ERADICATION OF THE LITTLE FIRE ANT, *WASMANNIA AUROPUNCTATA* (HYMENOPTERA: FORMICIDAE), FROM MARCHENA ISLAND, GALÁPAGOS: ON THE EDGE OF SUCCESS?

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Abstract

The development of effective techniques to eradicate populations of invasive ant species is crucial to the conservation of native biodiversity. An intensive program was initiated in 2001 to eradicate the invasive little fire ant, *Wasmannia auropunctata* (Roger) from ~21 ha on Marchena Island in the Galápagos Archipelago. Linear transects, approximately 10 m apart, were cut through the vegetation of the infested area and a buffer zone of 6 ha. Amdro® (Hydramethylnon) was applied manually up to three times in the treatment area at three-month intervals between March and October 2001. To date, five follow-up monitoring surveys have placed sticks painted with peanut butter in a grid 3-4 m apart. Two small populations (0.1% of the area originally occupied by *W. auropunctata*) were detected in April and October 2002 and were subsequently treated with Amdro®. No *W. auropunctata* ants were found in May 2003 and April 2004. Five nocturnal surveys carried out in the immediate area of introduction of *W. auropunctata* did not detect any individuals. Monitoring surveys will continue for an additional two years to ensure eradication of any remaining populations and verify the success of this program. This paper discusses the procedures used to kill *W. auropunctata* and monitor the efficacy of the eradication methods, the program's costs, and its applicability to other island ecosystems.

Key Words: Amdro®, ant control, dispersal, costs, invasive ants, monitoring

Resumen

El desarrollo de técnicas efectivas para erradicar poblaciones de hormigas invasoras es esencial para la conservación de la biodiversidad nativa. Un programa intensivo fue iniciado en 2001 para erradicar la hormiga colorada invasora *Wasmannia auropunctata* (Roger), de un área de ~21 ha en la Isla Marchena, Galápagos. Transectos lineales de aproximadamente 10 m entre cada uno, fueron hechos dentro de la vegetación del área infestada y en una zona de amortaguimiento de 6 ha. Amdro® (Hydramethylnon) fue aplicado manualmente hasta tres veces en el área de tratamiento a intervalos de tres meses entre marzo y octubre 2001. Hasta la fecha, se ha realizado cinco monitoreos para evaluar la eficacia del programa de erradicación colocando palitos pintados con mantequilla de maní en cuadriculas de 3-4 m. En abril y octubre 2002 se detectaron dos poblaciones pequeñas (0.1% del área ocupada originalmente por *W. auropunctata*) las cuales fueron tratados con Amdro®. No se encontró a *W. auropunctata* en Mayo 2003 y Abril 2004. Tampoco se encontró a la hormiga de fuego en cinco monitoreos nocturnos realizados en la zona de introducción de la hormiga. Los monitoreos continuarán por dos años adicionales para asegurar que no existen parches de hormigas y para verificar el éxito del programa. En este articulo se discute los procedimientos para erradicar *W. auropunctata* y para evaluar la eficacia de los métodos utilizados, los costos del programa y su aplicabilidad en otros ecosistemas isleños.

Translation provided by the authors.

Ants are highly successful invaders of both islands and continents (McGlynn 1999). Eradication of recently introduced populations is fundamental to preventing their dispersal and subsequent impacts on native biodiversity. Eradication is especially important for areas of high conservation value where the loss of endemic fauna, in particular invertebrates, is at risk. The probability of successful eradication decreases with increased distribution and when eradication is not feasible, control techniques are costly and time consuming. Moreover, they are often ineffective in preventing species from spreading through natural mechanisms, and most importantly with the aid of humans (Suarez et al. 2001). Historically, eradication has involved small, recently introduced populations at ports of entry. Techniques for eradication at a larger scale are still in the early stages of development and there have been few success stories: the removal of *Wasmannia auropunctata* (Roger) from 3 ha on Santa Fe Island in the Galàpagos (Abedrabbo 1994) is an example. Also, early results suggest that infestations of the ants *Pheidole megacephala* F. (up to 10 ha) and *Solenopsis geminata* (F.) (up to 3 ha) in Kakadu National Park, Australia may have been eradicated (Hoffman & O'Connor 2004). Yet, because of their unusual social organization and reproductive strategies (Passera 1994; Tsutsui & Suarez 2003), some species of ants are good candidates for eradication.

The little fire ant, *Wasmannia auropunctata* has been listed as one of the 100 worst invaders in the world by the Invasive Species Specialist Group of The World Conservation Union (IUCN) (Lowe et al. 2002). It is easily transported on fruits and vegetables, and growing trade between countries has facilitated this Neotropical insect's colonization in many parts of the world. In the last 25 years, at least seven Pacific island groups including Hawaii and recently Tahiti have been successfully colonized by *W. auropunctata* (Wetterer & Porter 2003; E. Loeve, Fenua Animalia, Tahiti, pers. comm.). Attributes that make *W. auropunctata* a successful invader include its adaptability to a wide range of habitats, polyphagous feeding habits, high interspecific aggression, and lack of intraspecific aggression which leads to unicoloniality (Ulloa-Chacón & Cherix 1990; Le Breton et al. 2004). Colonies are polygynous (Hölldobler & Wilson 1977), increasing the likelihood that small numbers of ants that are split off from the colony or are transported by man are able to found a new colony.

Introduced into the Galápagos archipelago between 35 and 70 years ago, *W. auropunctata* has colonized eight large islands; Santa Cruz, San Cristóbal, Isabela, Floreana, Santiago, Santa Fe, Pinzón, and Marchena (Silberglied 1972; Lubin 1984; Abedrabbo 1994). It also has been found recently on some of the smaller islands: Champion, Mao, Cousins, Albany, and Eden (C. E. C., unpubl. data). The ants were most likely transported between the inhabited islands on plants, food, and in soil. The uninhabited islands, on the other hand, are less frequently visited and then only by scientists and park rangers, and illegally by fisherman. Ants may have been transported in camping provisions and equipment or may have arrived on vegetation rafts.

Known locally as the "hormiga colorada", *W. auropunctata* has had a wide-ranging impact on biodiversity in the Galápagos, in particular to native invertebrates (Clark et al. 1982; Lubin 1984; Roque-Albelo et al. 2000; Mieles 2002). It also negatively affects the nesting activities and young of reptiles and birds and its painful sting makes it a significant pest to farmers and conservation workers (Lubin 1985; Roque-Albelo et al. 2000; C. E. C., unpubl. data). Additionally, *W. auropunctata* aids the build up and spread of populations of the cottony cushion scale (*Icerya purchasi* Maskell). Honeydew produced by this scale insect is exchanged for transportation and protection from predators (Causton 2001).

Mitigation of the impacts of *W. auropunctata* has been recognized as a priority for conservation organizations in Galápagos. On the larger islands the little fire ant is now distributed over thousands of hectares and is beyond the means of current methods of control. However, eradication programs are expected to be more successful on the smaller islands or areas that have been recently colonized where distributions are less than a few dozen hectares. This was demonstrated with the successful removal of *W. auropunctata* from Santa Fe Island (Abedrabbo 1994). Eradication was also considered feasible for the recently invaded Marchena Island, a near pristine island in the northern part of the Archipelago (Roque-Albelo et al. 2000).

Wasmannia auropunctata was first discovered in 1988 at a campsite on Playa Negra, a large black sand beach on the southwestern side of Marchena Island (Fig. 1) (Roque-Albelo et al. 2000). In 1992, the area infested by *W. auropunctata* was estimated at 0.5 ha (Fig. 2a, b) and a control program was initiated by the Galàpagos National Park Service (GNPS) and the Charles Darwin Research Station (CDRS) adopting the methodology previously used to eradicate *W. auropunctata* from Santa Fe Island (Abedrabbo 1994). Between 1993 and 1996, three attempts were made to eradicate *W. auropunctata* with Amdro® (Zuñiga 1994; Roque-Albelo et al. 2000). Follow up surveys indicated that the poison bait applications were only partially successful (Fig. 2a, b), probably because populations were missed and the area of infestation was underestimated. In 1996, *W. auropunctata* still occupied 1.5 ha, but the eradication program was suspended due to lack of funding. By 1998, an El Niño year, the area had increased to 17 ha (Fig. 2a, b) (Roque-Albelo et al. 2000). High precipitation rates during El Niño may have accounted for a rise in ant numbers. Lubin (1984, 1985), measured *W. auropunctata* spread at a rate of 170m/year in Santa Cruz Island, increasing to 500 m in El Niño years. Nevertheless, it is highly unlikely that El Niño was solely responsible for such dramatic population growth on Marchena, further suggesting that earlier assessments had missed some populations. Two years later in 2000, the infested area was estimated at 24 ha (Roque-Albelo et al. 2000). This proved to be an overestimate as later calculations showed the actual infested area to be 19.3 ha; an increase of approximately 2.3 ha. from 1998.

What was evident from surveys carried out post 1996 was that the distribution of *W. auropunctata* in Marchena was still expanding and

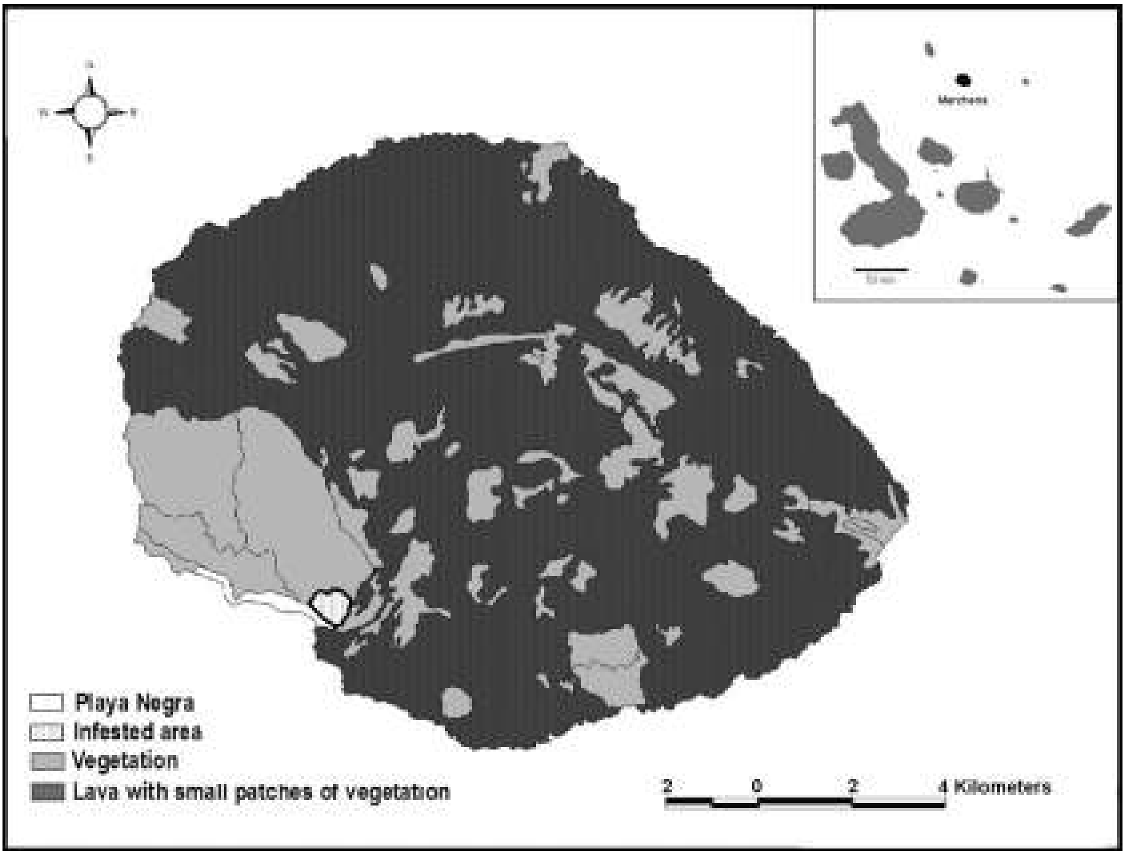

Fig. 1. Marchena Island (130 km^2, inset shows location within the Galápagos Archipelago) and location of *W. auropunctata* infestation in 2001 (~20.5 ha).

that there was a striking contrast between the composition of ant communities in habitats where *W. auropunctata* was present and areas that had not been invaded (Roque-Albelo et al. 2000). *Wasmannia auropunctata* typically infested only vegetated areas and in Marchena, vegetation covers only 25% of the total area of the island (130 km^2). If *W. auropunctata* continued to spread at the same rate, it could eliminate many of the native invertebrate species that occupy these habitats, especially those that are localized in distribution.

Paradoxically, the reproductive strategies that make *W. auropunctata* a successful colonizer and enable it to expand its distribution also facilitate the success of any program aimed at reducing population numbers. This is primarily because new colonies are typically formed by budding (Hölldobler & Wilson 1977), which restricts the dispersal capacity of *W. auropunctata* and contains it to areas immediately adjacent to existing colonies. As a consequence, eradication was still thought to be possible and in 2001 a program was initiated to eradicate *W. auropunctata* from Marchena Island. This paper evaluates the methods used in the current eradication program and discusses their applicability to other areas of conservation value.

Materials and Methods

Description of Area Infested by *W. auropunctata*

Colonies of *W. auropunctata* were found between 0-50 m elevation. Marchena Island is arid and the infested area was principally covered by areas of dry eroded soil and fresh lava fields. Vegetation was dense in parts, particularly in the rainy season, and was composed of dry forest dominated by *Bursera graveolens* (HBK) Trian. and Planch., *Croton scouleri* Hook. f., *Waltheria ovata* Cav., *Lantana peduncularis* Anderss., *Opuntia helleri* K. Scum., and *Castela galapageia* Hook. f. (Hamman 1981). In the Galàpagos, January to May is the warm/wet season with occasional rain and is followed by a cooler/dry season from May to December with little or no rain and lower temperatures. Annual meterological records do not exist for this island. Day time temperatures recorded during field trips from 2001 to 2004 ranged from 24°C to 44°C with a relative humidity of between 52 and 65%.

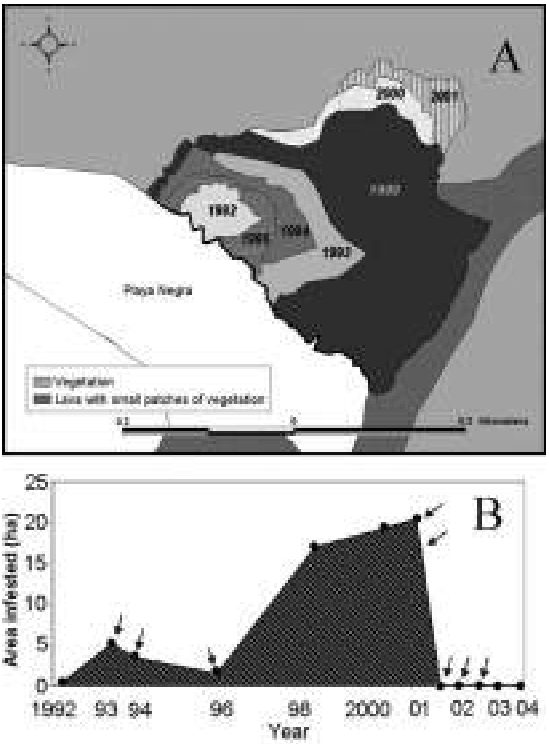

Fig. 2. A) Expansion of *W. auropunctata* at Playa Negra in Marchena Island, 1992-2001. B) Change in size of infested area. Arrows indicate applications of poison bait (Amdro®; see text for details).

Calculating the Size of the Treatment Area

In March 2001, 50 m longitudinal transects were cut outwards from the perimeter established in 2000 (Fig. 3) at 20 m intervals. Hot dogs (~5 mm thick, made of beef) were placed on the lower ends of 30-cm wire flags that were placed in the ground at 5-m intervals along these transects. Baits were checked after 45 min. In the event that *W. auropunctata* was recorded, transects were extended and additional baits placed at 5-m intervals until ants had not been detected for 50 m. The perimeter of the treatment area was established at least 50 m from the last infested point found in each transect to create a buffer zone between the infested and *W. auropunctata*-free areas (Fig. 3). The perimeter was tracked with a handheld GPS unit and the size of the area calculated with ArcView GIS (Version 3.2a, Environmental System Research Institute 1999). The area infested by *W. auropunctata* was estimated to be 20.5 ha. *Wasmannia auropunctata* was found up to 75 m away from where it was recorded in 2000. Including the buffer zone (6.1 ha), the area in which poison was applied and monitoring was conducted was estimated to be 26.6 ha. These measurements are two-dimensional and did not consider the topography of the area.

Preparation of Treatment Area

To enable the homogenous application of poison and facilitate monitoring, the treatment area was divided into five sectors (A, B, C, D, and I) based on the old perimeters and natural divisions provided by the terrain. In each sector 1.5-m wide longitudinal transects were cut with machetes through the vegetation at approximately 10-m intervals. By 2003, a total of 352 longitudinal transects had been cut in the treatment area ranging between 58 and 289 m in length (Fig. 3). Short latitudinal transects were cut in areas of especially dense vegetation. The sectors and transects were mapped by tracking with GPS units.

Control Techniques

Amdro® (Hydramethylnon with soybean oil, 0.88% active ingredient), a product developed for *Solenopisis* fire ants was used (Collins et al. 1992). This insecticide was the most attractive to *W. auropunctata* of four fire-ant products tested by Williams & Whelan (1992) and was also used to successfully control it on Santa Fe Island (Abedrabbo 1994). Amdro® was considered to be a minimum risk to non-targets because of its low toxicity to vertebrates, because it cannot be absorbed through the insect cuticle, and because it is not known to accumulate in the environment (Vander Meer et al. 1982; Extension Toxicology Network 1996; Bacey 2000). Some scavenging arthropods and arthropod predators, in particular ants, were expected to feed on the bait, but any localized non-target impacts that might occur would be negligible following re-colonization of the treatment area by invertebrates. However, the disadvantages of using this toxic bait are that it decomposes quickly and cannot be applied during or soon after rainfall (Vander Meer et al. 1982). Before each trip to Marchena, Amdro® was sampled at random and tested to ensure that it was still attractive to the *W. auropunctata*.

Amdro® was applied after 15.00 h to reduce exposure to sunlight. The bait was hand broadcast by groups of field assistants walking parallel to each other along adjacent transects. An average of 4.9 kg of Amdro® per hectare was applied to all sectors in the treatment area in March and June 2001 (Table 1). This was over double the quantity recommended by specialists (2.2 kg/ha) (D. Williams, University of Florida, Gainesville, pers. comm.), but was considered necessary because of the hilly terrain and the presence of caves and dense vegetation. Amdro® was only applied to sectors A and B in October 2001 after *W. auropunctata* had not been detected by the monitoring program in Sectors C, D and I in June and October 2001. In April and October 2002, the application of Amdro® was restricted to areas where small populations of *W. auropunctata* were found (Table 1).

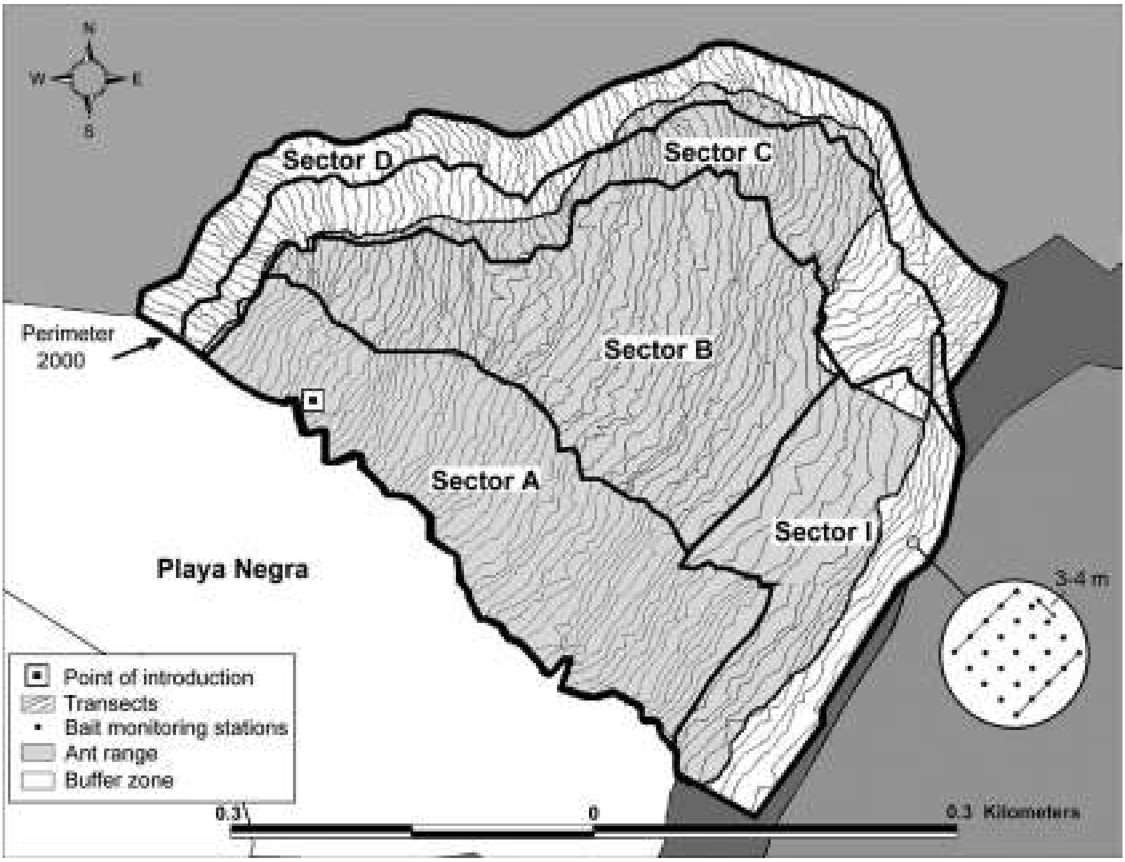

Fig. 3. Treatment area for eradicating *W. auropunctata* (Sector A: 6.8 ha, 65 transects; Sector B: 7.5 ha, 62 transects; Sector C: 4.4 ha, 103; Sector D: 3.9 ha, 107 transects; Sector I: 4.1 ha, 15 transects). The inset is a closer view of the 3-4 m grid of bait monitoring stations.

Monitoring the Effectiveness of Amdro® Applications

The intensity of monitoring increased as Amdro® applications decreased. In June 2001, three months after the first application, the primary objective was to detect any further spread of *W. auropunctata* in outlying Sectors C and D (Fig. 3). Hot dog baits were placed every 5 m in alternate transects and techniques were similar to those used to calculate the size of the treatment area in March 2001. Six months after the first application of Amdro® (October 2001), a more intensive monitoring program was begun placing peanut butter baits in grids 3-4 m apart (Fig. 3). In October 2001, the grid system was applied to sectors C, D, and part of I (bait stations were placed at 3-4 m intervals only along the length of the transects in the remaining sectors). For the last four trips (April and October 2002, May 2003, and April 2004), all sectors of the treatment area were monitored with a 3-4 m grid of bait stations. Additionally, in the area of introduction of *W. auropunctata* (Sector A, Fig. 3) and in the areas where small populations were found in April and October 2002, the distance between bait stations was reduced to every 1 m.

Peanut butter baits were used instead of hot dog baits because of the high proportion of hot dog baits that were eaten by lizards and hermit crabs on the first survey, and because peanut butter baits were easier to use in large numbers. The baits consisted of a wooden kebab stick (30 cm long) painted with a fluorescent marker on one end. Peanut butter was applied to the unpainted end from midway down. The pointed end of the stick was placed firmly in the ground to avoid removal by lizards and doves. Monitoring activities took place between 05:40-10:30 and 15:00-18:00 h and were not carried out on rainy days or during hours of intense sunlight when ants are less abundant. Bait stations were placed every 3-4 m along each of the longitudinal transects in the treatment area. Additional bait stations were placed every 3-4 m to the left and to the right of each of these bait stations until the bait stations on the adjacent transect were reached, thus forming a grid of 3-4 m squares (Fig. 3). The number of bait stations placed on each trip is shown in Table

TABLE 1. QUANTITY OF AMDRO® (KG) APPLIED TO TREATMENT AREA.

Date	Sectors	Area treated (ha)	Amdro (kg)	kg/ha
Mar 01	A, B, C, D, I	26.6	130	4.9
Jun 01	A, B, C, D, I	26.6	134	5.0
Oct 01	A, B	14.3	60	4.2
Apr 02[a]	Part of A and I	3.4	27	7.8
Oct 02[a]	Part of A, all of I	10.9	45	4.2
Total			396	

[a]Amdro® applied in response to finding colony fragments of *W. auropunctata*.

2. To ensure that the entire area was covered by bait stations, 4-5 groups of field workers worked parallel to each other along adjacent transects. Bait was left for one hour after which it was inspected for ants. Field workers were trained to identify and record *W. auropunctata* and the three most common ant species that they might encounter: *Tapinoma melanocephalum* (Fabricius), *Cardiocondyla emeryi* Forel and *Monomorium floricola* (Jerdon). When a field worker believed that they had detected *W. auropunctata*, the ants were collected and the site was marked. Bait sticks were counted at the end of each transect to check that all bait stations had been collected.

Nocturnal Monitoring

Nocturnal monitoring was carried out because it is possible that *W. auropunctata* resorts to feeding more actively in the night when diurnal temperatures are high and humidity is low (Meier 1994). Because time was limited, we surveyed only where *W. auropunctata* had initially been introduced in Sector A (Fig. 3). Bait stations were laid out on one night of each trip between 20:00 and 21:00 h. In October 2001, hot dog baits were placed every 10 m for 100 m along three transects with 30 m between transects. The number of transects was intensified on subsequent trips and peanut butter baits were used. Baits were placed at 5-m intervals along the first 50 m of 10 transects in April 2002, and along 42 transects in October 2002, May 2003, and April 2004 (Table 2). In all cases transects commenced at the beach. Additionally, baits were laid out every 1 m in the areas where colony fragments were found on previous trips.

Estimation of Colony Fragment Size

To determine the size of the remnant colonies discovered in April and October 2002, we placed peanut butter sticks in a grid with 1-m intervals centered on the bait station where *W. auropunctata* was detected. Baits were checked after an hour and in the event that ants were found, the flags were left in place and the grid amplified until ants had not been observed for 10 m in each direction.

RESULTS

Wasmannia auropunctata numbers

Wasmannia auropunctata was not detected at 700 non-toxic bait stations in Sectors C and D three months after the onset of the eradication program in June 2001 (Table 2). After two applications of Amdro® (October 2001), *W. auropunctata* was not recorded at 11,058 bait stations placed in all sectors of the treatment area. In April 2002, one year after the first toxic bait application *W. auropunctata* was recorded at three of 33,638 non-toxic bait stations (Table 2). A pop-

TABLE 2. MONITORING EFFORT IN THE TREATMENT AREA (26.6 HA) TO DETECT THE PRESENCE OF *W. AUROPUNCTATA* AND OTHER ANT SPECIES (CALCULATED NUMBER OF STATIONS REQUIRED FOR COMPLETE COVERAGE OF A TWO-DIMENSIONAL AREA WITH 3 M BETWEEN POINTS WAS 36,012).

	Monitoring dates					
	Jun 01	Oct 01	Apr 02	Oct 02	May 03	Apr 04
Man hours (in the field)	~392	~432	504	743	698	735
Total number of diurnal bait stations	700	11,058	33,638	36,251	44,142	40,100
Total number of nocturnal bait stations	—	33	110	570	780	780
Stations with *W. auropunctata*	0	0	3	2	0	0
Stations with other ant species	—	897	35	6,530	3,408	10,812

ulation of ants was located in a dry streambed in Sector I. Along a 1-m grid of non-toxic baits the infestation size was estimated to be at least 87 m^2 and measured about 6 m by 18 m. *Wasmannia auropunctata* was also found on two out of 36,251 non-toxic bait stations in October 2002 in Sector A (Table 2). With baits set in a 1-m grid, the colony was estimated to measure 99 m^2 in an irregular patch up to 10 m wide and 21 m long. After discovery of these populations, Amdro® was applied to the infested areas and the surrounding non-infested areas (Table 1). *Wasmannia auropunctata* was not registered in these areas on subsequent trips. In the last two monitoring surveys in May 2003 and April 2004, *W. auropunctata* was not detected at 44,142 and 40,100 bait stations, respectively. *Wasmannia auropunctata* was not recorded at any of the non-toxic bait stations placed at night during the monitoring surveys (Table 2).

Based on an equidistant point method on Arcview, the number of non-toxic bait stations placed in the last four monitoring surveys was calculated to be similar to or higher than the number of stations required for complete coverage with 3 m between points on a two-dimensional plane (Table 2).

Presence of Other Ant Species

Tapinoma melanocephalum, *C. emeryi*, and *M. floricola* were recorded from the non-toxic bait stations in varying intensities on all four monitoring trips. In October 2001 these ant species were present in approximately 8% of the total number of non-toxic bait stations, whereas in April and October 2002 ants occupied 0.1% and 18% of the stations, respectively. In May 2003 and April 2004, these ant species were again found in 7.7% and 27% of the stations (Table 2). It is possible that some of the identifications of *C. emeryi* may have been *Cardiocondyla nuda* (Mayr), as these two species are visually similar.

Discussion

Efficacy of Chemical Applications

To our knowledge, this is the largest eradication program that has been attempted for *W. auropunctata*. Monitoring results suggest that the application of Amdro® along a series of closely cut linear transects is an effective means of reducing *W. auropunctata* populations rapidly. Following three applications of poison bait over a 9-month period we have detected only two small patches of ants in approximately 0.1% of the area originally infested by *W. auropunctata*. A larger number of nest remnants was expected, especially given the difficult terrain. Negative results with intensive monitoring techniques in Sectors C and D suggests that *W. auropunctata* spread was contained and that ants may have been eradicated from this area after two applications of Amdro®. Nevertheless, as intensive monitoring was only carried out in all sectors beginning with the third application, we cannot make any determinations about the effectiveness of each individual application in eradicating ants from the entire infested area. It may have been that there were only a few survivors after the first application. However, because funding was limited and we wanted to guarantee that the populations were hit hard, additional bait applications and land clearing activities were given priority over monitoring surveys.

The apparent effectiveness of the chemical applications may have been augmented by the effect of extended dry periods following the first bait application in March 2001. The lower elevations of Marchena Island are typically very arid during the dry season (May to December) and there was very little green vegetation on subsequent trips in June and October 2001. *Wasmannia auropunctata* prefers moist habitats and only dominates arid zones when temperature and humidity are high (Clark et al. 1982; Lubin 1984, 1985). These dry conditions probably inhibited nest-founding activities in any nests that were not destroyed by the first chemical application. Both *W. auropunctata* density and the production of sexuals appear to be influenced by humidity, and decreases in both occur in the drier months (Clark et al. 1982; Ulloa-Chacón 1990). Furthermore, ant nests are typically found above ground (Lubin 1984, 1985; Ulloa-Chacón & Cherix 1990; Ambrecht & Ulloa-Chacón 2003) and are susceptible to drying out when humid nesting sites are less abundant (Lubin 1984).

During the monitoring surveys, two small populations of *W. auropunctata* were discovered. These may have been missed by the chemical applications because of the hilly and volcanic terrain, particularly at the beginning when the methodology was still being worked out and the distance between transects was larger. This is the most likely explanation for the small population discovered in Sector I in April 2002 where Amdro® was only applied twice. However, it is less likely that the population discovered in Sector A in October 2002 was missed, as Amdro® was applied three times in this area. The topography of the land may have influenced the success rate of some of the applications, while some poison bait may have been deactivated by high temperatures during shipment to the Galàpagos. Intensive monitoring along a 3-4 m grid was only initiated in these sectors in April 2002 and may explain why these populations were not detected earlier. It is also possible that nests that were partially hit by the Amdro® applications in 2001 may have taken some time to build up population numbers and initiate foraging activities. For example, in New Zealand non-toxic baits did not attract the Argentine ant, *Linepithema humile* (Mayr) nine months after treatment with toxic bait although searching revealed their presence (Harris et al. 2002).

Conversely, at least three sympatric ant species (*T. melanocephalum*, *C. emeryi*, and *M. floricola*) were collected from the non-toxic baits following the application of Amdro®. These introduced species were present in the *W. auropunctata* infested area before treatment began (Roque-Albelo et al. 2000; Mieles 2002) suggesting that they either were not affected as much by the toxic bait or had re-invaded rapidly after treatment. Fluctuations in ant numbers corresponded to patterns observed in non-infested areas during the same period and appear to be related to climate (Mieles 2002).

Effectiveness of Monitoring

The monitoring techniques used during this program should have been sufficient to detect the presence of *W. auropunctata*. Baits were made up of peanut butter, which has been shown to be highly attractive to *W. auropunctata* in the laboratory and in the field (Williams & Whelan 1992). Studies on the foraging behavior of *W. auropunctata* suggest that if ants were present in the area they would have been attracted to the non-toxic baits under most climatic conditions (including strong wind, heavy rain, and full sunlight) and at all times of the day, although ant numbers may vary (Clark et al. 1982; Meier 1994; Delsinne et al. 2001). The distance between bait stations should have permitted ants to reach the baits within an hour. *Wasmannia auropunctata* typically makes superficial nests under most environmental conditions (Lubin 1984, 1985; Ulloa-Chacón & Cherix 1990; Ambrecht & Ulloa-Chacón 2003). Although little is known about the foraging distances of *W. auropunctata*, workers have been observed to forage up to 2 m high in trees (de la Vega 1994; Meier 1994). With a mean foraging speed of between 15-18 cm/min (Meier 1994), and assuming that ants could detect baits up to ~2.1 m away (radius of the circle defined by the grid size), ants should have been recruited to the baits within an hour. Extended drought periods, however, are associated with lower ant abundance (Clark et al 1982; Ulloa-Chacon 1990) and have been known to cause hypogaeic nesting in other parts of the Galàpagos (Abedrabbo 1994; Meier 1994). Thus, some nests may not have been able to locate the bait within an hour under these conditions. Although, we did not find any nests below the ground in Marchena, we have restricted our monitoring efforts in the last two years to the end of the wet season when surviving colonies are expanding and food is in demand.

Populations that have been reduced by toxic baits also may be slow in reacting to the non-toxic bait stations. When peanut butter bait stations were set 3-4 m apart, *W. auropunctata* was detected at only three bait stations in April 2002 and two bait stations in October 2002. Yet, on both occasions, the area occupied by *W. auropunctata* proved to be larger (87 m^2 and 99 m^2, respectively), as was discovered when the distance between bait stations was reduced to 1 m. Approximately 13 bait stations should have picked up *W. auropunctata* at 3-m intervals. This may be because the populations were small and workers took longer to find and recruit to the baits at wider spacing.

Studies on the foraging behavior of *W. auropunctata* have not been repeated in different climatic conditions sufficiently to identify optimal conditions for monitoring. While it is likely that non-toxic bait stations were laid out when *W. auropunctata* was active, we suggest that repeated experimental trials be carried out with different population sizes (including those that have been partially hit by toxic bait applications) to determine foraging speed, distance, and peak foraging hours under all climatic conditions and that monitoring activities are modified accordingly. Nevertheless, provided that monitoring is maintained for several years it is likely that any surviving pockets of *W. auropunctata* should grow large enough to be detected at the level of intensity being employed in this study.

Could *W. auropunctata* Exist Outside the Containment Area?

Current evidence suggests that outlying populations are unlikely on Marchena unless independent introductions have occurred elsewhere on the island. *Wasmannia auropunctata* has not been collected from six batteries of pitfall traps randomly placed within a 500-m radius of the treatment area on six occasions between 2000 and 2004 (Mieles 2002; A. Mieles, CDRS, Galápagos, pers. comm.), nor has it been collected in surveys that have been initiated on other parts of the island (C. S., unpubl. data).

These findings seem to indicate that *W. auropunctata* has not used long distance dispersal as a means for spreading in Marchena. In areas where it has been introduced, *W. auropunctata* typically forms new colonies by budding, where inseminated queens are accompanied by workers on foot to a nearby site (e.g., Hölldobler & Wilson 1977; Lubin 1984). This leads to well-demarcated boundaries of infested versus non-infested areas as shown by Clark et al. (1982) and which were observed on Marchena Island. This dispersal strategy is corroborated by observations in the laboratory of intranidal mating and by the fact that queens were unable to establish new colonies in the absence of workers (Ulloa-Chacón 1990; Ulloa-Chacón & Cherix 1990). Furthermore, workers have been observed moving winged queens on Santa Cruz Island in the Galápagos (Clark et al. 1982), and until recently nuptial flights of *W. au-*

ropunctata had never been observed either in the field or laboratory (Spencer 1941; Sielberglied 1972; Lubin 1984; Ulloa-Chacón 1990). Mating flights have been reported, however, among *W. auropunctata* populations in Puerto Rico (Torres et al. 2001). It is evident that there are still many gaps in our knowledge of the population biology of *W. auropunctata*, highlighting the need for continued monitoring in Marchena and further studies in its native and introduced range to better understand the mechanisms used for colony reproduction.

Future Needs and Application to Other Island Ecosystems

Given the track record of *W. auropunctata*, it can only be a matter of time before it is introduced into other islands, especially in the Pacific. We strongly recommend that early warning systems are set up and that rapid response plans are available in the event that *W. auropunctata* is detected. We also recommend pre-approval of effective bait treatment because this can save months of delays if suitable treatment products are not currently registered for use.

Our results from Marchena indicate that these eradication techniques are effective for limiting the spread and possibly also for eradicating well established populations of *W. auropunctata* of up to at least 20 ha in size. Aerial application should be considered if the infestation is more than a few hectares and suitable aircraft are reasonably available. Amdro® is relatively safe to use in conservation areas but we recommend that toxicity studies be carried out on non-targets before applying the poison bait to areas where re-colonization of invertebrates from outlying areas is not possible. Caution should also be used in areas with water sources because of its toxicity to fish (Extension Toxicology Network 1996) and possible impact on aquatic invertebrates. Chemical applications are likely to be more successful at the beginning of the dry season when the reproductive potential of *W. auropunctata* is reduced and toxic bait applications are more effective. Post application surveys are crucial to the success of the program and are the only way to ensure that *W. auropunctata* has been eliminated. Surveys should be carried out only in the rainy season. Although labor intensive, there is no substitute for detailed mapping of the area with bait sticks. The smaller the grid size the greater the accuracy in evaluating the effectiveness of the poison bait applications.

Ultimately the intensity of the monitoring will depend upon financial and manpower constraints, but we believe that intense early monitoring may provide savings in the long run. To date, the program in Marchena has cost approximately $212,736 US (this includes time spent preparing for the field trips, field and laboratory work, and overhead). The cost for the purchase and shipping of Amdro® was approximately $10,700. Assuming that no more ants are found on the next two monitoring trips, the total projected cost for removing *W. auropunctata* for each hectare of infested area is estimated at $13,680. Personnel costs accounted for approximately 47% of the total spent on this program and can be reduced by using trained volunteers. Approximately 25% of these costs were for inter-island transport and surveys to evaluate the response of native invertebrate communities and may not be needed elsewhere. Additional studies on foraging behavior and the refinement of bait application and monitoring procedures should help us improve these techniques and make them less labor intensive and costly.

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