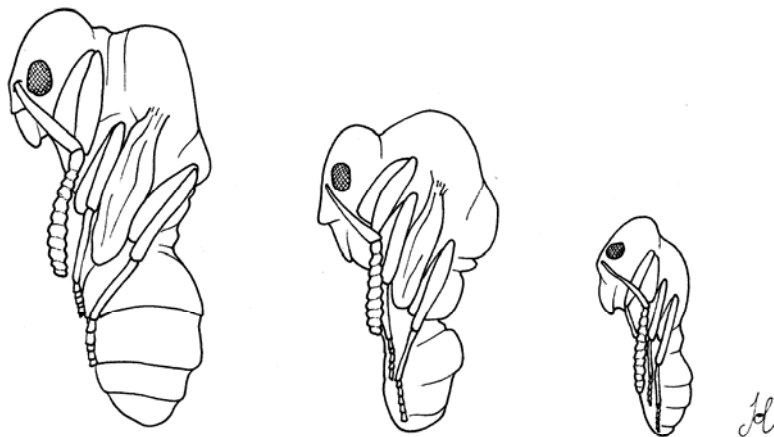
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“(...) La catàstrofe se produjo aquella mañana muy temprano. El cielo se oscureció de repente. Los Dedos rodeaban por completo la ciudad federal de Giu-li-kan. Las legiones de élite salieron en seguida, igual que los grupos de artilleras pesadas. Se intentó todo. Y todo fue en vano (...) Una gigantesca estructura plana y dura desgarró la tierra y se hundió al lado de la Ciudad, destrozando salas, pisoteando huevos, cortando corredores. La estructura plana se inclinó luego y levantó toda la Ciudad. (...) Todo ocurrió muy deprisa. Fuimos arrojadas a una especie de gran concha transparente y rígida. Nuestra ciudad fue puesta patas arriba. Las salas nupciales quedaron trastornadas, y las reservas de cereales saltaron por los aires. Nuestros huevos se desparramaron por todas partes. Nuestra reina fue capturada y herida. Yo debo mi salvación únicamente a una série de brincos rabiosos que me permitieron saltar a tiempo por encima del borde de la gran concha transparente.”

Bernard Werber, *El día de las hormigas*.



CHAPTER 2

The effect of seasonal dynamics on queen densities of the Argentine ant (*Linepithema humile*) in an invaded natural area of the NE Iberian Peninsula

Abril, S., Oliveras, J. and Gómez, C. *Sociobiology*. In Press.

The effect of seasonal dynamics on queen densities of the Argentine ant (*Linepithema humile*) in an invaded natural area of the NE Iberian Peninsula

Abstract

The annual elimination of large numbers of Argentine ant queens near the advance front of an invasion could be a useful tool for weakening the species' dispersion and, therefore, limiting its establishment in non-invaded areas. However, before carrying out trials to test the effectiveness of this method it would be essential to have sufficient knowledge of the effect of seasonal dynamics acting on the queens' densities of the species in order to determine the most favourable period of the year to act.

We analyzed the seasonal densities and nest dynamics of Argentine ant queens in an invaded Mediterranean natural ecosystem. We observed that the queens' density varied depending on the season of the year and that this variation was mainly due to the seasonal dynamics of nest aggregations in winter and ant dispersions in summer. The greatest densities per litre of nest soil were observed in winter (December to March approximately) and the lowest densities were observed in summer (June to July). This information is essential for improving current knowledge of the Argentine ant's biology and developing control methods based on the elimination of queens in invaded natural areas.

Introduction

The Argentine ant (*Linepithema humile* Mayr), well-known as an invasive ant species (McGlynn 1999), has spread world-wide (Hölldobler & Wilson 1990; Suarez et al. 2001) as a result of human commercial activities in habitats usually associated with human modification (Holway 1998a; Suarez et al. 2001). However, its ability to occupy non-altered habitats (Cole et al. 1992; Holway



1998a; Suarez et al. 2001; Gómez et al. 2003) has also been widely reported. Its distribution range includes areas with Mediterranean-type climates throughout the world (Passera 1994; Suarez et al. 1998). In these areas, the Argentine ant has impacted native ant faunas leading to changes in arthropod communities (Human and Gordon 1996; Human and Gordon 1997; Holway 1998b; Suarez et al. 1998), ant-vertebrate interactions (Suarez et al. 2000) and ant-plant relationships (Bond & Slingsby 1984; Visser et al. 1996; Gómez & Oliveras 2003; Blancafort & Gómez 2005). Its presence has also had economic effects, such as damage to infrastructure (Chang & Otta 1990), and negative effects on crops and plantations due to its mutualistic interactions with hemipterans which can affect the growth and production of the host plant (Buckley 1987; Ness & Bronstein 2004).

Attempts at eradicating established populations of Argentine ants have had little success. Current control methods focus on the use of toxic baits (Baker et al. 1985; Krushelnycky & Reimer 1998a, 1998b; Silverman and Roulston 2001; Klotz et al. 2004). However, these kinds of methods are not appropriate for controlling the plague in protected natural areas where the use of chemical methods is not allowed. Therefore, the development of other methods is necessary for controlling the invasion. This is a serious problem since the probability of eradication of an invasive species decreases drastically as its distribution range increases (Myers et al. 2000) and, in consequence, the elimination of the plague in areas where the Argentine ant population has been expanding for decades is practically impossible. In such areas slowing the rate of spread is the most viable method of control. The annual elimination of large numbers of queens near the advancing front of an invasion could prove a useful tool for weakening its dispersion and, therefore, limiting its establishment in other non-invaded areas. However, before carrying out trials on the effectiveness of this method it would be essential to have sufficient knowledge of the seasonal dynamics of the queens in order to determine the most favourable period of the year to act. To date, only few studies present any data about Argentine ant queen numbers in natural areas (Keller et al. 1989; Ingram 2002) and none analyze the seasonal dynamics of queen densities in natural nests. It is also important to take into account seasonal nest movements in colonies that can directly affect such densities. It seems that Argentine ant



colonies are relocated seasonally in accordance with their physiological requirements in relation to temperature and humidity (Newell & Barber 1913; Benois 1973; Heller & Gordon 2006). In winter they gather into large aggregations called “winter colonies” to increase nest temperatures. In spring, they bud into smaller nests and disperse to other areas with enough humidity to rear larvae. These smaller nests become totally established in summer and are called “summer colonies”. In autumn, the ants move back to the large aggregation formations (Newell & Barber 1913; Benois 1973; Heller & Gordon 2006).

The purpose of this study is to both confirm these seasonal nest dynamics in a Mediterranean invaded natural ecosystem and analyze the way they act on queen densities in natural nests. This is an essential first step to initiating control methods for slowing the rate of spread based on the elimination of queens without using chemical procedures.

Materials and methods

Study area. This study was carried out in an open cork oak secondary forest dominated by *Quercus suber*, *Quercus ilex*, *Erica arborea*, *Cistus monspeliensis*, *Cistus salvifolius* and *Arbutus unedo* on the southern edge of the Gavarres Massif, near the village of Castell d’Aro (NE Iberian Peninsula) (41° 49’ N 3° 00’ E). The study area is 4 km from the Mediterranean coast. This region has a Mediterranean sub-humid climate, with 750-800 mm annual rainfall.

Ascertaining Argentine ant seasonal migrations. To determine if the Argentine ant presented a seasonal migration pattern in the study area we placed a total of 140 artificial winter nests distributed over the invaded area (Figure 1) in the form of five blocks each containing 28 nests. The nests themselves were plastic containers (4.5 x 4.5 x 6cm high) full of soil from the study area and decomposing vegetable matter, since the bibliography refers to piles of decomposing vegetable matter as being the ideal location for Argentine ant winter colonies (Newell & Barber 1913). The artificial nest surface was covered with a stone as the Argentine ants in this area nest mainly under stones (pers. obs.). The nests were located at the end of August 2005 and were



checked for the presence/absence of Argentine ant colonies every 15 days in winter 2006 (December 2005-February 2006), summer 2006 (June-August 2006) and winter 2007 (December 2006-February 2007).

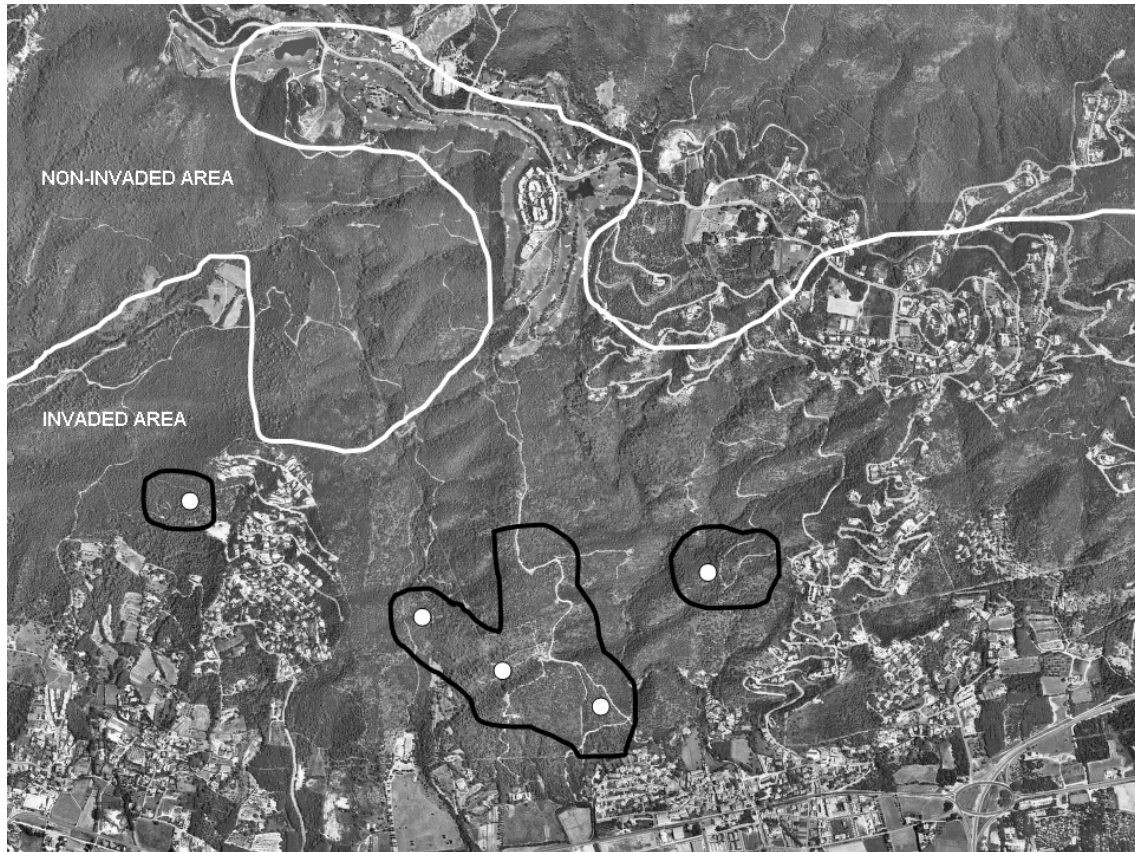


Figure 1 Geographical distribution of the Argentine ant invasion in the population area of Castell d'Aro (NE Iberian Peninsula) and location of both artificial winter nests and the areas where the Argentine ant nest soil was sampled. A white circle indicates each block of 28 artificial winter nests. The area delimited by the black line represents the territory sampled to assess queen densities. Modified from: Institut Cartogràfic de Catalunya (ICC). Scale map: 1:5000.

Seasonal queen densities of the Argentine ant. To assess queen densities in the study area, soil from natural nests was mechanically crumbled over 1000cm^3 plastic boxes and the total number of queens present in the boxes was later counted in situ. Random samples of soil were taken every 15 days from March 2006 to March 2007 from multiple areas of the study area (Figure 1). Soil samples were only collected when brood were visible, since queens were usually situated inside the nest near the brood (pers. obs.).



Differences between the queen densities of the winter colonies (according to our results from January to March) and the summer colonies (according to our results from June to August) were compared using a single factor ANOVA. Both variables were \sqrt{x} -transformed prior to analysis to improve homoscedasticity. All statistical analyses were run under the SPSS statistical package for Windows version 12.0.1 (Spss Inc., Chicago, IL, USA).

Results

Ascertaining Argentine ant seasonal migrations. Only 16 of the 140 artificial winter nests were occupied by Argentine ants during winter 2006. In summer 2006 all the occupied nests were abandoned. During winter 2007 a total of 20 nests were occupied, including 8 of the nests occupied the previous winter, that is to say fifty per cent of the nests occupied the previous winter were also occupied the next winter.

Seasonal queen densities of the Argentine ant. Queen densities varied depending on the season of the year (Figure 2). In April we observed a marked fall in densities, followed by a more gradual fall in May. In June, July and August we observed the lowest densities per litre of soil and from August to the end of the year there was a gradual rise, reaching a maximum in winter (January-March) (Figure 2). The annual mean (\pm SD) number of queens per litre of soil from natural nests was 5.04 ± 3.19 ($n = 240$). The month which reported minimum mean (\pm SD) queen densities was June (0.16 ± 0.25 ; $n = 12$), while the month which reported maximal mean (\pm SD) densities was February (9.56 ± 5.13 ; $n = 16$). The maximal queen density observed was in November with a total of 38 queens in a litre of soil. Queen densities from the winter colonies (mean \pm SD = 9.05 ± 6.5 ; $n = 60$) were significantly higher than the queen densities observed in the summer colonies (2.03 ± 3.71 ; $n = 51$) ($F_{1,110} = 75.366$, $P < 0.001$).



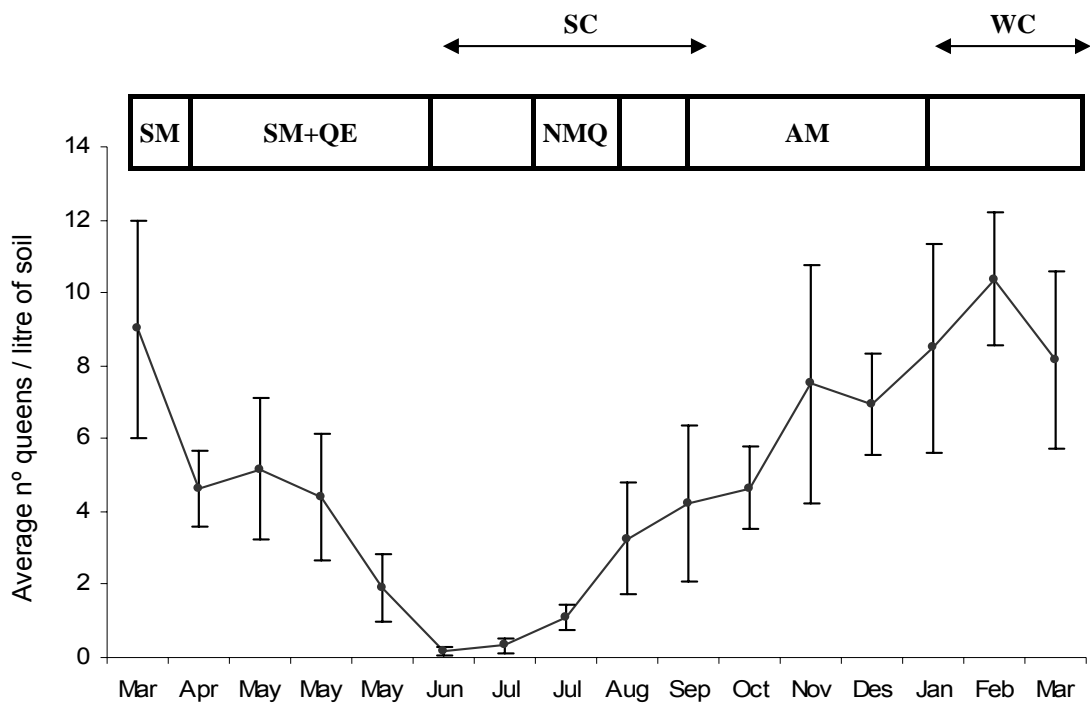


Figure 2 Seasonal densities of queens in natural nests of the Argentine ant. AM: autumn migration; NMQ: new mated queens; QE: queen execution; SC: summer colonies; SM: spring migration; WC: winter colonies. The error bars are the standard errors.

Discussion

The Argentine ant's seasonal dynamics observed in the study area were consistent with the studies carried out by Newell & Barber (1913), Benois (1973) and Heller & Gordon (2006) in New Orleans, southern France and California respectively. We observed that this species tended to relocate colonies depending on the season of the year. In winter we found them occupying artificial winter nests to increase nest temperatures and in spring dispersing to other areas to find more suitable conditions to rear the brood (Newell & Barber 1913; Benois 1973). This resulted in a total absence of colonies in the artificial winter nests in summer. The following winter we again found new colonies in the artificial nests, suggesting a new winter aggregation period.

The low occupation of nests observed in this study suggests that the study area, characterized by its high density of Argentine ant nests, still has a considerable availability of suitable nests sites. The accessibility of such sites in



areas with high concentrations of nests demonstrates once more the amazing capacity of this species to nest anywhere (Newell & Barber 1913).

The seasonal dynamics on queen densities observed in the present study can be largely explained by the seasonal cycle of nest aggregations in winter and nest dispersions in summer. The highest measurements of queen densities in winter are explained by the large nest aggregations occurring during this period (Newell & Barber 1913; Benois 1973; Heller & Gordon 2006), which constitute the “winter colonies” (Newell & Barber 1913). The marked decrease in queen densities in April is probably due to the dispersion occurring in spring (Newell & Barber 1913; Heller & Gordon 2006) which along with the seasonal killing of queens detected by Keller et al. (1989), causes a dramatic decrease in the number of queens per litre of nest soil in May. In summer (June-July) we find the lowest queen densities of the year. This low number of queens per litre of nest soil is probably related to the foundation of “summer colonies” characterized by their small size, and—as a result—their low concentration of biomass (Newell & Barber 1913; Heller & Gordon 2006). We must also take into account that during the months of June and early July the colony is still rearing the sexual brood and in consequence, the newly mated queens have not appeared yet (Benois 1973). This could also be a cause of the very low density of queens that was detected. In August, queen density measurements reach similar levels to those obtained in early May, before the time when workers kill around 90% of the queens (Keller et al. 1989). The rise in their numbers during this period can be related to the appearance of the newly mated queens (Benois 1973). From September to January there is a gradual increase in queen density measurements. This increase is probably related to a new aggregation period leading to the foundation of the “winter colonies” that seem to be present in the study area from January to March. Moreover, from April to June and from September to January we found a marked variation between queen densities in different samples, signifying that during the migration period the study area comprised both summer nests and winter nests. These results suggest that the migration period detected in spring and autumn is a slow process which takes place at different moments depending on the individual nest, and that this seasonal migration does not take place synchronously among all the nests of the invaded area.



According to Heller & Gordon (2006), the Argentine ant moves back to nest aggregation sites from the previous winter in late autumn. This explains the high proportion of artificial winter nests reoccupied in winter 2007.

To develop control methods based on the elimination of Argentine ant queens, we must take into account not only the seasonal variation of queen densities in the nest soil, which are directly affected by the dynamics of the species' nest aggregation and dispersion, but also the activity periods of both the Argentine ant and the native species. It seems that the presence of native ants in the advancing front can offer resistance to the invasion progress (Heller et al. 2006), and in the case of our study area, this is the most likely explanation for the backward movement in the advance front observed in April (Casellas 2004). Therefore, the best time of the year for attacking and eliminating the queens in the study area, and in other similar invaded ecosystems, would be winter, from January to March. The reasons for this are firstly because this is the period when queen densities in the nests are at their maximum, and secondly because in March the advance of the plague is greatest (Casellas 2004) due to the fact that the native ants of the study area are hibernating and cannot compete (Casellas 2004; Abril et al. 2007) and, therefore, offer resistance to the invasion. Systematically eliminating queens during the winter months from nests near the front of the invasion could work as a complementary way of offering resistance to the invasion in view of the lack of competition from native ants during this season (Casellas 2004). The elimination of queens could also work as a mechanism for reducing the high-level production of new individuals that takes place in spring and summer (Markin 1970; Benois 1973) and weaken the ants' dispersion by limiting their establishment in new areas.

This is the first study of Argentine ant queen densities in natural areas which report the number of queens using the amount of nest soil as a measure of queen density. The information given by the present study is essential to initiate control methods for slowing the rate of spread based on the elimination of queens without using chemical procedures in natural invaded areas.



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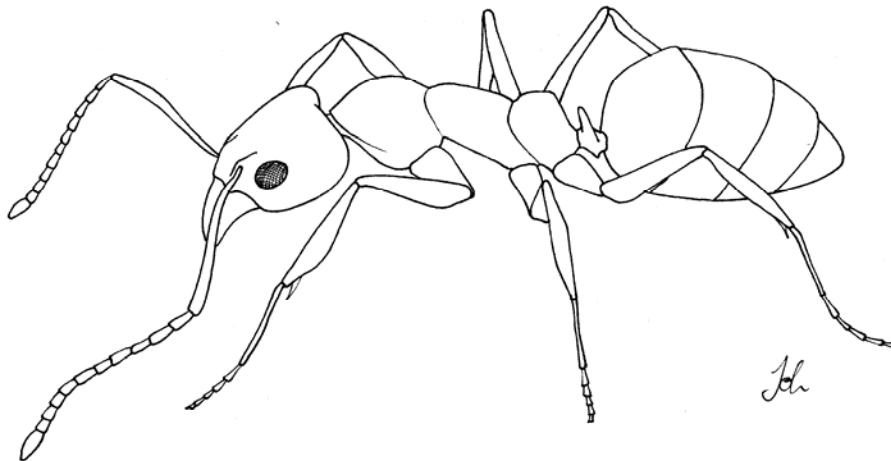


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“(…) Hace ya algunos días que Madre ha salido del gran sueño, y desde entonces no ha cesado de poner. Su abdomen, diez veces más voluminoso que lo normal, está recorrido por contínuos espasmos. En ese mismo momento, deja ir ocho huevos delgaduchos, de un color gris claro con reflejos nacarados. El futuro, redondo y deslizante, escapa de sus entrañas y rueda por la estancia, e inmediatamente las nodrizas se hacen cargo de él.”

Bernard Werber, *Las Hormigas*.



CHAPTER 3

Effect of temperature on the oviposition rate of Argentine ant queens (*Linepithema humile* Mayr) under monogynous and polygynous experimental conditions

Abril, S., Oliveras, J. and Gómez, C. *Journal of Insect Physiology* (2008) 54: 265-272

Effect of temperature on the oviposition rate of Argentine ant queens (*Linepithema humile* Mayr) under monogynous and polygynous experimental conditions

Abstract

Data concerning the influence of temperature on a species' physiological parameters can be a useful tool for predicting its potential distribution range, but in the case of the Argentine ant, data based on its physiological needs are too scarce and incomplete to make accurate predictions of this type. In the present study we offer new and complete data concerning the Argentine ant queen's oviposition rate under a wide range of temperatures in the laboratory.

We analyzed the oviposition rate of the Argentine ant (*Linepithema humile*) at twelve experimental temperatures: 10, 12, 14, 16, 18, 21, 24, 26, 28, 30, 32 and 34°C under monogynous conditions (1 queen per nest) and three different polygynous conditions (2, 4 and 8 queens per nest). We found that temperature affected their oviposition rate and that the effect was similar regardless of the number of queens in the nest. Egg-laying was at its maximum at 28°C, with variation in the upper and lower temperature limits at which oviposition took place depending on the degree of polygyny. Oviposition rates were negatively correlated with the number of queens in the nest. We also observed a marked variation in the oviposition rate of queens subjected to the same experimental conditions.

Introduction

The Argentine ant (*Linepithema humile* Mayr) is well-known as an invasive ant species (McGlynn 1999). As a result of human commercial activities it has spread world-wide (Hölldobler & Wilson 1990; Suarez et al. 2001) in habitats usually associated with human modification (Holway 1998a; Suarez et al. 2001). However, its ability to occupy non-altered habitats (Cole et al. 1992; Holway 1998a; Suarez et al. 2001; Gómez et al. 2003) has also been widely reported.



Its distribution range includes areas with Mediterranean-type climates throughout the world (Passera 1994; Suarez et al. 1998). In these areas, the Argentine ant has impacted native ant faunas leading to changes in arthropod communities (Human and Gordon 1996; Human and Gordon 1997; Holway, 1998b; Suarez et al., 1998), ant-vertebrate interactions (Suarez et al. 2000) and ant-plant relationships (Bond & Slingsby 1984; Visser et al. 1996; Gómez & Oliveras 2003; Blancafort & Gómez, 2005). Its presence has also had economic effects, such as damage to infrastructure (Chang & Otta 1990), and negative effects on crops and plantations due to its mutualistic interactions with hemipterans, which can affect the growth and production of the host plant (Buckley 1987; Ness & Bronstein 2004).

The distribution of the species is determined by climatic factors (Roura-Pascual et al. 2004) such as environmental temperature (Human et al. 1998; Holway et al. 2002). Temperature is also an important factor with a strong influence on the oviposition rate and brood developmental times of certain ant species (Porter 1988; Williams 1990). To date, knowledge is scarce about how different climatic factors influence the Argentine ant's reproductive success, and therefore its invasive potential. Up to now, studies of the influence of temperature on the physiological parameters of this species which can influence its reproductive success have been few and incomplete. Newell (1909) noticed that temperature influenced the oviposition rate of Argentine ant queens and Benois (1973) came to the same conclusion some years later. However, these data are very poor and were obtained with no control of the experimental temperature. Moreover, the Argentine ant is a polygynous species, i.e. nests contain multiple numbers of queens. Keller (1985, 1988a) observed that oviposition rates depended on the number of queens in a nest: the higher the number of queens, the lower the oviposition rate of each queen. However, there are no studies which document oviposition rate variation in relation to temperature under different polygynous conditions.

The relationship between temperature and oviposition rate is a useful component in models predicting the areas most suitable for the species to become established in, based on its physiological needs, and as a tool for predicting future changes in its present distribution range as a result of global climate change.



The purpose of this paper is to study changes in the oviposition rate of Argentine ant queens resulting from changes in temperature under different monogynous and polygynous experimental conditions, and to determine upper and lower temperature limits for oviposition, as well as the most favourable temperatures for egg-laying. This information will be helpful for developing prediction models of the distribution range of this tramp species based on its physiological needs.

Material and methods

Collection of field colonies. We collected 68 queens from 28 Argentine ant nests on the southern edge of the Gavarres massif, near the village of Castell d'Aro (NE Iberian peninsula) (41°49'N 3°00'E) in March 2006. In the laboratory we created 14 artificial monogynous nests each containing one queen and approximately 300 workers. We also created 14 artificial polygynous nests: 7 nests with 2 queens, 4 nests with 4 queens and 3 nests with 8 queens. Each polygynous nest had the same ratio of workers to queens—approximately 300 workers per queen. In all the artificial nests the queen/s and the workers came from the same natural nest. We did not take the queens' ages into account as the fecundity of this species is not influenced by this factor (Keller & Passera 1990). The artificial nests were a variant of those described by Passera et al. (1988) and were made up of rectangular plastic boxes (180x115x35mm high). The upper box was fitted with a layer of dry plaster of Paris connected laterally to a smaller plastic box (75x50x25mm high) by a wick of cotton wool permanently in contact with a piece of cotton soaked in water. To prevent escape the inner sides of the main plastic box were coated with liquid PTFE (Fluon). The ants were fed daily on a variant of the artificial diet described by Keller et al. (1989). We replaced the hashed beef meat with royal jelly and the sugar with honey. The food was not coated with paraffin and was placed directly on the nest floor. We knew this diet would be suitable for rearing Argentine ant colonies, having observed in previous experiments that it led to the production of healthy workers and high fecundity in queens. The nests were incubated mainly in the dark because in the field queens usually tend to reside inside the nest avoiding sunlight. We wanted to save them from situations that could



cause unnecessary stress and therefore affect their oviposition rate. The nests were exposed to the light for approximately one hour a day to carry out the colonies' feeding and cleaning. The relative humidity of the nests was around 80%. The total duration of the study was of three months (from March 2006 to May 2006) and comprised the maximal oviposition period of the queens (Benois 1973).

Oviposition tests. The artificial nests were kept in environmental chambers at one of twelve experimental temperatures ($^{\circ}\text{C} \pm \text{SD}$): 10 ± 0.1 , 12 ± 0.1 , 14 ± 0.1 , 16 ± 0.1 , 18 ± 0.1 , 21 ± 0.1 , 24 ± 0.1 , 26 ± 0.1 , 28 ± 0.1 , 30 ± 0.1 , 32 ± 0.1 and 34 ± 0.1 .

The queens' oviposition rates at each temperature and under the different monogynous and polygynous conditions studied were measured by isolating them individually and some of their workers for 24 hours in test tube nests at each of the experimental temperatures given above. After this time the eggs laid by each queen were counted under a binocular microscope. The oviposition rate of each queen was measured twice and then was used the mean number of eggs laid per queen to calculate the mean, which was then graphed.

The test tube nests consisted of a transparent plastic tube (70mm length x 10mm diameter) and a plastic top. The inner side of the plastic top was covered by a layer of dry plaster of Paris, connected by a wick of cotton wool to a small chamber filled with water. This structure kept the inside of the tube permanently humid during the experiment.

Data analysis. The average number of eggs oviposited per queen was estimated for each temperature and under the different conditions of monogyny and polygyny studied. Because our data for individual oviposition rate were not normally distributed all statistical analyses were performed using a generalized linear model (GLM) with quasi-Poisson error distribution and log-link. The occurrence of overdispersion meant that we used the more conservative F-ratio test instead of the χ^2 test. To compare the total number of eggs produced per colony we used a one factor-ANOVA. The statistics program R 2.5.1 (Copyright 2001, The R Development Core Team) was used for all analyses.



Results

Both temperature and the number of queens in the nest affected the queens' oviposition rate (GLM, quasi-Poisson errors: temperature: $F=102.9$, $P< 0.001$; number of queens: $F=92.92$, $P<0.001$) (Figure 1). Maximum egg oviposition resulted at 28°C and decreased with higher and lower temperatures (Figure 1). Temperature affected the queens' oviposition rate under both monogynous and polygynous conditions in a similar manner (Figure 1). However, the upper and lower inhibition of the oviposition rate occurred at different temperatures depending on the number of queens in the nest. The lower the number of queens, the higher the temperature which inhibited oviposition (Figure 1).

We also observed a marked variation in the oviposition rates of queens subjected to the same experimental conditions (Figure 1).

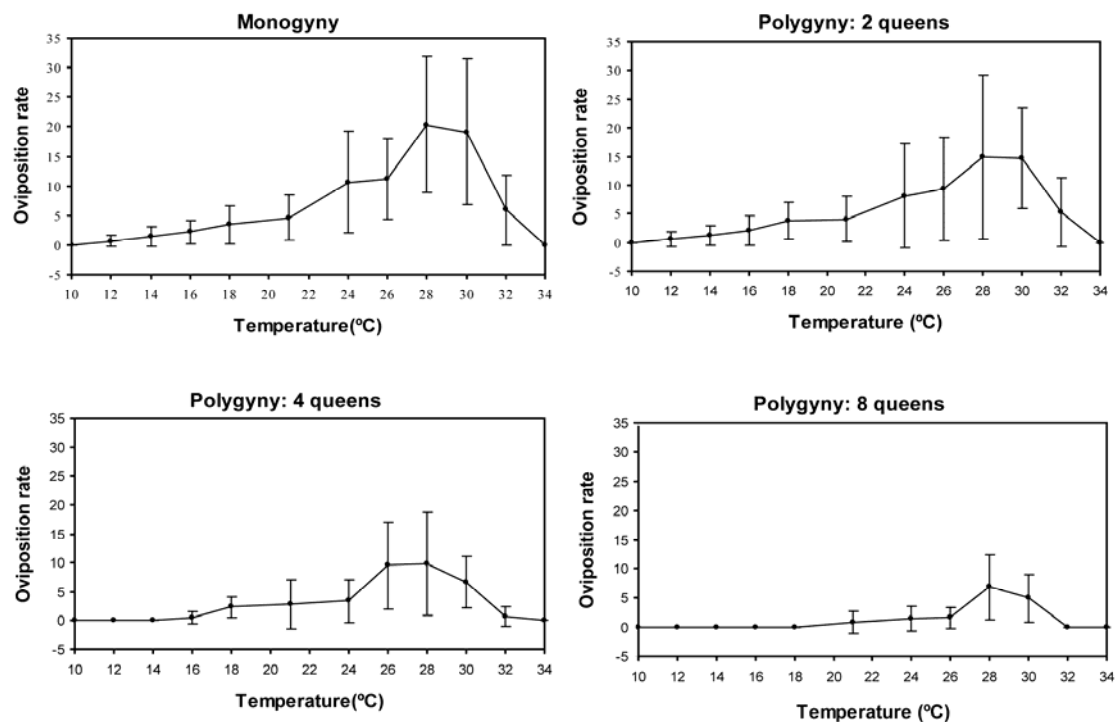


Figure 1 Effect of temperature on the oviposition rate of Argentine ant queens *Linepithema humile* under monogynous and polygynous conditions, with oviposition rate being defined as the mean number of eggs laid per capita in 24 hours. Bars denote standard deviation from mean.



The total number of eggs produced per colony differed with varying queen numbers ($F_{3,27}=3.153$; $P<0.005$). The lower the number of queens the lower the mean of the total number of eggs produced per colony (Figure 2).

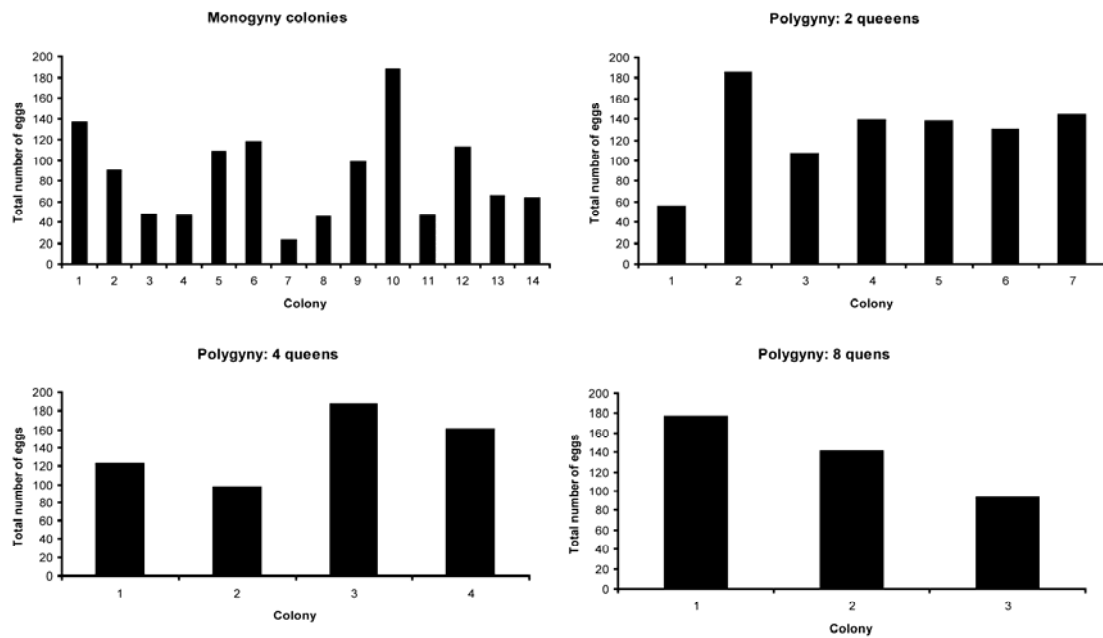


Figure 2 Total number of eggs produced per colony during the whole study under the monogynous and polygynous conditions tested.

So far as individual contributions to oviposition was concerned, we observed that regardless of the polygynous condition of the colony, there was always one main queen contributing to oviposition, with a lesser or even zero contribution from the other queens (Figure 3, 4 and 5).

Discussion

Our results show that temperature affects the number of eggs laid by Argentine ant queens. As we expected and as Keller (1985, 1988a) observed in previous works, oviposition rates are negatively correlated with the number of queens in a colony. In addition, the variations observed in the queens' oviposition rates resulting from temperature changes are quite similar in all the cases of monogyny and polygyny studied. Thus, it seems that temperature affects the egg-laying of queens irrespective of their number in a colony.

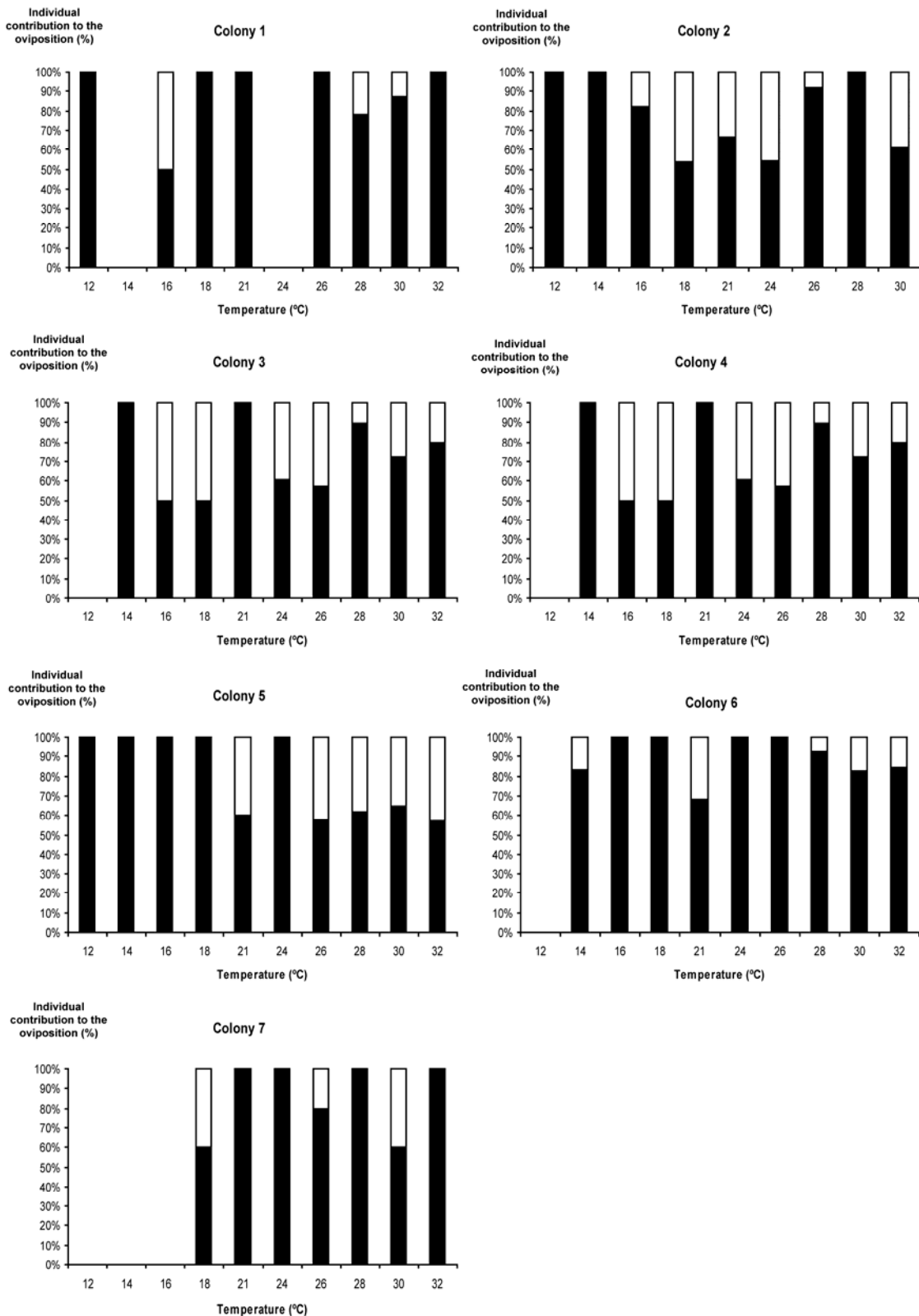


Figure 3 Individual contributions to egg-laying at different temperatures by the queens of each polygynous colony containing 2 queens. Each colour represents the proportion of eggs laid by one queen in relation to the total of eggs laid in the colony at each temperature.

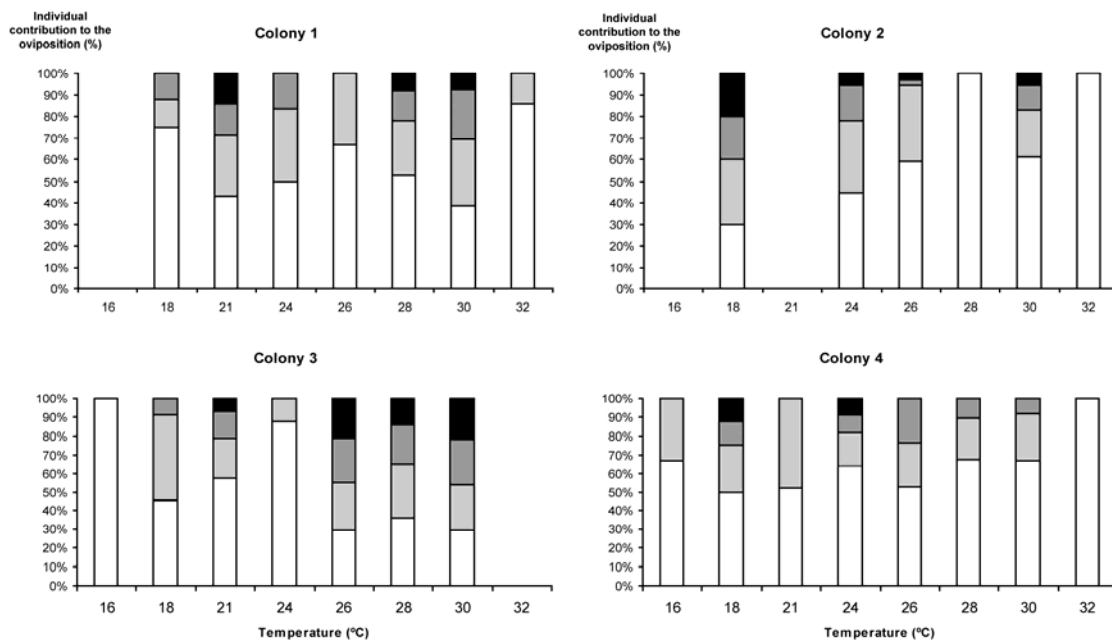


Figure 4 Individual contributions to egg-laying at different temperatures by the queens of each polygynous colony containing 4 queens. Each colour represents the proportion of eggs laid by one queen in relation to the total of eggs laid in the colony at each temperature.

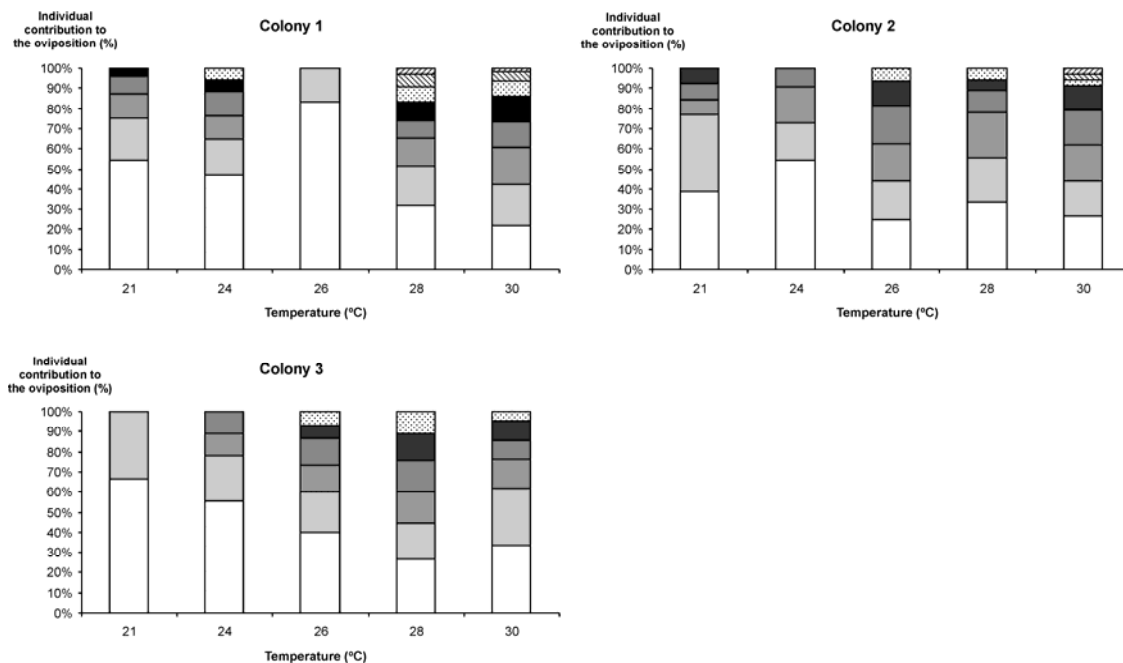


Figure 5 Individual contribution to egg-laying at different temperatures by the queens of each polygynous colony containing 8 queens. Each colour represents the proportion of eggs laid by one queen in relation to the total of eggs laid in the colony at each temperature.

Our results point to 28°C being the most favourable temperature for the Argentine ant queen's egg-laying. The sudden fall in the oviposition rate registered at 30°C indicates that queens are subjected to thermal stress caused by high temperatures. This stress is greatest at 32°C or 34°C (depending on the degree of polygyny) and results in a total absence of egg-laying. The lower limit of egg laying also varies depending on the degree of polygyny in the colony. Under monogynous and polygynous conditions with two queens, queens cease oviposition at 10°C, while under polygynous conditions with four and eight queens per colony the temperature at which oviposition ceases is a little higher: 14°C and 18°C respectively. These results prove that, depending on the degree of polygyny present in the nest, Argentine ant queens can still lay eggs below 17-18°C, which has been the lower temperature limit proposed up to now in the bibliography (Newell & Barber 1913; Benois 1973).

A possible explanation for the differences observed in the upper and lower temperature limits at which oviposition takes place is that queens are not only influenced by temperature but also by the amount of food they ingest as a consequence of their attractiveness to workers. Almost 20 years ago, Keller (1988b) observed that queens from monogynic societies are more attractive to workers than queens from polygynous colonies and, in consequence, the former receive more food than the latter even if they have the same ratio of workers caring for them. He also observed that the differences in the amount of food ingested caused differences in the amount of eggs laid (Keller 1988a,b), and therefore queens from monogynous colonies laid more eggs than queens from polygynous colonies (Keller 1988a,b). This difference in the queens' attractiveness to workers seems to be related to a lower nestmate recognition in polygynous colonies. Keller et al. (1988) hypothesized that the mixture of pheromone sources produced by several queens in polygynous colonies may result in a less distinct colony odour than one from a single pheromone source in monogynous colonies, and that this factor may affect the coordination and efficiency of workers in polygynous societies, leading to differences in the feeding rates of the queens and, in consequence, their fecundity. In the case of our study, the differences observed in the upper and lower limits of oviposition can be related to this factor: the more queens in the colony, the greater the mixture of pheromone sources and, in consequence, the lower the efficiency of



workers in taking care of the queens. Thus, in the colonies with a higher degree of polygyny there were greater differences in the amount of food ingested among the queens than in the colonies with a lower degree of polygyny. This would affect the fecundity of the queens and could also affect the lower and higher limits at which oviposition takes place.

Therefore, it seems clear that increasing the number of Argentine ant queens in the nests causes lower oviposition rates per capita and limits the queens' egg-laying capacity to a narrow range of temperatures. On this basis, the greater number of Argentine ant queens concentrated into natural nests in the field seems to make no sense in terms of egg production. One possible explanation to this apparently contradictory situation is that, as we observe in the present study, although the oviposition rate per capita is lower in highly polygynous colonies, the total number of eggs produced per colony is greater. On the other hand, our results prove that, in general, there is always one queen in polygynous colonies that contributes proportionally more to egg-laying and that the other queens contribute less or even do not contribute at all. As our data do not differentiate between queens, further research is necessary to discover whether or not it is always the same queen that mainly contributes to the egg-laying. In any case, the marked differences between individual contributions to egg production in the colonies observed in this study can be related to differences in feeding rates caused by the queens' different attractiveness to workers mentioned above.

The marked variation in egg-laying between individuals seems to respond to genetic and/or physiological differences, as Keller (1988b) suggests in a previous work. This characteristic is not unique to the Argentine ant but has also been detected in *Wasmannia auropunctata* queens (Ulloa-Chacón 2003). Moreover, in this ant species a marked variation was also observed between the queens' fecundity in relation to the number of queens in the colony: the higher the number of queens, the lower the queens' oviposition rate per capita (Ulloa-Chacón 2003). This seems to be related to the workers' relative lack of coordination and efficiency in taking care of the queens, as Keller et al. (1988) suggested for the Argentine ant.

The results obtained in the present work are essential not only for improving current knowledge of the Argentine ant's reproduction physiology, but

also for application as a component in models that predict how this species' physiological needs affect the geographical limits of its distribution.

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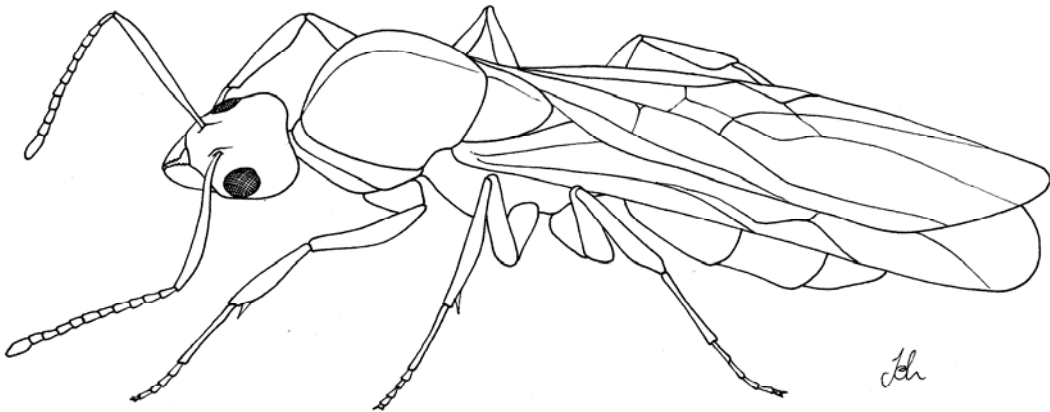


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“(...) Es una larga metamorfosis; con los lametones de las nodrizas y el paso del tiempo, los huevecitos crecen y amarillean. Se transforman en larvas de dorados pelos al cabo de un tiempo (...) Montones de larvas, cuyos diez segmentos corporales empiezan a marcarse, gritan pidiendo alimento. Agitan la cabeza en todas direcciones, estiran el cuello y gesticulan hasta que las nodrizas les entregan un poco de melaza o carne de insecto (...) Cuando ya han “madurado” lo suficiente, las larvas dejan de comer y de moverse. Fase de letargia en la que se preparan para el esfuerzo. Reúnen sus energías para segregar el capullo que las transformará en ninfas (...) La ninfa que está a punto de eclosionar es llevada a parte, en compañía de las otras que comparten el mismo estado. Las nodrizas agujerean con cuidado el velo del capullo, liberando una antena, una pata, hasta liberar una especie de hormiga blanca que tiembla y se tambalea. Su quitina aún húmeda y clara, será roja dentro de unos días, como la de todos los belokanianos.”

Bernard Werber, *Las Hormigas*.



CHAPTER 4

Effect of temperature on the Argentine ant's development and survival – an experimental study

Abril, S., Oliveras, J. and Gómez, C. Submitted.

Effect of temperature on the Argentine ant's development and survival – an experimental study

Abstract

Data about the influence of temperature on a species' brood development times can be a useful tool for predicting its potential distribution range. In the case of the Argentine ant, such data are too scarce and incomplete to make accurate predictions based on its physiological needs. In the present study we offer comprehensive new data about brood survival and developmental times at a wide range of temperatures in the laboratory. We observe that temperature affects both the complete brood development from egg to adult worker and each of the immature stages separately. The higher the temperature, the shorter the development times. The total brood survival from egg to adult is low, the maximum survival rate we report being only 16% at 26°C.

Temperature also affects each of the immature stages in a different manner: eggs are negatively affected by high temperatures, while larvae are negatively affected by low temperatures and the survival of pupae is apparently independent of environmental temperature. At 32°C no eggs survived, while at 18°C less than 2% of the eggs emerged into larva.

Introduction

The Argentine ant (*Linepithema humile* Mayr) is well known as an invasive ant species (McGlynn 1999). Native to South America (Tsutsui et al. 2001), it has now been introduced into various parts of the world as a result of human commercial activities (Hölldobler & Wilson 1990; Suarez et al. 2001). Its distribution includes areas with Mediterranean-type climates (Passera 1994; Vega & Rust 2001). Its rapid expansion in invaded zones has been facilitated by habitat disturbance (Suarez et al. 1998), but there is increasing evidence of its ability to occupy non-altered habitats (Cole et al., 1992; Holway 1998). In such areas its capacity to negatively affect native ant faunas (Camell et al. 1996;



Human and Gordon 1997; Suarez et al. 1998; Holway 1999; Gómez & Oliveras 2003; Sanders et al. 2003) through intense interference and exploitative competition has been widely reported (Holway 1999). It is also recognized that climatic factors are the key elements that determine its distribution in invaded areas (Roura-Pascual et al. 2004; Hartley et al. 2006), especially environmental temperature (Human et al. 1998, Holway et al. 2002). Temperature has a strong influence on some reproductive traits of the species: for example, the queens' oviposition rate (Newell 1909; Benois 1973; Abril et al. 2008). The Argentine ant's brood development rate also seems to be strongly affected by environmental temperature (Newell & Barber 1913; Benois 1973), although data concerning this aspect of its biological cycle are very scarce and incomplete. Such data are essential for predicting, for example, the timing of plague outbreaks or the geographical limits of an insect's distribution (Hartley & Lester, 2003). To date, we have little knowledge about how the Argentine ant's biological needs influence its distribution range, since most of the prediction models that have been made are only based on its climatic requirements (Roura-Pascual et al., 2004). Up to now only few models based on the Argentine ant physiological needs have been made (Hartley & Lester, 2003; Krushelnycky et al. 2005), probably due to the poor data available about the influence of abiotic factors on the specie's biological cycle.

The relationship between temperature and brood development rates is a useful component in models predicting the areas most suitable for the species to become established in, based on its physiological needs, and as a tool for predicting future changes in its present distribution range as a result of global climate change (Helmuth et al. 2005). However, such models need a considerable amount of data in constant temperature environments to be accurately calibrated (Blanck et al. 2000) and the currently available data on the Argentine ant's brood development rates have a lack of replicates for each measured temperature and daily averages instead of fixed temperatures were used to obtain brood developmental times (Newell & Barber 1913; Benois 1973).

In order to offer new physiological data in an area which is clearly data deficient, the purpose of this study is to obtain new data about the Argentine ant's brood development times in relation to environmental temperature not only



to improve current knowledge of this species' biology, but also to provide valuable information which will allow the creation of accurate prediction models based on its physiological needs.

Material and Methods

Ant collection and laboratory colonies. Argentine ants used in the study were collected in March 2006 from an invaded natural area situated on the southern edge of the Gavarres Massif near the village of Castell d'Aro (NE Iberian Peninsula) (41° 49'N, 3°00'E).

We collected 14 queens from 14 nests and numerous workers to create 14 artificial monogynous nests each containing one queen and approximately 300 workers. The nests were incubated at 28°C, which is the optimal temperature for queen oviposition in the Argentine ant (Abril et al. 2008). The nests were a variant of those described by Passera et al. (1988), made up of a regular plastic box (180 x 115 mm and 35 mm high). The upper box was fitted with a layer of dry plaster of Paris and was connected laterally to a smaller plastic box (75 x 50 mm and 25 mm high) by a cotton wool wick permanently in contact with a piece of cotton soaked in water. To prevent escape, the inner sides of the main plastic box were coated with liquid PTFE (Fluon). The ants were fed daily with a variant of the artificial diet described by Keller et al. (1989). We replaced the hashed beef meat with royal jelly and the sugar with honey. The food was not coated with paraffin but placed directly on the nest floor. We knew this diet to be very suitable for rearing Argentine ant colonies because we had observed that it allowed a high fecundity in queens (Abril et al. 2008), and the production of healthy workers and sexuals, both males and queens. The incubation time of these colonies was two weeks. After this period each queen was subjected to an oviposition test to obtain the eggs for the study following the same procedure as in Abril et al. (2008).

Total brood developmental times and survival rates of the worker caste. In order to study the total immature development period of the worker caste from egg to adult, a total of 100 eggs from the oviposition tests mentioned above were placed in artificial queenless colonies containing approximately 600



workers. In the case of 18°C and 32°C we tested a total of 400 eggs instead of 100 to obtain reliable data in those extreme conditions.

The brood was totally removed to avoid confusion with the growing test brood. The artificial colonies were kept in environmental chambers at one of seven experimental temperatures ($^{\circ}\text{C}\pm\text{SD}$): 18 \pm 0.1, 21 \pm 0.1, 24 \pm 0.1, 26 \pm 0.1, 28 \pm 0.1, 30 \pm 0.1 and 32 \pm 0.1.

Observations were carried out daily and the exact worker development times at each of the seven experimental temperatures were noted. We calculated the brood survival rate at each temperature from egg to adult form (including the sexuals) by means of the data obtained.

Development times and survival rate of each immature stage. An ant's development from egg to adult form includes three different stages: egg stage, larval stage and pupal stage. We also studied the effect of temperature on development and survival in each of these three stages.

Due to the small size of the eggs and the difficulty of observing them in the artificial nests (because the workers tended to carry them quickly through the nest when we started observing them under the binocular microscope), the eggs were incubated in test tube nests instead of in artificial queenless colonies at each of the seven experimental temperatures. The number of eggs tested varied depending on the queen's egg-laying at each temperature and ranged from 105 to 354. The eggs' incubation in the test tube nests was performed without the presence of any workers because previous observations had shown us that there were no differences between the viability of eggs in the care of workers and the viability of eggs without such care, and the observation and individualization of the eggs were easier without workers. The eggs were observed daily from the first day of egg-laying to the appearance of the larva. In this way, we were able to obtain more accurate data by noting the exact number of days from egg to larvae for each egg that hatched into larvae. We also calculated the survival rate of the eggs at each of the seven experimental temperatures.

The small size of new born larvae made it difficult to obtain reliable data about development times in this stage. Therefore, we estimated the times by taking the difference between the total brood development time and the sum of



the egg stage and the pupal stage development times. The survival rate of this stage was also estimated from this data.

The duration of the pupal stage at each of the seven experimental temperatures was measured taking the data from the daily observations of the artificial queenless colonies set up to obtain the total brood development times of the species. We also calculated survival rates in this stage at each of the experimental temperatures.

Results

Total brood development times and survival rates of the worker caste. As it was expected, temperature substantially affects brood development in the Argentine ant. The higher the temperature, the shorter the brood development times, and vice versa (Table 1). As egg development stopped at 32°C we assumed that this was the upper limiting temperature for total brood development from egg to adult form. In consequence, the larval and pupal development times at this temperature were not analyzed. At 18°C the survival of the eggs were almost zero: only 1.7% of incubated eggs achieved emergence. For this reason we assumed that total development from egg to adult worker at 18°C would be negligible and therefore did not analyze the larval and pupal development times at this temperature.

Another significant point is that the range of days to development is also affected by temperature. As the temperature rises, the range of days decreases, and vice versa (Table 1).

The brood survival rate, expressed in percentage terms, is at its maximum at 26°C and decreases with higher and lower temperatures (Figure 1).



Table 1 Total developmental times of the Argentine ant, *Linepithema humile* from egg to adult worker.

Temperature (°C)	21	24	26	28	30
Developmental range (days)	80-230	59-135	68-135	40-87	39-81
Mean of total developmental times ± S.E. (days)	147.7 ± 56.1	100.4 ± 24.2	110.1 ± 24.1	66.2 ± 14.4	59.7 ± 11.5
n	17	12	8	15	11

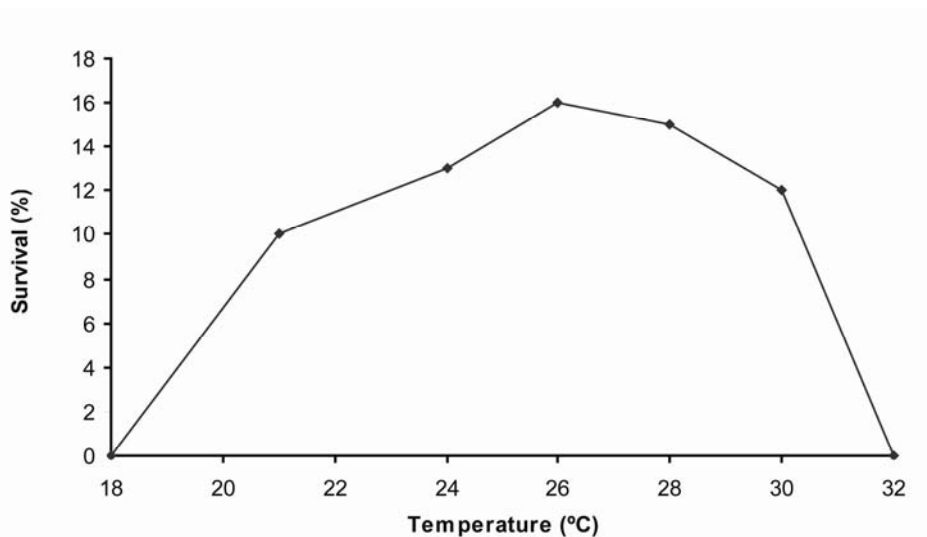


Figure 1 Effect of temperature on brood survival from egg to adult form in the Argentine ant, *Linepithema humile*.

Developmental times and survival rate of each immature stage. The duration of the egg stage declined from 58 days at 18°C to less than 15 days at 30°C (Table 2). Eggs at 32°C in the test tube nests (a total of 105) failed to develop because all died in the first two weeks of incubation. At 18°C virtually none of the incubated eggs into the test tube nests survived: only three of 173 eggs emerged into larva and the rest died. The eggs placed in the artificial queenless colonies at 32°C (a total of 400) also died in the first two weeks of



testing, undoubtedly killed by the extreme temperatures. The incubation range of days decreased as temperature increased and vice versa (Table 2).

The survival rate of the eggs of this species is negatively affected by high temperatures since only about 13% emerge into larva at 30°C, in comparison with about 52% which emerge into larva at 21°. Below 21°C the eggs' survival rate is again negatively affected by the temperature since only 1.7% of the eggs achieve the larval stage at 18°C (Figure 2).

Table 2 Duration of the egg stage of the Argentine ant, *Linepithema humile*.at different temperatures.

Temperature (°C)	18	21	24	26	28	30
Developmental range (days)	56-62	28-36	17-24	15-22	14-19	14-19
Mean of eggs developmental times ± S.E. (days)	58.3 ± 3.2	32.0 ± 1.7	20.3 ± 1.9	17.7 ± 1.6	15.9 ± 1.3	15.7 ± 1.6
n	3	49	69	47	74	45

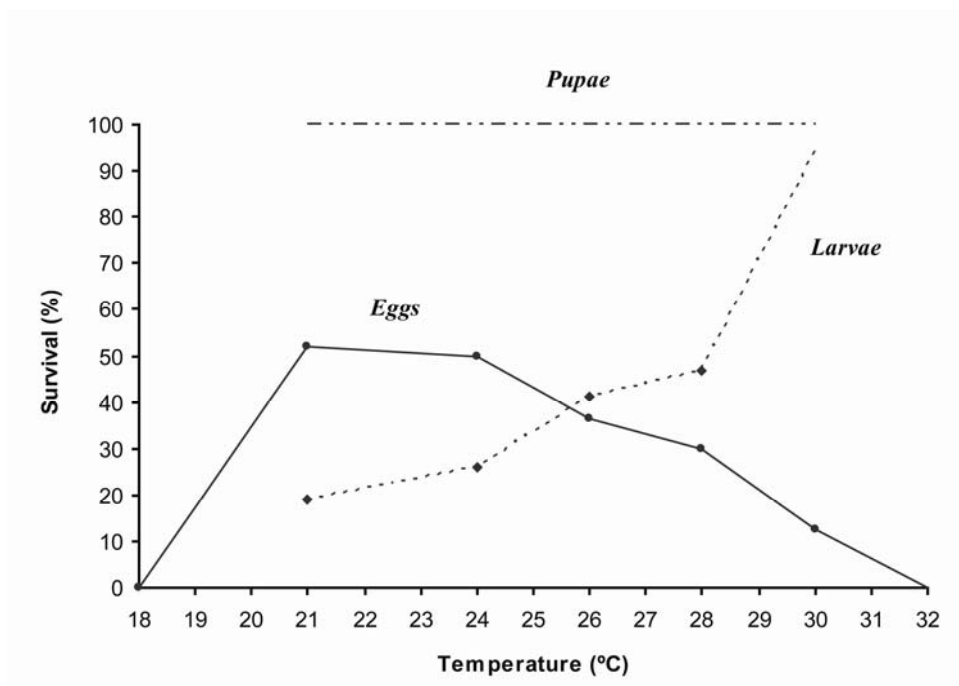


Figure 2 Effect of temperature on the survival of each of the immature stages of the Argentine ant, *Linepithema humile*.



The estimated duration of the larval stage decreased as temperature increased (Table 3). The range of days to pupal emergence decreased with high temperatures. We also observed a marked variation in the duration of this stage between samples incubated at the same experimental temperatures (Table 3).

Table 3 Duration of the larval stage of the Argentine ant, *Linepithema humile*.at different temperatures.

Temperature (°C)	21	24	26	28	30
Developmental range (days)	24-172	23-100	40-106	14-61	15-59
Mean of larval developmental times ± S.E. (days)	84.2 ± 55.5	58.5 ± 26.3	81.1 ± 24.0	40.2 ± 14.1	35.6 ± 11.9
n	17	12	8	15	11

Table 4 Duration of the pupal stage of the Argentine ant, *Linepithema humile*.at different temperatures.

Temperature (°C)	21	24	26	28	30
Developmental range (days)	24-26	15-18	11-12	7-10	7-9
Mean of pupal developmental times ± S.E.	25.5 ± 0.6	16.1± 0.8	11.4 ± 0.5	8.9 ± 1.1	8.0 ± 0.8
n	17	12	8	15	11

The survival rate of this stage seems to be negatively affected by low temperatures since only about 19% of the larvae pupated at 21°C compared with the 94% estimated at 30°C (Figure 2).

The duration of the pupal stage declined from about 25 days at 21°C to about 8 days at 30°C (Table 4). The range of days of the emergence to adult worker varied little, from one to three days of difference (Table 4). In contrast to



eggs and larvae, the survival of pupae was always 100% and apparently independent of temperature (Figure 2).

Discussion

Our results show that, as it was expected, temperature affects the development times of both the complete brood development from egg to adult worker and each of the immature stages in the same manner: the higher the temperature, the shorter the developmental times. This relation was reported by Newell & Barber (1913) almost one hundred years ago. However, our data show longer developmental times than they presented. These differences can be explained by their lack of data: there are few replicates for each measured temperature. Moreover, they measured developmental times by means of average daily temperature, while in the present study we used fixed temperatures to incubate the brood.

The effect of environmental temperature on Argentine ant brood development times that we have observed in this study has also been observed for other ant species (Porter 1988; Arcila et al. 2002). In comparison with the results obtained for the Argentine ant, the developmental times for some ants like *Solenopsis invicta* (Porter 1988), the Crazy ant (*Paratrechina fulva*) (Arcila et al. 2002) or *Anoplolepis longipes* (Rao & Veeresh 1991 cited by Arcila et al. 2002) are in general shorter, while other species of ants like *Prenolepis imparis* (Tschinkel 1987 cited by Hölldobler & Wilson 1990) have longer developmental times than the Argentine ant at similar conditions of temperature and humidity (27°C; 80% relative humidity).

The duration of the Argentine ant immature stages also varies. The larval stage has the longer developmental times, while the pupal stage is characterized by its short duration.

The upper limit of egg development is 32°C; at this temperature the eggs' survival is zero. The lower temperature limit seems to be around 18°C; at this temperature the survival of the eggs is practically zero: only three eggs out of 173 emerged into larva.

In the case of larval developmental times, there is a marked variation between the development times of samples subjected to the same temperature



treatment. This could be due to the fact that this form is the only one that is fed by workers (Markin 1970a). This being the case, it would seem that larval developmental times are not only affected by environmental temperature, but also by the food they ingest. This would explain why such a marked difference is only observed in this phase.

Brood survival rate also varies as a function of environmental temperature. While eggs are negatively affected as the temperature rises, larvae are negatively affected by low temperatures. This can be explained in the light of the biological cycle of this species in its natural environment. In spring and autumn when the environmental temperature is cool, the Argentine ant queens are at their maximum egg-laying period and in consequence we can find the maximum egg densities in the nest at this time, while maximum larvae densities appear at the end of springtime when the temperatures are warmer (Markin 1970b; Benois 1973). Therefore, it seems that the Argentine ant's biological life cycle as far as brood development is concerned, is adapted to the physiological temperature needs of each immature form almost during their maximal densities in natural nests. It would allow the maximal survivor of the different brood stages, and in consequence, the maximal reproductive success of the colony.

The high survival rate observed in pupae at all the experimental temperatures tested in this study indicates to us that this phase is the most resistant to temperature variation. This is in accordance with the observations made by Porter (1988) who observed high levels of survival in pupae of the ant species *Solenopsis invicta* not related to temperature.

The total brood survival from egg to adult observed in this study was quite low. Only sixteen out of one hundred eggs reached adult form under the optimal survival temperature of 26°C. In that sense, the percentages of brood survivor observed in the present study are in agreement with the ones observed under experimental conditions and similar rearing conditions of temperature and humidity (27°C and 80%, respectively) by Arcila et al. (2002) for the ant species *Paratrechina fulva*. She find a survival of 30% in eggs and of approximately 50% in larvae (Arcila et al. 2002), the same percentages obtained in that study at 28°C for the Argentine ant. This fact gives us confidence in the results obtained. Moreover, studies carried out fifty years ago in Portugal (Silva Dias



1955) on the relationship between brood development times of the Argentine ant and environmental temperature, revealed again this low brood survival under experimental conditions. They tried to obtain complementary data to that obtained by Newell & Barber (1913) concerning development from larvae to pupae and from pupae to adult worker. But in the end they were only able to obtain three more pieces of data at different experimental temperatures. We think that this was probably due to the low brood survival rate of this species, at least under experimental conditions, that we have observed in our study.

We believe that brood survival would probably be higher in natural nests due to the thermal gradient present in the nest and the fact that environmental temperature is not constant but changes throughout the day. The overall likelihood is that this would be used by the workers to incubate the different brood stages at their optimal survival temperature and, in short, assure the maximum reproductive success of the colony. Even though further research is necessary to confirm this, the data given in the present study are very valuable, not only because there are no other studies which offer comprehensive, accurate data about the brood developmental times of the Argentine ant at a wide range of fixed temperatures, but also because these data are essential for developing prediction models about the distribution range of this tramp species based on its physiological needs in relation to temperature.

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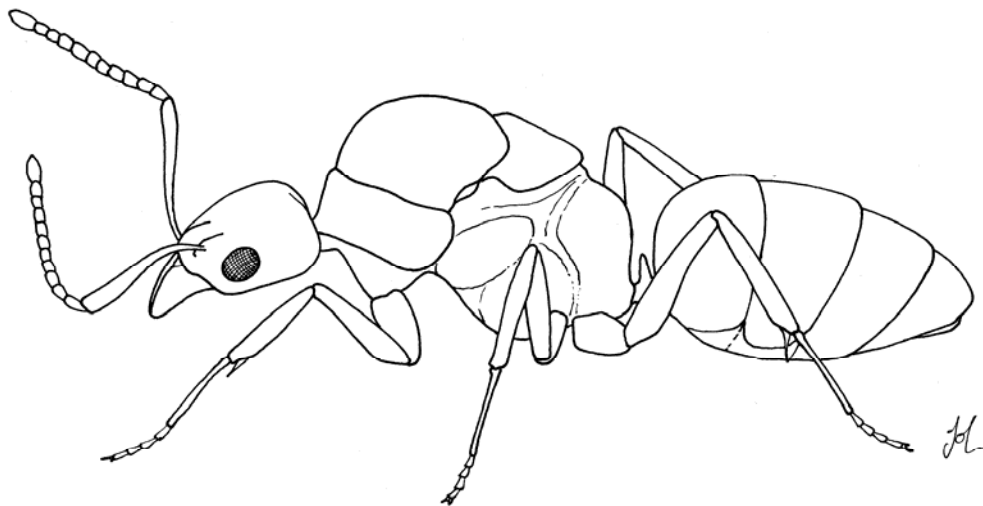


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"(...) Las enanas vivían hace mucho tiempo en otro país, a cientos de miles de cabezas de distancia. Este país era muy diferente del bosque de la Federación. Había en él grandes frutos, llenos de colorido y muy azucarados. Por otra parte no había invierno ni tampoco hibernación (...) Su nido estaba al pie de un laurel rosa. Entonces, ocurrió que el laurel rosa y la arena que lo rodeaba fueron un día arrancados del suelo para ser depositados en una caja de madera. Las enanas intentaron huir de la caja, pero ésta fue depositada en el interior de una estructura gigantesca y muy dura. Y cuando llegaron a las fronteras de esa estructura, cayeron al agua. Había agua salada hasta donde alcanzaba la vista (...) El cambio resulto decepcionante. Los frutos, las flores, los insectos eran más pequeños y tenían menos colorido. Habían dejado un país rojo, amarillo y azul para ir a parar a otro verde, negro y marrón."

Bernard Werber, *Las Hormigas*.



CHAPTER 5

Predicting the potential distribution range of the Argentine ant (*Linepithema humile* Mayr) in Catalonia (NE Iberian Peninsula) on the basis of its physiological needs

Abril, S., Oliveras, J. and Gómez, C. In Preparation.

Predicting the potential distribution range of the Argentine ant (*Linepithema humile* Mayr) in Catalonia (NE Iberian Peninsula) on the basis of its physiological needs

Abstract

To address the lack of a physiological approach in current predictions of the Argentine ant's potential distribution, we used both old and recently published data on development rates to evaluate degree-day models for Catalonia (NE Iberian Peninsula), and published data on brood survival and oviposition rates of queens to create a worker productivity model. The degree-day model generated using recent data on brood development indicated that complete development from egg to adult worker required approximately 599 degree-days above a threshold of 18.4 °C. Neither the degree-day model generated using old published data nor the model obtained using recently published data matched well with the currently known distribution of the Argentine ant in Catalonia. This can be explained in terms of the limitations of the model on the data used to calibrate it. On the other hand, the predicted net productivity of Argentine ant workers generated by the worker productivity model, on the other hand, closely matches its present known distribution in Catalonia. In general, the present work shows the utility of physiological data for predicting the distribution range of the species. The information provided could be used to improve current prediction models, which up to now have taken little account of the physiological needs of the species in terms of its establishment and dispersion in an invaded area.

Introduction

The Argentine ant (*Linepithema humile* Mayr) is a well-known invasive species (McGlynn 1999). Native to northern Argentina, southern Brazil, Uruguay and Paraguay (Tsutsui et al. 2001; Wild 2004), it has spread world-wide in areas with Mediterranean-type climates (Hölldobler and Wilson 1990; Passera 1994;



Suarez et al. 1998; Suarez et al. 2001) usually associated with disturbed habitats as a result of human activities (Holway 1998a; Suarez et al. 2001). However, its ability to occupy natural habitats has also been reported (Cole et al. 1992; Holway 1998a; Suarez et al. 2001; Gómez et al. 2003). In its introduced range, the Argentine ant has impacted native ant faunas leading to changes in arthropod communities (Human and Gordon 1996; Human and Gordon 1997; Holway 1998b; Suarez et al. 1998), ant-vertebrate interactions (Suarez et al. 2000) and ant-plant relationships (Bond and Slingsby 1984; Visser et al. 1996; Gómez and Oliveras 2003; Blancafort and Gómez 2005). Its presence has also had negative effects on crops and plantations due to its mutualistic interactions with hemipterans, which affect the growth and production of the host plant (Buckley 1987; Ness and Bronstein 2004).

Predicting areas that will prove suitable for the successful establishment of Argentine ants, as well as the rate of spread of the invasion once they have been introduced are essential steps towards managing the invasion in affected areas. Up to now, several studies have assessed the potential distribution of the Argentine ant using a correlative approach, which relates occurrence data to environmental variables susceptible to influence its distribution (Hartley et al. 2006; Roura-Pascual et al. 2004, 2006). However, the establishment of an invasive species in a new geographic area is a complex phenomenon that includes other elements responsible for the restriction or enhancement of its distribution range such as the physiological requirements of the species for the growth and survival of the colony. Few studies have assessed the distributional range of the species on a local scale while taking into account the physiological basis of its establishment (Hartley and Lester 2003; Krushelnycky et al. 2005). Their predictions were made using a degree-day model based on the assumption that temperature is the main factor influencing the developmental rate of insects. By means of this relationship this model predicts the most suitable areas for the species' development and, therefore, for its survival and successful establishment. This information is essential for estimating the potential range of the Argentine ant in invaded areas on a physiological basis, and should be taken into consideration in future predictive models, together with the combination of climatic factors responsible for its introduction (Helmuth et al. 2005).



To generate a degree-day model, data on the species' developmental rates at different temperatures is needed. Previous studies used the development data available at that time (Newell and Barber 1913; Benois 1973). Although these data contain enough observations on the brood development of the species to develop a degree-day model, they have certain limitations. For example, the accurate calibration of a degree-day model requires a considerable amount of data from constant temperature environments (Blanck et al. 2000). However, the data of Newell and Barber (1913) and Benois (1973) are missing replicates for each temperature measured, and daily averages were used instead of fixed temperatures to measure brood developmental times.

The new data available on the brood development and survival of the Argentine ant at different temperatures is an improvement on previously published data on this subject: there are more replicates for each temperature measured and the brood is incubated at fixed temperatures (Abril et al. unpublished data). The present study, therefore, attempts to gauge the usefulness of new models to determine the potential distribution of the Argentine ant across Catalonia (NE Iberian Peninsula) using recent data on brood development and survival (Abril et al. unpublished data), and on the oviposition rates of queens (Abril et al. 2008), in order to address the lack of physiological data in predictive models.

Material and methods

To predict the potential distribution of the Argentine ant, including data on the development and survival of the species, we adopted two different modelling approaches: (1) a degree-day model, based on the fact that an insect's growth is closely linked to temperature; and (2) a worker productivity model, relating the net productivity of workers per queen to the mean temperature of the productive months.



Degree-day model

Data sources and analysis. The development rate of insects is mainly linked to temperature: to develop from one point in their life cycle to another, insects require a certain degree of heat (Chiang 1985). The measurement of this degree of heat is called “physiological time”, which is expressed in units called “degree-days” (°D). A degree-day is the heat experienced in 24 hours by an insect when the temperature is one degree above its lowest development threshold (Chiang 1985). Each species requires a specific number of degree-days to complete its development (Chiang 1985). A degree-day model predicts this number of degree-days on the basis that the development rate is a linear function of temperature (Hartley and Lester 2003). This is expressed as follows:

$$DT = k + t_0D$$

where D = duration of development in days, T = temperature, t_0 = a baseline temperature of theoretically zero development, and k = a thermal constant for development measured in units of degree-days.

To search for differences between models calibrated using different development data, we applied the degree-day model developed by Hartley and Lester (2003) using data on development rates in relation to temperature from Newell and Barber (1913) and Benois (1973) to predict the potential distribution of the Argentine ant in Catalonia, and compared this prediction with the one obtained using data from Abril et al. (unpublished data). We estimated “ t_0 ” and “k” values necessary for developing the degree-day model from the slope and intercept respectively of a linear regression of DT vs. D, as Hartley and Lester (2003) did in their study. The standard errors of “ t_0 ” and “k” were calculated from the standard errors of the slope and intercept.

Mapping the degree-day model. Following Hartley and Lester (2003), we calculated the average annual accumulation of degree-days above t_0 from 136 meteorological stations located across Catalonia. The meteorological data was supplied by the Meteorological Service of Catalonia (SMC) (<http://www.meteocat.com>), and consisted of daily air temperature values recorded between 2000 and 2005. Missing values were ignored, and only time



series with a minimum of 1500 days of measurements were used. We used an ArcView GIS (version 3.2) to assemble and analyse the resulting data.

Worker productivity model

Data sources an analysis. The increased availability of occurrence data on the effects of temperature on the Argentine ant's physiology (Abril et al. 2008; unpublished data) allows the development of a worker productivity model.

A worker productivity model can be a useful tool for predicting the potential distribution range of the species under a new approach: the colony's temperature requirements to produce new individuals and, therefore, to maintain its presence in an area.

To develop such a model and calculate the Argentine ant's net productivity of workers per queen, we used data on oviposition rate and brood survival from egg to adult worker at different temperatures (Abril et al. 2008; unpublished data).

Data on the oviposition rate of the species were reported under different monogynous and polygynous experimental conditions (Abril et al. 2008). As the oviposition rate can vary depending on the number of queens in a colony (Keller and Cherix 1985; Keller 1988; Abril et al. 2008) and it is widely recognised that there is a high level of polygyny in natural nests of this species (Passera 1994; Keller 1995; Ingram 2002), we used data on oviposition rates from the highest level of polygyny studied (eight queens per nest), to calculate the net productivity of workers per queen.

We used generalized linear models (GLM) with Poisson error distribution and a log-link function to evaluate net worker productivity as a function of temperature. Because the relationship between the workers' net productivity and the temperature was curvilinear, we generated a quadratic function of our response variable. We measured the goodness-of-fit of the model generated by the change in deviance as calculated by the χ^2 test (Dobson 1999) with a 5% significance level. We also calculated the percentage of explained deviance (Dobson 1999).

It has been reported that Argentine ant queens have a period of physiological rest during the winter months (December to March) in which egg



production stops (Benois 1973). Therefore, having obtained the model summarising the relationship between net productivity and temperature, we estimated the potential net productivity of Argentine ant workers in Catalonia. We only considered the months from March to November, when the queens lay eggs.

All the analyses were run using the statistics program S-PLUS (version 6.1).

Mapping the productivity model. To predict the workers' annual net productivity of Argentine ants, we calculated the mean value of March, April, May, June, July, August, September, October and November mean air temperature –measured over the past 15 years- from the Climatic Atlas of Catalonia (<http://magno.uab.es/atles-climatic/>). Months were selected according to the Argentine ant queen's oviposition period (Benois 1973). To map the potential productivity predicted by the model, we used an ArcView GIS (version 3.2).

Results

Degree-day model

The relationship between duration of development (D) and duration of development multiplied by temperature (DT) appeared fairly linear among data on development time from egg to adult worker from Abril et al. (unpublished data) (Figure 1).

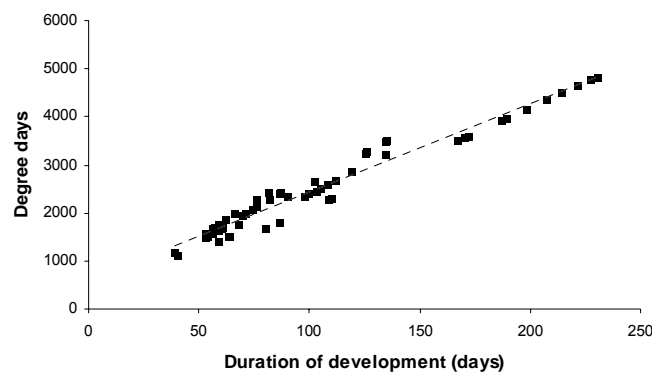


Figure 1 Relationship between duration of development and degree-days from egg to adult worker in the Argentine ant.



The degree-day model generated with data from Newell and Barber (1913) and Benois (1973) estimated that a total of 445,4 (± 18.9 SE) degree-days above a developmental threshold of 15.9 (± 0.8 SE) °C would be required for total development from egg to adult worker (Hartley and Lester 2003). However, the degree-day model using new data on brood development taken from Abril et al. (unpublished data) suggested that a higher annual accumulation of degree-days above a higher developmental threshold would be necessary to complete development, specifically 599.5 (± 58.5 SE) degree-days above a developmental threshold of 18.4 (± 0.5 SE) (Table 1).

Table 1 Estimated parameter values for the degree-day models.

Complete development egg→adult worker	Threshold temperature, $t_0 \pm s.e. (^{\circ}C)$	Cumulative degree-days, $k \pm s.e. (^{\circ}C \text{ days})$	Temperature range of linear relationship ($^{\circ}C$)	<i>n</i>
Degree-day model ^a	15.9 \pm 0.8	445.4 \pm 18.9	17-30	52
Degree-day model ^b	18.4 \pm 0.5	599.5 \pm 58.5	18-32	63

a Degree-day model using data from Newell and Barber(1913) and Benois(1973)

b Degree-day model using data from Abril et al. (unpublished data)

Figure 2 illustrate the estimated degree-days calculated at various locations within Catalonia using data from Newell and Barber (1913) and Benois (1973) (Figure 2a), and Abril et al. (unpublished data) (Figure 2b). The two predictions are rather different: Figure 2a indicates that a total of 92.6% of the locations studied are suitable for the survival and establishment of the Argentine ant, in contrast to the only 39.7% shown in Figure 2b. Comparing these two degree-day models with the present-day known distribution of the specie in Catalonia (Figure 3) (Roura-Pascual 2006), we can see that the model based on data from Newell and Barber (1913) and Benois (1973) predicts the survival and establishment of the species in locations where the species is not present (e.g. Cervera, Falset, Lleida or Sort). On the other hand, the degree-day model generated with data from Abril et al. (unpublished data) predicts that many locations where the Argentine ant is currently present are not suitable in terms of temperature needs for its survival and establishment (e.g. Castell d'Aro, Barcelona, Cassà de la Selva or Malgrat de Mar).



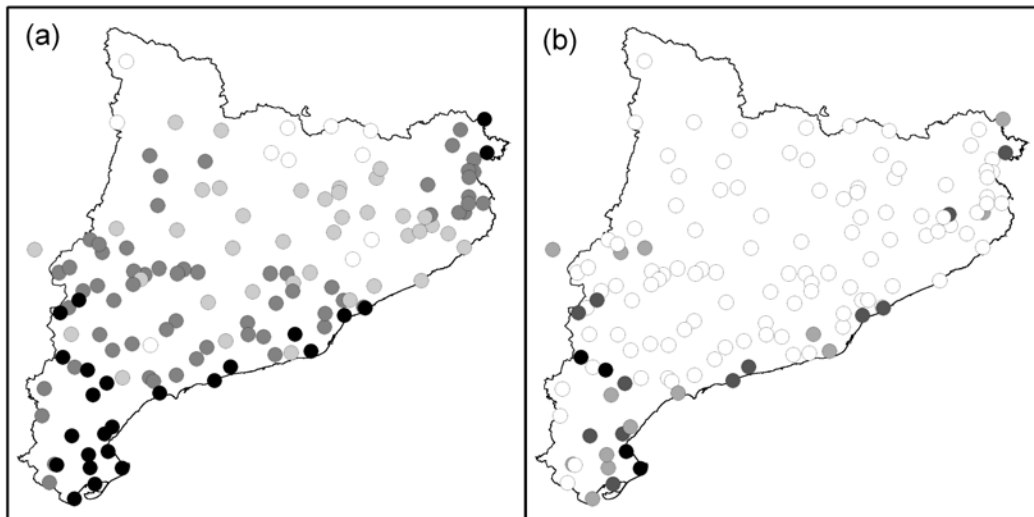


Figure 2 Predicted potential distribution of the Argentine ant in Catalonia according to the degree-day model based on data from (a) Newell and Barber (1913) and Benois (1973), and on data from (b) Abril et al. (2008; unpublished data). Each point represents a meteorological station. White circles indicate predicted unsuitable areas for the establishment and survival of the species, while grey and black circles indicate predicted suitable areas. The darker the circle, the higher the predicted suitability of the area.

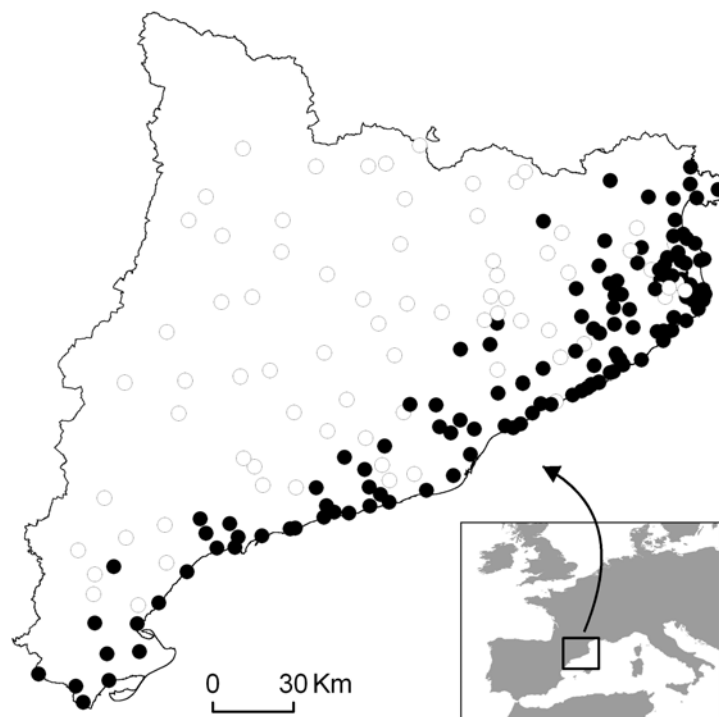


Figure 3 Current distribution of the Argentine ant across Catalonia (Roura-Pascual 2006)



Worker productivity model

The final model was:

$$y = -0.0367 x^2 + 1.9331 x - 23.388$$

where y is the net worker productivity per queen and day of laying and x is the air temperature.

The GLM model shows a change in deviance of 46.745, which differs significantly from the Null Deviance of the model (Null Deviance: 129.0197, df: 189; χ^2 test (P| Chi | < 0.001). This model explains 36.23% of the total deviance. The predicted net productivity of Argentine ant workers in Catalonia generated by the model (Figure 4) fits fairly well with the current distribution of the species (Figure 3), showing a marked peripheral distribution range at least over the whole coastal band.

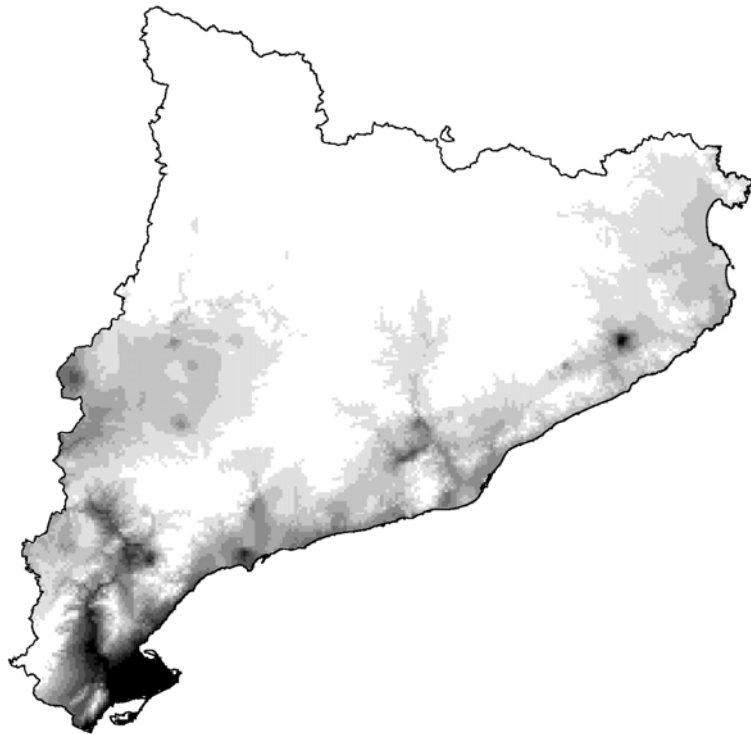


Figure 4 Predicted potential distribution of the Argentine ant in Catalonia according to the worker productivity model based on data from Abril et al. (2008; unpublished data). Darker shades indicate higher predicted productivity of workers.



Discussion

The degree-day model generated using data from Newell and Barber (1913) and Benois (1973) predicted the establishment and survival of the species in many locations in Catalonia where it has never been detected. This could be due to the fact that the species may not yet have had an opportunity to invade these areas since it is reported that the species is still expanding its distribution range in certain areas (Holway 1998a; Casellas 2004). Another possible explanation, however, is that these places are unsuitable for its establishment and survival because of ecological conditions that hamper its expansion, e.g. low temperatures or insufficient relative humidity (Espadaler and Gómez 2004), and that the model overestimates the potential distribution of the Argentine ant in Catalonia (Figure 2a).

In comparison with the degree-day model created with data from Newell and Barber (1913) and Benois (1973), the model created using recent data on brood developmental rates (Abril et al. unpublished data) might be calibrated more accurately because it predicts a theoretical development threshold closer to the real threshold observed, which seems to be around 18°C (Abril et al. unpublished data). However, this model predicts that the species would not be able to survive in locations such as Castell d'Aro or Barcelona (Figure 2b), where the invasion is serious and quite extensive. A possible explanation for these mismatches between the model output and the species' present distribution is that there are other processes involved in determining growth and survival of the colony, e.g. foraging activity or the fecundity of queens, which allow the establishment and survival of the species in such places even when the air temperature is not suitable for its brood development.

On the other hand, there are some important limitations that may reduce the trustworthiness of the degree-days' predictions within Catalonia. Firstly, environmental temperature inside the nests may well be different from the outside temperature, thereby making the use of data on soil temperatures essential in the creation of the model, and such data are unfortunately not available in Catalonia, where the meteorological stations of the Meteorological Service of Catalonia (SMC) only report air temperatures. Secondly, it only takes into account the effect of temperature on rates of development, when there may



be other temperature-dependent processes that could be affecting the growth and survival of the colony (Helmuth et al. 2005). Additionally, we think that the most significant limitation of a degree-day model when it comes to predicting the potential distribution of the Argentine ant on a physiological basis is that it does not take into account the importance of humidity in the survival of the species. The Argentine ant is heavily influenced by humidity. It is reported that it has significantly higher rates of water loss and cuticular permeability than other ant species (Shilman et al. 2005; 2007). This is probably the reason why low humidity environments negatively affect its foraging activity under the influence of high air temperatures (Human et al. 1998; Holway et al. 2002; Abril et al. 2007). It is also reported that the excess of humidity can also negatively affect the species (Krushelnycky et al. 2005; Wetterer et al. 2006). Thus, the low tolerance of the Argentine ant to both dry and excessive damp environments can restrict its distribution at regional scales (Krushelnycky et al. 2005; Wetterer et al. 2006; Shilman et al. 2007). As the degree-day model does not take into account humidity as a factor in predicting the most suitable areas for the species' establishment and survival, the degree-day models generated in the present study predict the potential presence of the species in western Catalonia, where warm, dry summers may not allow the survival of the species in non-anthropogenic environments. This is the most likely explanation for the differences observed between the degree-day models and the present known distribution of the species within the western part of Catalonia.

In view of the new data available on brood survival and oviposition rates of queens in relation to temperature, we decided to generate a model predicting the net productivity of workers across Catalonia in relation to temperature to predict the potential distribution range of the Argentine ant in this region under a novel approach.

To predict the potential range of the Argentine ant using data on oviposition rates and brood survival, we made three main assumptions. Firstly, we assumed that all the nests of this species contain eight queens, since our data on net worker productivity are based on the oviposition rates of queens in polygynous colonies containing eight queens (Abril et al. 2008). If we take into account that the oviposition rate of a queen varies according to the number of queens in the colony, and that the Argentine ant is a highly polygynous species



(Keller and Cherix 1985; Keller 1988; Passera 1994; Keller 1995; Ingram 2002; Abril et al. 2008), the fecundity and thus the predicted productivity of workers per queen could be substantially different from the data presented. Secondly, we assumed that the air temperature was equal to the temperature inside the nest. Obviously, nest temperature is probably different from air temperature but, as was the case with the degree-day models generated, meteorological stations in Catalonia do not report soil temperatures. We were therefore forced to use air temperatures rather than soil temperatures to generate the productivity model. Finally, we also considered that the percentage of brood survival obtained under experimental conditions (Abril et al. unpublished data), which we used to calculate net worker productivity, was not different from the percentage of brood survival in the field, although brood survival would probably be higher in natural nests due to the thermal gradient present in the nest and the fact that the environmental temperature is not constant but changes throughout the day.

Although we recognise the above limitations, our analysis indicates that the predicted productivity of Argentine ants in Catalonia, and the species' consequent distribution, may well be limited by temperature, showing a peripheral distribution similar to that observed in other parts of the world as a reflection of the species' need for a temperate climate and high humidity (Majer 1994; De Kock and Giliomee 1989; Suarez et al. 2001; Espadaler and Gómez 2003). Moreover, the model fits quite closely with the present known distribution of the Argentine ant in Catalonia (Figure 3) and also with the predictions of Roura-Pascual et al. (2004), which indicates the importance of the physiological factors in its distribution range. However, as with the degree-day model, its most important limitation is that it does not take into account the importance of humidity for the Argentine ant's survival, and therefore, its invasive potential. This may explain the main differences between the present known distribution of the species and the predictions of the productivity model in western Catalonia, where the low humidity present in the area probably makes it unsuitable the establishment of this pest species.

Predicting the potential distribution of an invasive insect is a complex undertaking, but the information that it can give us is extremely valuable in relation to managing the control of the invasion in the introduced areas. This study is an example of the enormous possibilities that physiological data offer in



the creation of new prediction models combining both climatic and physiological factors to determine the potential distribution range of the Argentine ant in great detail. But it also reveals the need for data on the effect of humidity on the physiology of the species (i.e. the effect of humidity on the oviposition rate of queens, or on the development and survival of the brood), if confidence in future predictions is to be improved.

In conclusion, the information obtained from these models only refer to areas in Catalonia with a higher risk of being invaded (if they have not been invaded already) by the Argentine ant in response to the species' physiological requirements in terms of temperature, and is only applicable to non-altered habitats since human related habitats such as crops or cities usually offer ideal conditions for establishment of the plague (Hölldobler and Wilson 1990; Holway 1998a; Suarez et al. 2001; Espadaler and Gómez 2003). But their real importance is that they provide new information to be taken into account in future prediction models of the Argentine ant's potential distribution in invaded areas; this opens the door to improvements in these valuable tools, which are essential for effective management of the Argentine ant invasion.

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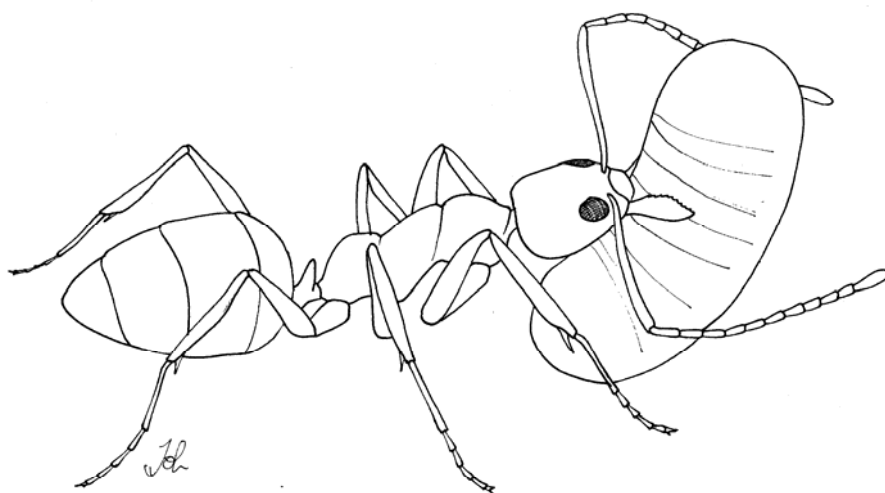


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DISCUSSIÓ GENERAL I CONCLUSIONS

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Discussió general

Els diferents estudis desenvolupats en aquesta tesi proporcionen informació nova i valuosa de cara al possible control de l'expansió de la formiga argentina en ecosistemes naturals fora de l'àrea nativa, així com també una millora en els coneixements que fins ara es tenien sobre la biologia de l'espècie. A continuació es discuteixen els diferents resultats obtinguts en relació a la seva aplicació en el control de la plaga com a pas previ a tenir en compte a l'hora de dissenyar protocols de gestió de l'espècie en medis naturals envaïts.

Al capítol 1 de la tesi es donen les bases biològiques per combatre la invasió en ecosistemes naturals envaïts mitjançant tractaments amb esquers tòxics d'efecte retardat. Tenint en compte que, l'estat fisiològic i reproductiu de la colònia i la qualitat nutricional de l'aliment que actua com a esquer, són dos dels factors clau que poden afectar l'eficàcia dels esquers tòxics (Krushelnycky & Reimer 1998; Rust et al. 2000; Soeprono & Rust 2004), els resultats obtinguts al capítol 1 d'aquesta tesi ens permeten donar resposta a les preguntes de "com" i "quan" es pot atacar la plaga mitjançant aquest mètode. Per altra banda, resulta obvi que atacar la plaga eliminant obreres no és un sistema massa efectiu, ja que el seu nombre és exageradament elevat i la quantitat de reines que hi ha als nius permet una constant regeneració d'aquesta casta. Ara bé, atacar la plaga eliminant les reines i la posta, pot arribar a afeblir enormement la colònia i, de retruc, el seu potencial d'expansió. Al capítol 1 es va observar que malgrat que la formiga argentina és una espècie omnívora (Newell & Barber 1913; Human et al. 1998), la qualitat de l'aliment que s'introdueix al niu varia depenent de l'època de l'any, i sembla estar estretament relacionada amb les necessitats tròfiques de la colònia en cada moment. Així, els mesos de màxima introducció d'aliment líquid (molt probablement melassa, ja que les obreres l'obtenien de la capçada de les sureres) coincidien amb els mesos de màxima densitat de mascles i obreres al niu (Markin 1970a; Benois 1973) que són els principals consumidors de carbohidrats de la colònia. Mentre que els mesos de màxima introducció de preses sòlides al niu, i per tant de proteïnes, coincidien amb els mesos de



màxima posta de les reines i de màxima densitat de larves (Benois 1973), que són els individus de la colònia que més requereixen d'aquest nutrient per pondre ous i créixer (Markin 1970b; Rust et al. 2000). Així doncs, el millor moment per atacar és quan la introducció de proteïnes al niu és màxima, ja que aquestes majoritàriament aniran destinades a la posta de les reines i al creixement de les larves. Aquest període comprèn els mesos de març a juny i de setembre a novembre a la nostra àrea d'estudi (capítol 1). Ara bé, el fet que les reines i larves també s'alimentin, encara que en proporció molt més petita de carbohidrats (Markin 1970b), ens suggereix que l'esquer pot resultar més atractiu a les obreres si també conté una part d'aquest nutrient. D'altra banda, el fet que la formiga argentina s'alimenti en més d'un 90% de la seva dieta d'aliment líquid (Markin 1970a; capítol 1), ens indica que seria convenient que l'esquer presentés una consistència líquida o gelatinosa per tal de maximitzar la seva atractivitat.

El capítol 1 també ens dóna informació valuosa sobre el ritme diari d'activitat de les obreres farratjadores a les sureres. Es va veure que aquest ritme d'activitat depenia majoritàriament de la temperatura i la humitat. Així doncs, es va observar que l'activitat diària de la formiga argentina es potencia durant el vespre, nit i matinada durant els mesos de primavera, estiu i tardor, quan la humitat és elevada i les temperatures són suaus, mentre que a l'hivern (mesos de desembre a febrer a la nostra àrea d'estudi), malgrat que no s'observa cap indicatiu d'hibernació en aquesta espècie, sí que s'observa, tal i com també va observar Markin (1970c) en un cultiu de llimoners a Califòrnia, que la seva activitat diària es veu limitada per les baixes temperatures (concretament les obreres deixen de cercar aliment per sota de 5°C). Per tant, a l'hora de dur a terme mètodes de control basats en esquers en àrees naturals envaïdes, s'hauria de tenir en compte també el fet que l'activitat diària de l'espècie varia depenent del moment del dia en què ens trobem i de l'estació de l'any.

Pel que fa a la dieta sòlida, s'ha observat que la formiga argentina s'alimenta bàsicament d'insectes i d'aquests la majoria són àfids (capítol 1). Aquesta informació pot ser rellevant en termes de l'atractivitat de l'esquer per les obreres farratjadores i s'hauria de tenir en compte a l'hora de dissenyar els esquers, per exemple fent que la proteïna que contingui provingui d'insectes.



La observació més interessant d'aquest apartat és el fet que malgrat que la majoria d'organismes capturats per la formiga presentaven signes de deshidratació, la qual cosa ens suggereix que segurament ja estaven morts quan les obreres els van recol·lectar, alguns dels individus encara estaven vius quan les obreres els transportaven al niu, posant en evidència el fet que la formiga argentina també pot actuar com a caçadora de petites preses com ara larves petites de lepidòpter o formes àpteres d'àfids (capítol 1).

Per altra banda, no deixa de ser sorprenent el fet que, tenint en compte que la formiga argentina pot arribar a presentar una activitat fins a 5 cops superior a la presentada per les formigues natives, l'extracció total anual d'aliment líquid de sureres per part de la formiga argentina en les àrees envaïdes no difereix significativament del que duen a terme les principals espècies de formigues natives recol·lectores de melassa observades a l'àrea d'estudi: *Crematogaster scutellaris* i *Lasius cinereus*. Aquesta situació es dona bàsicament pel fet que les formigues natives, com que són més grosses que la formiga argentina, acaben extraient de les sureres molt més aliment líquid per càpita, compensant així les diferències existents en nombre d'individus (capítol 1).

La formiga argentina presenta fenòmens estacionals de recol·locament de les colònies en resposta a les seves necessitats fisiològiques de temperatura i humitat (Newell & Barber 1913; Benois 1973; Heller & Gordon 2006; capítol 2). Així, a l'hivern les colònies d'aquesta espècie presenten una elevada densitat d'individus per tal d'incrementar la temperatura del niu. Per aquest motiu, sembla que les "colònies d'hivern" (Newell & Barber 1913) es col·loquen preferentment amb orientació sud, per tal de rebre la incidència directa del sol, i s'ubiquen en zones amb presència de matèria vegetal en descomposició, ja que aquest fenomen també allibera calor (Newell & Barber). A la primavera, amb l'ascens de les temperatures, es duu a terme una migració de dispersió (Newell & Barber 1913; Benois 1973; Heller & Gordon 2006; capítol 2), en la qual les diferents colònies d'hivern, es desglossen en colònies més petites que es dispersen fent avançar el front d'invasió. D'aquesta manera s'acaben constituint les anomenades "colònies d'estiu" (Newell & Barber 1913), que es caracteritzen per estar ubicades en zones fresques i humides per afavorir el desenvolupament de la posta (Newell & Barber 1913). A la tardor, es



torna a dur a terme una segona migració en la qual les diferents colònies es tornen a reagrupar generant novament les colònies d'hivern (Newell & Barber 1913; Benois 1973; Heller & Gordon 2006; capítol 2). Aquesta dinàmica estacional de disgregació i reagrupació de les colònies, juntament amb altres factors com la matança estacional de reines per part de les obreres o el naixement de noves reines, són els principals causants de la variació estacional de la densitat de reines als nius (capítol 2). D'aquesta manera, les màximes densitats de reines es poden trobar a l'hivern, quan les "colònies d'hivern" estan ben formades, mentre que les densitats més baixes es troben a l'estiu (capítol 2). La migració primaveral, juntament amb la matança massiva de reines al mes de maig (Keller et al. 1989), fa disminuir dràsticament la densitat de reines, mentre que aquesta torna a ascendir a la tardor, molt probablement gràcies a l'aparició de les noves reines fèrtils de la colònia i a la reagrupació de nius (capítol 2). Aquesta informació és molt útil a l'hora de dur a terme mesures de control de la plaga en àrees naturals envaïdes que es basin en la eliminació directa de reines sense utilitzar material químic. Ara bé, per tal d'iniciar mesures de control d'aquest tipus, també s'ha de tenir en compte tant l'activitat estacional de les formigues argentines com l'activitat de les formigues natives. Sembla ser que la presència de formigues natives al front d'avanç ofereix resistència a la invasió (Casellas 2004; Heller et al. 2006). Això explicaria el fet que a la nostra àrea d'estudi s'observi un retrocés del front d'avanç al mes d'abril, probablement causat per la sortida de la hibernació de les formigues natives (Casellas 2004; capítol 1). Malauradament, aquest retrocés es veu superat per l'avanç de la invasió al mes de març, durant el qual les formigues argentines aprofiten que les natives encara hibernen per poder envair noves zones lliures de la resistència de les espècies natives (Casellas 2004). Per aquest motiu el millor moment per atacar la invasió seria durant els mesos d'hivern (gener-març a la nostra àrea d'estudi); primerament perquè durant aquesta època hi ha les densitats de reines més elevades de l'any (capítol 2) i això permetria eliminar grans quantitats d'aquesta casta, en segon lloc perquè també permetria eliminar grans quantitats de posta hibernant, que segons Benois (1973) és la que generarà principalment reines a finals de primavera i estiu, i en tercer lloc, perquè atacar l'espècie durant aquesta època permetria oferir una via complementària de resistència a l'expansió de la invasió durant el



període de màxim avanç de la plaga. Així doncs, l'eliminació de reines i la consegüent reducció de la producció de nous individus al front d'avanç podria arribar a afeblir la dispersió de l'espècie i, per tant, limitar el seu establiment a noves àrees.

Per altra banda, aquesta tesi també aporta noves dades sobre els efectes de la temperatura en el desenvolupament i la supervivència de les diferents fases de la posta de la formiga argentina així com en la taxa de posta de les reines, aspectes força desconeguts fins ara en aquesta espècie. Així doncs, el capítol 3 ens dóna informació sobre l'efecte de la temperatura en la taxa de posta de les reines. Com era d'esperar, la taxa de posta de les reines de formiga argentina es veu afectada per la temperatura (Newell 1909; Benois 1973; capítol 3), així com també pel nombre de reines a la colònia. Mentre més alt és el grau de poligínia, més baixa és la taxa de posta per càpita (Keller 1985; Keller 1988a, capítol 3). El que no es sabia fins ara era com afectava exactament la variació de temperatura a la taxa de posta, ni tampoc a quines temperatures s'arribava a limitar la posta de les reines. Els nostres resultats indiquen que la temperatura més favorable per la taxa de posta de les reines és d'uns 28°C, sigui quin sigui el nombre de reines a la colònia (capítol 3), i que les temperatures que inhibeixen la taxa de posta varien depenent del nombre de reines. A mesura que aquest augmenta, el rang de temperatures en què les reines poden arribar a pondre ous es fa cada vegada més estret (capítol 3). És especialment interessant el fet que fins ara es creia que les reines deixaven de pondre ous a 17-18°C (Newell & Barber 1913; Benois 1973), mentre que en aquest estudi s'ha observat la posta d'ous per sota d'aquestes temperatures. Concretament, en colònies monogíniques (1 reina) o digíniques (2 reines), les reines podien arribar a pondre ous fins a 12°C (encara que en quantitats molt baixes). Això, juntament amb el fet que les obreres es mantenen actives a temperatures properes als 5°C (capítol 1) demostra la gran resistència d'aquesta espècie a les baixes temperatures. D'altra banda, també s'ha vist que dins les colònies poligíniques sempre hi ha una reina que tendeix a contribuir proporcionalment més a la posta de la colònia que la resta de reines del niu, algunes de les quals, malgrat ser fèrtils, poden arribar a no pondre a temperatures a les quals no hi hauria d'haver cap problema perquè ponguessin ous (capítol 3). Aquest fet es podria explicar en termes de l'atractivitat de les



reines a les obreres. Així, en colònies poligíniques, les reines atrauen en diferent grau a les obreres, de tal manera que aquestes tendeixen a alimentar més a unes reines que a les altres. En conseqüència, les reines més ben alimentades ponen més ous que les que són menys alimentades (Keller 1988a,b; Keller et al. 1988).

La temperatura també afecta la taxa de desenvolupament de les diferents fases de la posta de la formiga argentina (Newell & Barber 1913; Benois 1973; capítol 4). El capítol 4 presenta noves dades del temps de desenvolupament de la formiga argentina en funció de la temperatura, les quals presenten algunes millores metodològiques en comparació a les dades conegudes fins ara (Newell & Barber 1913; Benois 1973). D'una banda, s'ha mesurat la durada exacta (en dies) des de la posta dels ous fins a l'aparició de les obreres en funció de la temperatura, dades que fins ara només es podien arribar a obtenir mitjançant la suma de les mitjanes obtingudes de la durada de cada un dels estadis de la posta. Al capítol 4, s'han obtingut aquestes dades mesurant la durada exacta en dies des de la posta dels ous fins a l'aparició de les obreres. A més, la utilització d'estufes i incubadors va permetre obtenir una relació precisa entre el temps de desenvolupament i la temperatura, millorant així les dades obtingudes en altres estudis realitzats anteriorment per Newell i Barber (1913) i Benois (1973), en què les colònies es mantenien a temperatura ambient i les dades dels temps de desenvolupament es relacionaven amb la mitjana diària de temperatura. Això impedia també que pogués haver-hi una bona replicació de les temperatures sota estudi. En canvi, el capítol 4 presenta dades amb rèpliques per a cada temperatura. Aquestes diferències metodològiques podrien explicar el fet que els temps de desenvolupament a les diferents temperatures estudiades al capítol 4 siguin en general més llargs que els obtinguts anteriorment per Newell & Barber (1913) i Benois (1973).

D'altra banda, s'ha observat que el temps de desenvolupament de les larves era el més llarg a les diferents temperatures estudiades, en relació al temps de desenvolupament dels ous i les nimfes (capítol 4). Molt probablement, aquestes diferències s'expliquin pel fet que la larva és la única fase de la posta que és alimentada per les obreres (Markin 1970b), de manera que el temps de desenvolupament d'aquesta fase no només depèn de la temperatura, si no també de l'alimentació que rebí. Pel que fa a l'efecte de la temperatura sobre la



supervivència de les diferents fases de la posta de la formiga argentina (capítol 4), s'ha vist que mentre que els ous sobreviuen millor a temperatures suaus (21-24°C), les larves sobreviuen millor a temperatures més elevades (28-30°C). La supervivència de les nimfes, en canvi, no es veu afectada pels canvis de temperatura dins el rang de temperatures estudiat (21°C-30°C). Això repercuteix en els percentatges de supervivència observats en la totalitat del procés d'ou fins a obrera adulta, ja que en mantenir una temperatura constant per a tot el cicle, sempre hi havia una de les fases que presentava dificultats per a sobreviure. Aquest fet acabava generant percentatges de supervivència força baixos a totes les temperatures observades. La temperatura més favorable per a dur a terme el cicle complet fins a obrera adulta va ser de 26°C, ja que aquesta permetia una supervivència relativament alta tant dels ous, com de les larves. Probablement, els percentatges de supervivència de la posta en condicions naturals siguin més elevats ja que la temperatura a la natura no és un factor constant, sinó que va canviant al llarg del dia i de l'any (com a mínim a les zones envaïdes), i també perquè com que els nius presenten una tercera dimensió, que és la profunditat, es pot donar un gradient vertical de temperatura que permeti a les obreres recol·locar la posta a les condicions més òptimes per assegurar la màxima supervivència a cada fase de desenvolupament, i assolir així el màxim èxit reproductiu de la colònia. No obstant això, tot i les possibles diferències que hi pugui haver entre les condicions experimentals i les naturals, les dades obtingudes sobre la supervivència de la posta en relació a la temperatura són, ara per ara, les úniques que hi ha per aquesta espècie. Aquestes dades poden arribar a ser molt útils a l'hora de generar models predictius, ja que descriuen, de manera precisa, la relació que hi ha entre la temperatura i la supervivència de la posta. De la mateixa manera, les dades sobre el temps de desenvolupament obtingudes al capítol 4 d'aquest treball, són fins ara les que millor representen la relació entre la temperatura i el temps de desenvolupament de la posta, la qual cosa pot ajudar a crear models predictius millor calibrats que els obtinguts anteriorment. En resum, els capítols 3 i 4 de la tesi deixen clar que la temperatura és un factor clau per a la supervivència de la colònia, ja que influeix fortament tant en el temps de desenvolupament de la posta com en la supervivència de la mateixa. A més, la relació establerta entre la temperatura i



el desenvolupament i supervivència de la posta, és un dels múltiples factors que determinen que l'espècie es pugui establir en unes zones i no en unes altres, i per tant, és una informació essencial que cal tenir en compte a l'hora de generar models de predicció de la seva distribució potencial a les àrees envaïdes. D'aquesta manera, al capítol 5 s'utilitzen aquestes dades per a crear models de predicció de les àrees de Catalunya tèrmicament més favorables per què la formiga argentina pugui dur a terme el seu cicle vital. La informació obtinguda en aquests models ens indica les zones de Catalunya potencialment més favorables per a la productivitat d'obrerres o pel desenvolupament del seu cicle vital en ecosistemes naturals. Tanmateix, això pot no ésser suficient a l'hora de predir la distribució potencial de l'espècie en aquesta regió, ja que l'establiment de la mateixa en una àrea determinada no només depèn de factors fisiològics sinó també d'altres factors biològics i ecològics. A més, no hem d'oblidar que es tracta d'una predicció amb les seves respectives limitacions. Ara bé, aquesta nova informació obre les portes a la millora dels models de predicció de la distribució potencial d'aquesta espècie, que fins ara no tenien en compte la importància de la seva fisiologia a l'hora de predir la seva distribució potencial a les àrees envaïdes, la qual cosa pot arribar a ser molt útil a l'hora de prevenir i/o gestionar la invasió en ecosistemes naturals.

Futures línies d'investigació

La informació obtinguda en aquesta tesi doctoral en referència a l'estudi de diferents aspectes biològics de la formiga argentina com a base per a iniciar protocols de gestió de la plaga en ecosistemes naturals, obre les portes a noves línies d'investigació relacionades amb aquest tema. En aquest sentit, seria interessant poder determinar amb més detall la dinàmica de moviment espacial i estacional de nius en general, i reines en particular, de la formiga argentina en ecosistemes naturals amb la intenció de determinar espacialment i temporal quan i on poder actuar sobre les reines com a element de control de l'avançament de la invasió, així com determinar exactament quines són les característiques que determinen la ubicació dels nius (insolació, cobertura, temperatura del sòl...) i relacionar aquests factors amb la densitat d'individus i sobretot de reines. Així mateix, tenint en compte que la formiga argentina



presenta una taxa d'expansió superior en zones naturals cremades que en zones no cremades (obs. pers.), també seria interessant estudiar si també existeixen diferències en quant a la ubicació dels nius i la seva densitat en funció de si les àrees envaïdes han estat afectades per un incendi o no.

En segon lloc, també seria interessant determinar si la taxa d'expansió es troba influenciada per la dinàmica estacional de moviment de nius i per la densitat poblacional d'aquests i, com en el punt anterior, si existeixen diferències en aquest sentit entre zones naturals cremades i zones no pertorbades per aquest factor.

En tercer lloc, i tenint en compte tant la informació obtinguda en aquesta tesi com la obtinguda en estudis posteriors com els comentats anteriorment, seria convenient determinar els efectes de l'extirpació de nius en l'avançament del front d'invasió i en la fauna d'artòpodes tant en zones naturals cremades com zones no cremades, i comparar-los amb l'efecte de l'ús d'esquers tòxics d'efecte retardat.

Conclusions

1. La millor època per atacar la plaga mitjançant esquers tòxics d'efecte retardat en ecosistemes naturals és durant l'època de màxima introducció de proteïnes al niu, ja que aquest aliment va destinat majoritàriament a l'alimentació de reines i larves. L'eliminació d'aquestes castes pot afeblir la producció d'individus i, de retruc, el seu potencial per expandir-se. Aquesta època comprén els mesos de primavera i principis d'estiu (març a juny a la nostra àrea d'estudi) i de tardor (setembre a novembre a la nostra àrea d'estudi).
2. Per a la utilització d'esquers tòxics d'efecte retardat és important tenir en compte que l'activitat diària de cerca d'aliment de la formiga argentina en ecosistemes naturals de clima estacional depèn de l'estació de l'any d'acord amb les variacions de temperatura i humitat. A l'hivern, finals de tardor i principis de primavera l'activitat es concentra durant les hores de màxima temperatura ambiental i desapareix per sota de 5°C. La resta de l'any l'activitat de cerca d'aliment comprén les 24 hores del dia i es concentra durant la nit i matinada, quan la temperatura és més suau i la humitat més elevada.



3. Altres factors que podrien afectar l'atractivitat de l'esquer és la consistència del mateix. Tenint en compte que la formiga argentina s'alimenta principalment d'aliment líquid, l'esquer podria resultar més atractiu si es presenta en forma líquida o gelatinosa. A més, el fet que les reines i larves s'alimentin principalment de proteïnes i que la formiga argentina obté aquestes proteïnes principalment d'insectes morts o depredats, l'esquer podria resultar més atractiu si presentés com a nutrient principal proteïnes d'insecte, juntament amb una petita part de carbohidrats, ja que les reines i larves també s'alimenten, encara que en proporció més petita, d'aquest nutrient.
4. Per tal de dur a terme metodologies d'eliminació massiva de reines de manera mecànica i sense ús d'agents químics, s'ha de tenir en compte que la densitat de reines per litre de sòl en ecosistemes naturals varia depenent de les migracions estacionals de l'espècie i d'altres factors propis de la seva biologia, com la matança massiva de reines al maig o el naixement a l'estiu de noves reines. Per tant, el millor moment per eliminar aquests individus és durant els mesos d'hivern (gener a març a la nostra àrea d'estudi), perquè és l'època en què hi ha una densitat més elevada de reines, perquè també permetria eliminar la posta de larves hivernant que generalment és la que genera reines a l'estiu, i perquè permetria oferir una via complementària de resistència a l'expansió de la invasió durant el període de màxim avanç de la plaga.
5. La taxa de posta de la formiga argentina es veu fortament influenciada, tant per la temperatura com pel nombre de reines a la colònia. Així, mentre més elevat és el grau de poligínia del niu, més estret és l'espectre de temperatures en el qual les reines poden pondre ous. La temperatura òptima de posta d'ous en qualsevol condició de poligínia és de 28°C en condicions experimentals.
6. La temperatura és un factor clau que determina els temps de desenvolupament i la supervivència de les diferents fases de la posta de la formiga argentina. Així, mentre que la supervivència dels ous es veu afectada negativament a mesura que s'incrementa la temperatura, la supervivència de les larves es veu afectada negativament a mesura que la temperatura decreix. L'estadi nimfal és el que presenta més resistència a la variació de la temperatura. La temperatura òptima en condicions experimentals per a dur a terme el cicle complet des d'ou fins a obrera adulta en la formiga argentina és de 26°C.



7. Les noves dades aportades sobre les necessitats tèrmiques de la formiga argentina en relació a la posta d'ous, temps de desenvolupament i supervivència de les seves fases juvenils permeten la creació de models de predicció de la potencial distribució de la formiga argentina en ecosistemes naturals envaïts en base a aquests aspectes de la seva fisiologia, que fins ara no es tenien en compte a l'hora de generar models predictius. La combinació d'aquesta informació, juntament amb la obtinguda fins ara en models predictius basats en factors climàtics, obre les portes a la creació de futurs models que permetin una millor predicció de la distribució potencial de la invasió en ecosistemes naturals envaïts de tot el món.

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