Comparing Methods of Evaluating the Spread of Argentine Ants in Natural Habitats: Pitfall Traps vs. Baiting

by

David Casellas[1 *], Crisanto Gómez[1] & Miguel Clavero[1, 2]

Abstract

Two methods of trapping Argentine ants in natural habitats are compared. Both methods are used on the boundaries of an invaded area with the goal of assessing the spread of the invasion front. Pitfall surveys take longer to obtain results than bait surveys, but bait surveys are only a "snapshot" of the moment, with less chance of detecting Argentine ant workers. Significant differences are found between the methods in terms of the number of traps occupied by Argentine ants, native ants or a combination of both. Differences in the richness of native ant species are found as well, showing that pitfall surveys are necessary to assess such richness. Despite this, no differences in the assessment of spread are found between the methods. Bait surveys are an easier and faster method to assess the spread of Argentine ants, spread being one of the most important characteristics of biological invasions.

Key words: Argentine ant, bait, *Linepithema humile*, methods of survey, pitfall, colonial spread.

Introduction

Biological invasions threaten global biodiversity by altering the structure and functioning of ecosystems (MacDougall & Turkington 2005). Invasion is a multi-step process consisting of three stages: initial dispersal, establishment of self-sustaining populations within the new habitat, and spread of the organism to nearby habitats (Kolar & Lodge 2001; Williamson & Fitter 1996). Most studies in recent years have focused on the establishment of invasive species and not on initial dispersal or spread (Puth & Post 2005). The relationship between the generalized steps in the invasion process and

[1*]Departament de Ciències Ambientals, Universitat de Girona. Campus de Montilivi, 17071 Girona, Catalonia (Spain) Author for correspondence: Email: david.casellas@udg.edu.

[2]Grup d'Ecologia del Paisatge, Àrea de Biodiversitat, Centre Tecnològic Forestal de Catalunya. Carretera vella de Sant Llorenç de Morunys km 2. 25280 Solsona, Catalonia (Spain).

the management of invasive species implies that sufficient knowledge of the spread will allow better control of the invasive species and the area invaded (sakai *et al.* 2001)

The invasive Argentine ant is native to south America and has spread almost worldwide (roura-Pascual *et al.* 2004; suarez *et al.* 2001), especially in Mediterranean-type communities around the world (Majer *et al.* 1994). Where it is introduced, many ant and other arthropod populations decrease drastically (bond & slingsby 1984; Cole *et al.* 1992; Human & Gordon 1996; suarez *et al.* 1998).

Within introduced populations, Argentine ant colonies have a unicolonial structure with large, multiple-queen colonies that lack clear boundaries due to a general absence of intraspecific aggression (Giraud *et al.* 2002; suarez *et al.* 1999; tsutsui *et al.* 2000). This invasive species exhibits two ways of dispersal: jump dispersal over longer distances, associated with human-mediated transport (suarez *et al.* 2001), and over shorter distances through colony budding, where queens and workers disperse on foot to form new nests (Keller 1995).

The lack of a mating flight and dispersion over shorter distances allows spread to be studied at short scale. In the case of Argentine ants, this spread ranges from 0 to 275 m/yr (suarez *et al.* 2001). other examples of local spread in invasive ants are: 146-294 m/yr for the crazy ant, *Anoplolepis gracilipes,* in the seychelles Islands (Gerlach 2004); 71.6 m/yr in Gabon (Walsh *et al.* 2004); 170 to 500 m/yr on santa Cruz in the Galapagos Islands for *Wasmannia auropunctata* (Lubin 1984; Lubin 1985) and 1.2-134 m/yr in Hungary and spain for *Lasius neglectus* (Espadaler *et al.* 2007). There are several studies that have investigated the spread of the Argentine ant and ant and arthropod abundances at a local scale using pitfalls (Cole *et al.* 1992; Heller *et al.* 2006; Krushelnycky *et al.* 2004; Menke *et al.* 2007; suarez *et al.* 2001). This method obtains a good indication of the presence of any species, including their abundance and, depending on the time of the survey, the results may include information about variation in activity patterns. other studies have used baits to study the spread of the Argentine ant (Holway 1998b). baits are usually used to assess the presence of a particular species, or recruitment; it is a faster method of obtaining results, but has the disadvantage of being like a "snapshot" and therefore not taking into consideration differences in activity patterns. There are several examples of studies comparing the two methods to test for the presence or absence of the Argentine ant (stanley *et al.* 2008).

In the present paper we compare the two methods (pitfall survey and bait survey) with the main objective of assessing potential differences between them in measuring the rate of spread in the case of the Argentine ant.

MATERIALS AND METHODS

The study was conducted in a Mediterranean forest massif (Les Gavarres), near the village of Castell-Platja d'Aro (41°49'N, 3°00'E). Four transects were established on the boundary of the invaded area in such a way that a part of each transect was placed inside the invaded area and the other part in the non-invaded area, so that the movement of the Argentine ant invasion front could be periodically observed.

Two types of survey were carried out, a pitfall survey and a bait survey. both consisted of 30 traps (pitfall or bait, depending on the type) being placed in a straight line at four-meter intervals along a 120-meter long transect. The fact that the distance between the intervals was known allowed us to assess the variation in the spread of invasion. Each pitfall trap consisted of a plastic conical tube 10cm long and 2cm in diameter containing 10ml of ethylene glycol at 70% as a killing and preserving solution (similar to the pitfall trapping in Holway 1998a). This pitfall was placed inside an empty tube driven into the ground, which remained there throughout the study to avoid the attraction effect for arthropods that the removed soil could have had. The bait trap consisted of a 76 x 26mm microscope slide painted white and covered with a mixture of tuna fish and peach jam, representing sources of lipids, proteins and carbohydrates (Fellers 1987).

The study was conducted from October 2004 to October 2005, and the traps were placed every month, so that by comparing the data monthly we could assess the movement of the invasion front. Each trap was numbered and placed at the same point each month.

To compare the two types of survey, we placed each pitfall trap next to a corresponding bait trap (so there was the same number of each). because the time that each type of survey needed to run in order to work was different, we first drove the pitfalls into the ground and then one week later we put table 1. statistical analysis of the variables. The comparisons between transects (n=4), methods (n=2; pitfall and bait), months (n=13, from october 2004 to october 2005) and method*month are shown.

	spread (meters)		% of *L.humile*		% of native ants		% *L.humile* + native ants		abundance of *L.humile*		species richness	
	F-statistic	*P*-value	*F*-statistic	*P*-value	*F*-statistic	*P*-value	*F*-statistic	*P*-value	*F*-statistic	*P*-value	*F*-statistic	*P*-value
transect	11.1	***	27.2	***	5.6	**	8.7	***	11.9	***	2.9	*
Method	2.2	0.15	4.3	*	100.0	***	73.2	***	1.5	0,23	128.8	***
Month	6.6	***	12.3	***	24.7	***	5.6	***	25.5	***	23.4	***
Method * Month	0.5	0.84	2.2	*	3.3	**	5.4	***	3.5	**	2.4	*

the top on the pitfall, and then placed the bait trap close to the corresponding pitfall. two hours later we collected the pitfalls and we made a note of the ants present on each bait trap. before carrying out the bait survey we put the top on the pitfalls to avoid the attraction effect that the bait could have produced on the pitfalls, and the subsequent overestimation of pitfall captures.

From the bait survey we took data about the type of ant (native or *L. humile*) present on each bait trap and, if possible, the species of native ant. From the pitfall survey we identified in the laboratory the species of ant present in each pitfall and classified the remaining arthropods to order taxa.

The data obtained was processed with sPss version 15. The percentage of items occupied by *L. humile*, or native ants, or both or neither of them, was compared between survey types. We used the same program to compare the two types in the assessment of the spread of the invasion: the number of items occupied by *L. humile* during each month of study, and the number of meters occupied by the invasive ant.

rEsuLts

With regard to the spread of the invasion front, there were no significant differences between the methods (Fig.1, F = 2.2, P = 0.15; table 1). We found a difference in the months where the number of meters of the transect occupied by *L. humile* was maximum: June in the pitfall survey and April in the bait survey (table 1; F = 6.6, P<0.001).

significant differences were detected in the percentage of units (both pitfall and bait) occupied by *L. humile*, by native ants or a combination of both (*L. humile* plus native ants) (table 1). The differences by methods of survey were more significant in the case of native ants (F = 100.0, P<0.001) and a combination *L. humile* and native ants (F = 73.2, P<0.001), than in the case of *L. humile* (F = 4.3, P = 0.04). The reason is that in the cases of both native ants and a combination, the pitfall survey always showed them reaching a higher proportion, whereas in the case of *L. humile* the pitfall survey showed higher numbers in some months and in other months it was the bait survey (Fig. 2). In fact, for the combination, we only found data in the pitfall survey, i.e. no interaction of *L. humile* and native ants in the bait survey was observed.

The differences between methods in terms of the abundance of *L. humile* were not significant (table 1, F = 1.5, P = 0.23), despite the fact there was a period of the year, from september to February (the period with least ant activity), when the abundance was higher when measured by pitfall survey

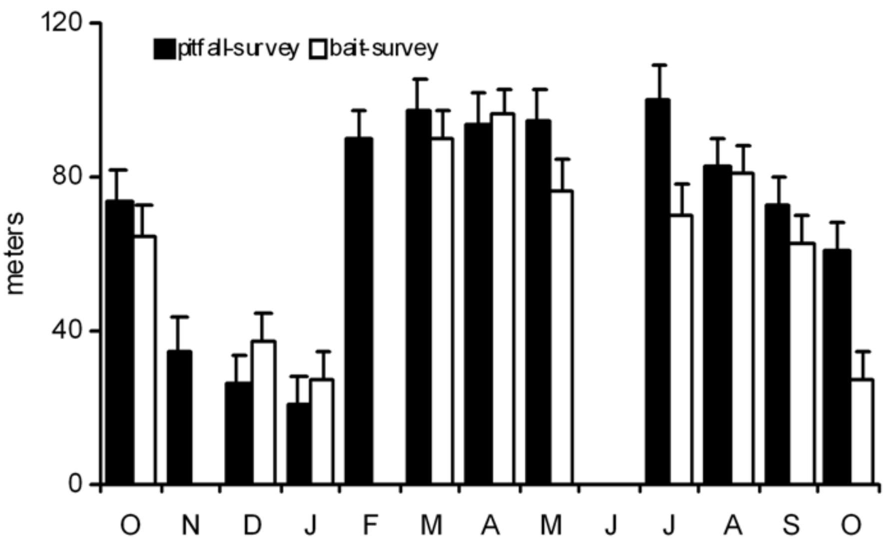
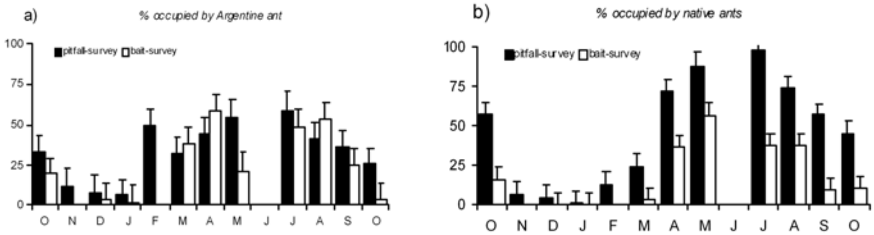


Figure 1. spread of the Argentine ant invasion front: comparison of two survey methods. bait surveys were not conducted in november or February, and in June neither bait nor pitfall surveys were conducted.

and from March to August (with the exception of May) when the bait survey indicated higher abundances (Fig. 3).

There were significant differences between the methods of survey in terms



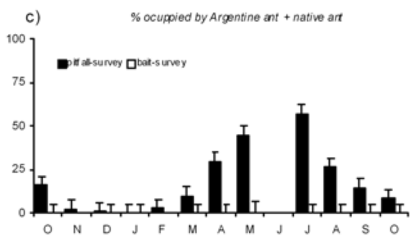


Fig. 2. Percentage of traps (pitfall or bait) occupied by Argentine ants (a), native ants (b) or a combination of both Argentine and native ants in the same trap (c).

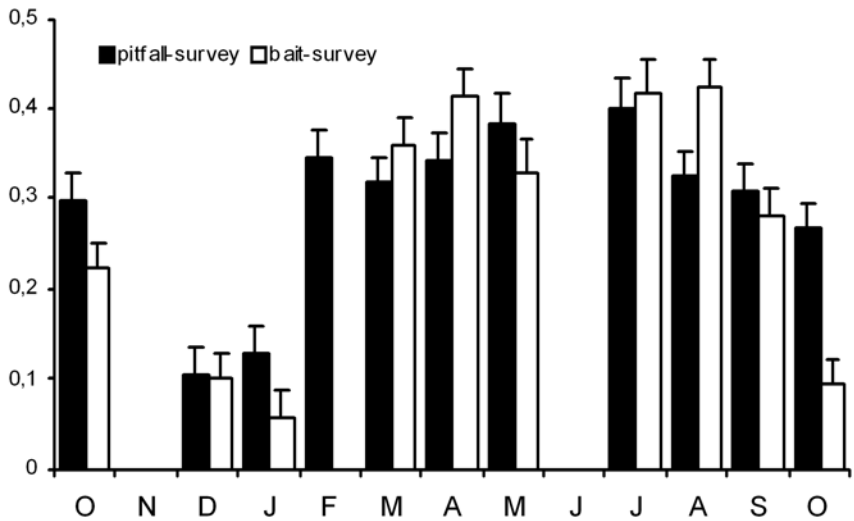


Fig. 3. Abundance of Argentine ants. The values are shown on a relative scale resulting from standardization (the value of abundance multiplied by 30, which is the total number of each transect's traps, divided by the number of traps with data; logarithmic transformation was then applied).

of species richness (table 1, F = 128.8, P<0.001). The pitfall survey seemed to be the better of the two (Fig. 4).

For all aspects of the analysis (spread, percentage of ants, abundance of *L. humile* and species richness) there were great differences between months (table 1, P<0.001).

DIsCussIon

Spread of *Linepithema humile*

Despite the fact there were no significant differences between the methods of survey in terms of testing spread, seasonal variation was observed. In the coldest period of the year (December and January, and perhaps november and February although there were no bait-data for these two months), the bait survey achieved higher values than the pitfall survey. During the rest of the year, with the exception of April, the pitfall survey data had higher values. Although they avoid temperature extremes, Argentine ants are active in a wide range of abiotic conditions (Human *et al.* 1998), so in colder months it is more probable that the active ants on the surface will be Argentine ants (Casellas 2004). In addition to this, in colder months the colony networks

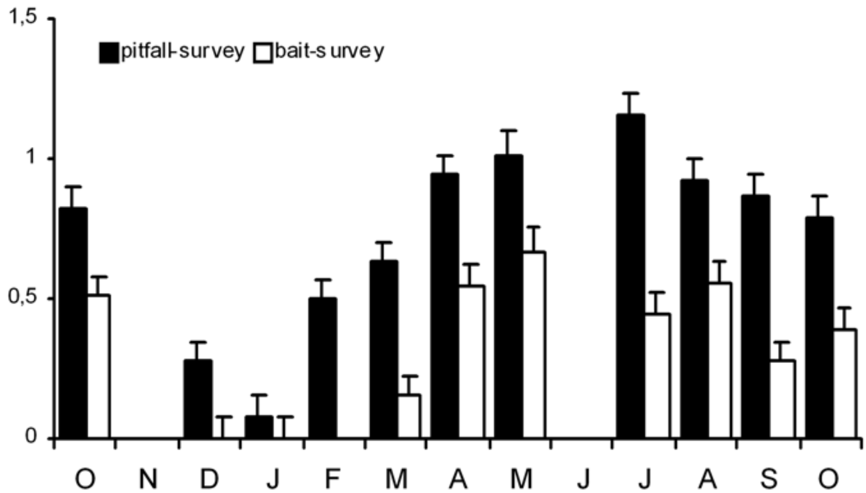


Fig. 4. richness of native ants. The values are shown on a relative scale resulting from standardization (the value of abundance multiplied by 30, which is the total number of each transect's traps, divided by the number of traps with data; logarithmic transformation was then applied).

contract (Heller & Gordon 2006) and foraging activity is lower due to the low temperatures (Holway *et al.* 2002; Human *et al.* 1998; Krushelnycky *et al.* 2005; oliveras *et al.* 2005; rust *et al.* 2000). All these characteristics lead to the conclusion that the pitfall survey could be a snapshot of the area occupied by the contracted colony networks and that the bait survey could attract foragers beyond the borders of the colony (our baits represented an easily available food resource). The rest of the year (from March to october), the pitfall survey showed a greater advance in the spread than the bait survey, with the exception of April (although the difference is so slight it was probably due to random processes). The results from March to october could be the consequence of encounters between Argentine ants and native ants. July is the month that shows the most marked difference in the results of the two survey methods, with the pitfall survey reaching the higher values; Fig. 2c shows that July was also the month when the most pitfalls were occupied by native and Argentine ants. Interactions between Argentine and native ants on the surface led to the displacement of the latter (sanders *et al.* 2001) because of the superior competitive ability of the Argentine ant (Human & Gordon 1996; 1997). The interactions were located and detected by bait surveys because the ants involved were workers, probably recruited by foragers that had previously found the food source (the tuna fish and jam bait); this bait could have been found by native ants more or less at the same time. As explained in the materials and methods section, we put the top on the pitfall the same day but before we placed the bait. If we hadn't done this, there probably wouldn't have been any difference between the two methods. The bait survey detects the new area that is going to be invaded (after a successful interaction with native ants) or the boundaries of the invaded area, whereas the pitfall survey detects the area currently invaded by Argentine ants.

Activity in *Linepithema humile* and native ants

significant differences between the methods are observed in the percentage of traps (pitfall or bait) occupied by ants. In terms of Argentine ant activity, in March, April and August the bait survey had higher values than the pitfall survey; curiously, March and April showed the most native ant activity, due to temperatures being more suitable after the winter, while the maximum level of activity of *Pheidole pallidula*, the native ant most abundant in the study area (personal observations, unpublished data) was in August. The differences between the methods were most appreciable in the traps occupied by native ants or a combination of native ants plus Argentine ants (Figs. 2b and 2c; table 1). The foraging activity of ants and other small invertebrates is particularly sensitive to climatic fluctuations (Fellers 1989) and the Mediterranean ant communities show this temporal variation in their daily activity patterns (Cros *et al.* 1997). The "snapshot" of the bait survey meant that the activity of some native ants could not be registered in the survey because the time available to observe the baits didn't match up with the time of native ant activity. The pitfall method allowed us to minimize the effect of this "snapshot" in the bait survey because this method ran over a period of one week, so it was a better way to register the activity of the ants. surprisingly, no bait with both Argentine and native ants was found at the same time, further evidence of temporal variations in activity patterns. These interactions did appear in the pitfall survey, which showed a higher number of encounters from April to August than in the rest of the year due to the increase in Argentine ant activity, which made encounters more probable.

Abundance of *Linepithema humile* and native ant richness

no difference between the two methods was observed in the abundance of *L. humile*, indicating that both methods are suitable for assessing the numbers of this ant in field studies. Differences appeared in months showing the temporal variability in the seasonal cycle of Argentine ant activity (Fig. 3, table 1).

native ants' species richness showed significant differences (Fig 4. table 1). The best method of assessing species richness is the pitfall survey because, as previously explained, native ants have different daily patterns of activity, and the bait survey only ran for about two hours. The native species richness graph (Fig. 2b) and the percentage of native ants graph (Fig. 4) are very similar (pitfall survey correlation: 0.956; bait survey correlation: 0.878), indicating that the amount of native species (richness) and the number of traps occupied by native ants are correlated. This means that there is a spatial and temporal variation in the activity patterns of native ants (Cros *et al.* 1997).

The clear differences existing between months throughout the analysis reflect seasonal variations in ant activity in general and *Linepithema humile* in particular.

ACKnoWLEDGMEnts

This study has been financed by the spanish Ministry of Education and science (CGL2004-05240-C02-02/bos and MEC/FEDEr2007-64080-C02-02/ bos).

rEFErEnCEs

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

Casellas, D. 2004. tasa de expansión de la hormiga argentina, Linepithema humile (Mayr 1868), (Hymenoptera, Dolichoderine) en un área mediterránea. boletín de la sociedad Española de Entomología 28:207-216.

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.

Cros, s., X. Cerdá & J. retana 1997. spatial and temporal variations in the activity patterns of Mediterranean ant communities. Ecoscience 4:269-278.

Espadaler, X., A. tartally, r. schultz, b. seifert & C. nagy 2007. regional trends and preliminary results on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae). Insects sociaux 54:293-301.

Fellers, J.H. 1987. Interference and exploitation in a guild of woodland ants. Ecology 68:1466-1478.

Fellers, J.H. 1989. Daily and seasonal activity in woodland ants. oecologia 78:69-76.

Gerlach, J. 2004. Impact of the invasive crazy ant Anoplolepis gracilipes on bird Island, seychelles. Journal of Insect Conservation 8:15-25.

Giraud, t., J.s. Pedersen & L. Keller 2002. Evolution of supercolonies: the Argentine ants of southern Europe. Proceedings of the national Academy of sciences, early edition, 1-5.

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.

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

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

Holway, D.A., A.V. suarez & t.J. Case 2002. role of abiotic factors in governing susceptibility to invasion: a test with Argentine ants. Ecology 83:1610-1619.

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.

Human, K.G., s. Weiss, A. Weiss, b. sandler & D.M. Gordon 1998. Effects of abiotic factors on the distribution and activity of the invasive Argentine ant (Hymenoptera: Formicidae). Environmental Entomology 27:822-833.

Keller, L. 1995. social-Life - the Paradox of Multiple-Queen Colonies. trends in Ecology & Evolution 10:355-360.

Kolar, C.s. & D.M. Lodge 2001. Progress in invasion biology: predicting invaders. trends in Ecology & Evolution 16:199-204.

Krushelnycky, P.D., s.M. Joe, A.C. Medeiros, C.C. Daehler & L.L. Loope 2005. The role of abiotic conditions in shaping the long-term patterns of a high-elevation Argentine ant invasion. Diversity and Distributions 11:319-331.

Krushelnycky, P.D., L.L. Loope & s.M. Joe 2004. Limiting spread of a unicolonial invasive insect and characterization of seasonal patterns of range expansion. biological Invasions 6:47-57.

Lubin, Y.D. 1984. Changes in the native fauna of the Galapagos Islands following invasion by the little red fire ant, *Wasmannia auropunctata*. biological Journal of the Linnean society 21:229-242.

Lubin, Y.D. 1985. studies of the little fire ant, *Wasmannia auropunctata*, in a niño year. El niño en las Islas Galápagos: el evento de 1982-1983: 473-493.

MacDougall, A.s. & r. turkington 2005. Are invasive species the drivers or passengers of change in degraded ecosystems?. Ecology 86:42-55.

Majer, J.D., J.H.C. Delabie & M.r.b. smith 1994. Arboreal Ant Community Patterns in brazilian Cocoa Farms. biotropica 26:73-83.

Menke, s.b., r.n. Fisher, W. Jetz & D.A. Holway 2007. biotic and abiotic controls of Argentine ant invasion success at local and landscape scales. Ecology 88:3164-3173.

oliveras, J., J.M. bas, D. Casellas & C. Gómez 2005. numerical dominance of the Argentine ant vs native ants and consequences on soil resource searching in Mediterranean cork-oak forests (Hymenoptera: Formicidae). sociobiology 45:1-16.

Puth, L.M. & D.M. Post 2005. studying invasion: have we missed the boat?. Ecology Letters 8:715-721.

roura-Pascual, n., A.V. suarez, C. Gomez, P. Pons, Y. touyama, A.L. Wild & A.t. Peterson 2004. Geographical potential of Argentine ants (*Linepithema humile* Mayr) in the face of global climate change. Proceedings of the royal society of London series b-biological sciences 271:2527-2534.

rust, M.K., D.A. reierson, E. Paine & L.J. blum 2000. seasonal activity and bait preferences of the Argentine ant (Hymenoptera: Formicidae). Journal of Agricultural and urban Entomology 17:201-212.

sakai, A.K., F.W. Allendorf, J.s. Holt, D.M. Lodge, J. Molofsky, K.A. ,With s. baughman, r.J. Cabin, J.E. Cohen, n.C. Ellstrand, D.E. McCauley, P. o'neil, I.M. Parker, J.n. Thompson & s.G. Weller 2001. The population biology of invasive species. Annual review of Ecology and systematics 32:305-332.

sanders, n.J., K.E barton & D.M. Gordon 2001. Long-term dynamics of the distribution of the invasive Argentine ant, *Linepithema humile*, and native ant taxa in northern California. oecologia 127:123-130.

stanley, M., D. Ward, r. Harris, G. Arnold, r. toft & J. rees 2008. optimizing pitfall sampling for the detection of Argentine ants, *Linepithema humile* (Hymenoptera : Formicidae). sociobiology 51:461-472.

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

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 98:1095-1100.

suarez, A.V., n.D. tsutsui, D.A. Holway & t.J. Case 1999. behavioral and genetic differentiation between native and introduced populations of the Argentine ant. biological Invasions 1:43-53.

tsutsui, n.D., A.V. suarez, D.A. Holway & t.J. Case 2000. reduced genetic variation and the success of an invasive species. Proceedings of the national Academy of sciences 97:5948-5953.

Walsh, P.D., P. Henschel & K.A. Abernethy 2004. Logging speeds little red fire ant invasion of Africa. biotropica 36:637-640.

Williamson, M. & A. Fitter 1996. The varying success of invaders. Ecology 77:1661-1666.