# Effect of Seasonal Dynamics on Queen Densities of the Argentine Ant (*Linepithema Humile*) in an Invaded Natural Area of the NE Iberian Peninsula

by

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## 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.

KEYWORDS: Argentine ant, *Linepithema humile*, queen densities, seasonal nest dynamics

## INTRODUCTION

The Argentine ant (*Linepithema humile* Mayr), well-known as an invasive ant species (McGlynn 1999), has spread world-wide (Hölldobler & Wilson

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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 & Gordon 1996, Human & 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,b, Silverman & 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 analyse 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 (Fig.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).

## 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 (Fig.1). Soil samples were only collected when brood were visible, since queens were usually situated inside the nest near the brood (pers. obs.).

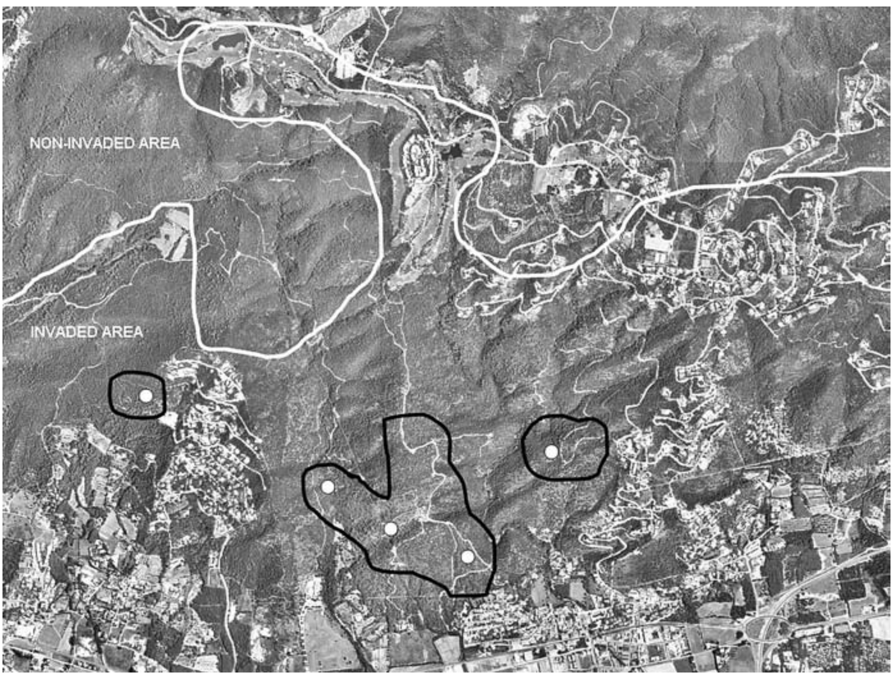


Fig.1. Geographical distribution of the Argentine ant invasion in the population area of Castell d'Aro (NE Iberian Peninsula) and location of both artificial winter nests and the areas where the Argentine ant nest soil was sampled. A white circle indicates each block of 28 artificial winter nests. The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

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 (Fig.2). In April we observed a marked fall in densities, followed by a more gradual fall

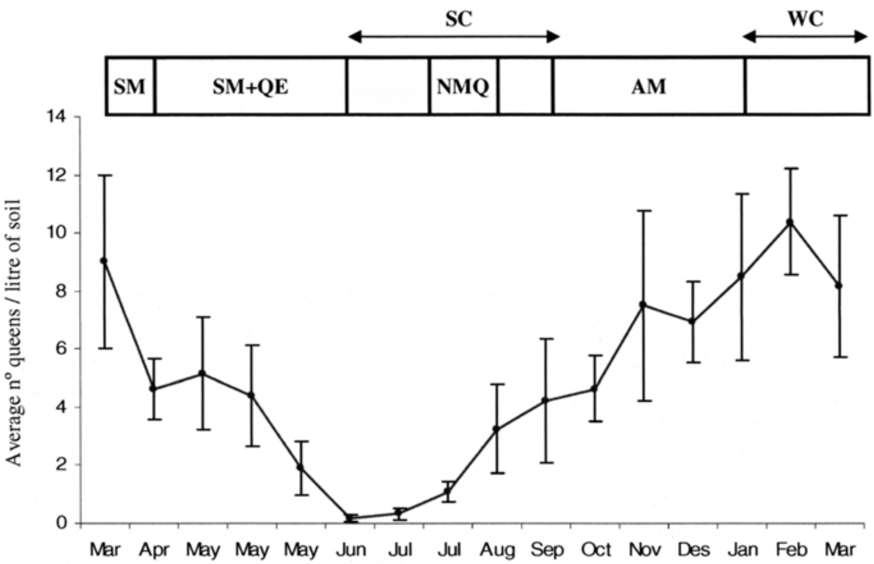


Fig.2 .Seasonal densities of queens in natural nests of the Argentine ant. AM: autumn migration; NMQ: new mated queens; QE: queen execution; SC: summer colonies; SM: spring migration; WC: winter colonies. The error bars are the standard errors.

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) (Fig.2). The annual mean (± SD) number of queens per litre of soil from natural nests was 5.04 ± 3.19 ($n$ = 240). The month which reported minimum mean (± SD) queen densities was June (0.16 ± 0.25; $n$ = 12), while the month which presented maximal mean (± 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 ± 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) ($F_{1,110} = 75.366$, $P < 0.001$).

## 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 and 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 nest 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 reached 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 reports 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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## REFERENCES

Abril, S., J. Oliveras & C. Gómez 2007. Foraging activity and dietary spectrum of the Argentine ant (Hymenoptera: Formicidae) in invaded natural areas of the northeast Iberian Peninsula. Environmental Entomology 36(5): 1166-1173.

Baker, T.C., S.E. Van Vorhis & L.K. Gaston 1985. Bait preference tests for the Argentine ant (Hymenoptera: Formicidae). Journal of Economical Entomology 78: 1083-1088.

Benois, A. 1973. Incidence des facteurs écologiques sur le cycle annuel et l'activité saisonnière de la fourmi d'Argentine, *Iridomyrmex humilis* Mayr (Hymenoptera, Fomicidae), dans la région d'Antibes. Insectes Sociaux 20: 267-295.

Blancafort, X. & C. Gómez 2005 Consequences of the Argentine ant, *Linepithema humile* (Mayr), invasion on pollination of *Euphorbia characias* (L.) (Euphorbiaceae). Acta Oecologica 28: 49-55.

Bond, W. & P. Slingsby 1984. Collapse of an ant-plant mutualism: the Argentine ant (*Iridomyrmex humilis*) and myrmecochorous proteaceae. Ecology 65: 1031-1037.

Buckley, R. 1987. Ant-plant-Homopteran interactions. Advances in Ecological Research 16: 53-85.

Casellas, D. 2004. Tasa de expansión de la hormiga argentina, *Linepithema humile* (Mayr 1868), (Hymenoptera, Dolichoderine) en un área mediterránea. Boletín de la Asociación española de Entomología 28(1-2): 207-216.

Chang, V. & A.K. Otta 1990. Ant control in Hawaiian drip irrigation systems, *In:* R.K. Vander meer, K. Jaffe, and A. Cedeno [eds.], Applied myrmecology- a world perspective. Westview Press, Boulder, CO: 708-715.

Cole, F.R., A.C. Medeiros, L.L. Loope & W.W. Zuehlke 1992. Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. Ecology 73: 1313- 1322.

Gómez, C. & J. Oliveras 2003. Can the Argentine ant (*Linepithema humile*, Mayr) replace native ants in myrmecochory? Acta Oecologica 24: 47-53.

Gómez, C., P. Pons & J.M. Bas 2003. Effects of the Argentine ant *Linepithema humile* on seed dispersal and seedling emergence of *Rhamnus alaternus*. Ecography 26: 532-538.

Heller, N.E. & D.M. Gordon 2006. Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant (*Linepithema humile*). Ecological Entomology 31: 499-510.

Heller, N.E., N.J. Sanders & D.M. Gordon 2006. Linking temporal and spatial scales in the study of an Argentine ant invasion. Biological invasions 8: 501-507.

Hölldobler, B. & E.O. Wilson 1990. The Ants. Belknap Press Harvard, Cambridge, Massachussetts, USA.

Holway, D.A. 1998a. Factors governing rate of invasion. A natural experiment using Argentine ants. Oecologia 115: 206-212.

Holway, D.A. 1998b. Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. Oecologia 116: 252-258.

Human, K.G. & D.M. Gordon 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. Oecologia 105: 405-412.

Human, K.G. & D.M. Gordon 1997. Effects of Argentine ants on invertebrate biodiversity in northern California. Conservation Biology 11:1242-1248.

Ingram, K.K. 2002. Plasticity in queen number and social structure in the invasive Argentine ant (*Linepithema humile*). Evolution 56(10): 2008-2016.

Keller, L., L. Passera & J.P. Suzzoni 1989. Queen execution in the Argentine ant, *Iridomyrmex humilis*. Physiological Entomology 14: 157-163.

Klotz, J.H., M.K. Rust & P. Phillips 2004. Liquid bait delivery systems for controlling Argentine ants in citrus groves (Hymenoptera: Formicidae). Sociobiology 43: 419-427.

Krushelnycky, P.D. & N.J. Reimer 1988a. Bait preference by the Argentine ant (Hymenoptera: Formicidae) in Haleakala National Park, Hawaii. Environmental Entomology 27:1482-1487.

Krushelnycky, P.D. & N.J. Reimer 1988b. Efficacy of maxforce bait for control of the Argentine ant (Hymenoptera: Formicidae) in Haleakala National Park, Maui, Hawaii. Environmental Entomology 27: 1473-1481.

Markin, G.P. 1970. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in southern California. Annals of the Entomological Society of America 63(5): 1238-1242.

McGlynn, T.P. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. Journal of Biogeography 26:535-548.

Myers, J.H., D. Simberloff, A.M. Kuris & J.R. Carey 2000. Eradication revised: dealing with exotic species. Trends in Ecology and Evolution 15: 316-320.

Ness, J.H. & J.L. Bronstein 2004. The effects of invasive ants on prospective ant mutualists. Biological Invasions 6: 445-461.

Newell, W. & T.C. Barber 1913. The Argentine ant. US Department of Agriculture, Bureau of Entomology Bulletin 122:1-98.

Passera, L. 1994. Characteristics of tramp species. Pp 23-43 in D.F. Williams (Ed.) Exotic Ants: Biology, Impact and Control of Introduced Species. Westview Press. Boulder. Colorado.

Silverman, J. & T.H. Roulston 2001. Acceptance and intake of gel and liquid sucrose compositions by the Argentine ant (Hymenoptera: Formicidae). Entomological Society of America 94: 511-515.

Suarez, A.V., D.T. Bolger & J.T. Case 1998. Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79: 2041-2056.

Suarez, A.V., J.Q. Richmond & T.J. Case 2000. Prey selection in horned lizards following the invasion of argentine ants in Southern California. Ecological Applications 10(3): 711-725.

Suarez, A.V., D.A. Holway & T.J. Case 2001. Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. Proceedings of the National Academy of Sciences of the USA 98: 1095-1100.

Visser, D., M.G. Wright & J.H. Giliomee 1996. The effect of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), on flower visiting insects of *Protea nitida* Mill. (Proteaceae). African Entomology 4: 285-287.