An Artificial Aril Designed to Induce Seed Hauling by Ants for Ecological Rehabilitation Purposes

Natalia Henao-Gallego,1 Selene Escobar-Ramírez,1 Zoraida Calle,2 James Montoya-Lerma,1 and Inge Armbrecht1,3

Abstract
Ants are effective at moving seeds toward their nests, something that may benefit the seeds. We evaluated whether seed movements that may be useful for the rehabilitation of degraded pastures in Colombia can be enhanced by local ants. An artificial aril was prepared and then evaluated in six open cattle pasture farms. Twenty paper disks (each holding seeds with an artificial aril, honey, tuna oil, and control) were set up along linear transects at each farm, and monitored five times in 48 hours. A total of 340 out of 480 seeds were moved from the experimental units by ants. Seeds with tuna oil and an artificial aril were removed twice as frequently as the control and honey smeared seeds. Ectatomma ruidum, Solenopsis geminata, and Pheidole sp. removed the majority of seeds. Advantages of the artificial aril over tuna oil are discussed. This inexpensive technique can enhance seed movement by generalist ants in degraded pastures, likely contributing to regeneration and ecological rehabilitation.

Key words: ecological services, myrmecochory, seed dispersion.

Introduction
Dispersal of seeds by ants, also called myrmecochory (myrmex = ant, chorus = transport), may be either mutualistic or opportunistic (Weiss 1908; Hölldobler & Wilson 1990). A key factor in developing one of such interactions is the presence of attractive nutritious tissues attached to the seeds (e.g. arils) (Baskin & Baskin 1998), which influence animal behavior (Brew et al. 1989). As social insects, ants are especially suited to disperse seeds (Horvitz 1981; Howe & Smallwood 1982). Ants have been shown to actively disperse seeds possessing arils and elaiosomes with different biochemical compositions (Weiss 1908; Horvitz 1981; Pizo & Oliveira 2001), even in ecologically degraded tropical habitats (Escobar et al. 2007; Domínguez-Haydar & Armbrecht 2011).

Hence, ants might play an outstanding ecological function in natural ecosystems and agro-ecosystems by modulating the ecological processes of seed dispersal and post-dispersal, which frequently determine the spatial distribution of plants (Passos & Oliveira 2003). Workers of different ant species are attracted to seeds with nutritious rewards (Ness et al. 2010). Although many of the seeds either are predated by the ants or abandoned on their trails, under logs, or in soil tunnels (Horvitz & Schemske 1986), a proportion of these seeds can eventually be moved to trash piles, after the arils or elaiosomes have been removed. Therefore, germination may occur in suitable microhabitats if ants placed the seeds in their nests or refuse piles, where humidity, organic matter, or soil aeration may influence the success of seedlings and saplings (Passos & Oliveira 2002).

In degraded or abandoned lands, generalist ants act simultaneously as seed predators (Majer 1980, 1983b) and dispersers, presumably showing a transition from antagonism to mutualism in certain ecological contexts (Rico-Gray & Oliveira 2007). Ants may also act as important post-dispersal agents. In tropical ecosystems, seeds are usually dispersed by frugivore vertebrates, whose droppings and wastes are frequently visited by ants and other insects (Howe & Smallwood 1982). Dispersal or post-dispersal of seeds by ants may occur in both natural ecosystems and managed lands such as cattle pastures (Escobar et al. 2007) and rehabilitated mine sites (Majer 1983a; Domínguez-Haydar & Armbrecht 2011).

Conventional cattle grazing systems based on treeless monocultures of invasive African grasses, such as Pennisetum clandestinum, Cynodon plectostachyus, and Brachiaria decumbens, currently occupy most of the deforested lands in Latin America (Murgueitio et al. 2011). Recently, more sustainable systems based on the combined use of leguminous native species for shade, soil protection, and cattle foraging have been developed. However, the incorporation of trees and shrubs is not always straightforward because most dispersers of tree seeds (mammals, birds, etc.) quickly disappear in impoverished...
ant behavior to carry out this service as part of an ecological rehabilitation strategy.

Methods
The study was carried out in three stages: (1) a pilot study in which ant preference for two natural arils was tested; (2) laboratory design of an artificial aril; and (3) a field trial to determine ant preference for the artificial aril.

Why an Artificial Aril?
Not all plant species form arils or elaiosomes. For instance, certain legumes and pioneer herbs, valuable for restoration purposes, lack these attractive tissues. For managers wanting to accelerate pastureland rehabilitation, the next step is to test whether it is possible to affect the probability of a seed being moved by ants in a degraded land plot (agroecosystem or natural ecosystem). An artificial aril needs to meet certain conditions: it should be attached to a target seed in such a way that it cannot be easily detached in situ; it must be safe for the seed; and it has to be carried easily by ants. On the other hand, the ingredients should be natural, readily available, of low-cost, and resistant to decomposition.

The seeds chosen for the purposes of this study should be orthodox, that is, able to survive either desiccation, freezing, or both factors over long time periods. We chose seeds of the Fabaceae *Senna spectabilis* (DC.) H.S. Irwin & Barneby plant, because it is useful for land rehabilitation given (1) its fast growth and high biomass production, (2) its low fertility requirements and high nitrogen-use efficiency (3) its ready adaptation to harsh conditions, and because (4) ants showed very low preference for its seeds. In a previous pilot study, ants moved only 10% of the seeds of this plant species in an open pasture (102/1200).

Study Sites
This study was carried out between May and November 2009 in seven cattle pastures (plots) located in the Cauca and Valle del Cauca departments, southwestern Colombia, with altitudes between 960 and 1004 m a.s.l., a mean annual temperature of 24°C, annual rainfall below 1100 mm, and relative humidity of 70%. All sites are within the Tropical Dry Forest life zone according to the Holdridge system (Espinal 1967). The most frequent grass species are *Brachiaria decumbens*, *Cynodon plectostachyus*, *Panicum maximum*, *Brachiaria humidicola*, *B. brizantha*, and *B. arrecta*. All the studied pastures were treeless or open, thus directly exposed to solar radiation and wind. The geographic locations of the farms in the Cauca department are; (1) Limonar (03°08’10.1”N; 76°27’42.2”W); (2) La Josefina (3°5’17.3”N; 76°28’18.5”W); (3) Cachimbalito 3°9’1.00”N; 76°27’46.00”W). Locations in the Valle del Cauca department are (4) Sachamate (3°16’27.49”N; 76°33’28.00”W); (5) Lituania (3°20’48.5”N; 76°30’30.6”W); (6) Marañon (3°20’48.30”N; 76°31’23.91”W); and (7) Universidad del Valle (3°22’41.04”N; 76°32’6.24”W).

Natural Arils
A preference trial was carried out in April 2009 in two cattle pastures (plots), located at the campus of the Universidad del Valle and at Lituania farm. Ten Experimental Units (EU), separated 10 m from one another, were placed on a 100 m transect at each plot, the first station being at least 20 m away from the edge of the cattle pasture. Each EU consisted of five arils of *Cupania latifolia* (Sapindaceae) and five of *Passiflora ligularis* (Passifloraceae) haphazardly and equidistantly placed on a 10 cm diameter paper disk. Both arils have a fleshy and smooth consistency (orange and grayish, respectively). All EUs were protected with a rigid metallic mesh cage (openings of 1.5 cm) firmly clamped to the ground surface to avoid vertebrate interference. Visual observations were made every 5 minutes for two consecutive hours. The response variable was the number of arils moved out from the paper border.

Preparation of an Artificial Aril
The aril was prepared with local ingredients at the Experimental Station Laboratory, Universidad del Valle (Cali), based on the chemical analysis of the natural arils used in the pilot study. Through a trial and error approach, the proportions and concentrations of different ingredients were altered in order to improve hardness and storage capacity. The final formula was: 30 ml water, 15 g of unflavored gelatin, 8 g of oat flakes, 40 g of white sugar, and 0.5 ml of tuna oil.

The artificial arils were attached to *S. spectabilis* seeds. In order to prepare 200 such arils, a mold with 200 cavities was made with paraffin. While still warm, circular depressions slightly larger than the *S. spectabilis* seeds were made on the surface, each with two lateral canals. In order to prepare the arils, the ingredients were mixed in a container. Each seed was placed in one depression of the mold, always with the micropile upward. The depressions plus seeds were filled with the jellylike mixture, without covering the micropiles, and were allowed to cool down at room temperature. Finally, the arils + seeds were carefully removed with forceps, and left to dry for several hours while covered with mosquito mesh to prevent insect contamination or attack.
Field Study: Preference for the Artificial Aril

To examine whether the artificial aril improves the probability of a seed being removed from a specific location within a pasture, we used *S. spectabilis* seeds treated with different phagostimulants, in six active cattle pasture plots (three in Valle del Cauca and three in Cauca Department). In each plot, a total of 20 paper disks were distributed along a transect, 10 m apart from each other. On each disk, the *S. spectabilis* seeds were randomly arranged, one for each treatment: (1) with the artificial aril attached; (2) smeared with tuna oil; (3) smeared with honey; and (4) untreated, as a control. Hence, each EU consisted of the 20 paper disks per farm plot. After 48 hours, all seeds were counted and the proportion of seeds removed was estimated. The same procedure was followed in each of the six plots. Vertebrates were excluded in the same way described for the pilot study. The EUs were always set at 07:30 hours and only on sunny days (or at least with no continuous rain). Interactions among ants and the different seeds were observed and recorded in the field trial during 48 hours at different time intervals (2, 4, 8, 24, and 48 hours). Only in those cases where it was physically possible, the distance of seed movement by ants was recorded. Ants moving any seed out of the disk were collected, preserved in 75% ethanol and identified to genus or species (Palacio & Fernández 2003) in the Laboratory at Universidad del Valle, Cali, Colombia.

Data Analyses

In order to compare ant preferences for the two types of natural arils, a paired *t* test was performed comparing the means of the number of seeds removed by ants. Normality and homogeneity of variances were previously tested with Shapiro Wilks and Levene’s tests, respectively. For the artificial aril field trial, the proportion of seeds removed by ants in the four treatments was compared by using a random blocks Analysis of Variance with a Mixed Effects Model, where the treatments were considered to be fixed effects and the plots random effects. An arcsine transformation was applied to the square root of the response variable. This analysis was not intended to compare seed removal averages at different times. The analyses were performed with SAS software (SAS Institute, Inc., Cary, North Carolina, USA).

Results

Preference Trial for Two Natural Seed Arils

Chemical analyses showed that both *P. ligularis* and *C. latifolia* arils contained fiber, although the first had higher water and protein contents than *C. latifolia* (Table 1).

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<th>Arilated Seed</th>
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<td>*C. latifolia&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>6.2</td>
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<td>*P. ligularis&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> Based on a 100 g sample.

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<sup>a</sup> Based on a 100 g sample.
Table 2. Ant species observed interacting with the seeds of any treatment during both the pilot and field experiments, in Valle and Cauca departments.

<table>
<thead>
<tr>
<th>Ant Species</th>
<th>S</th>
<th>LT</th>
<th>M</th>
<th>LM</th>
<th>J</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectatomma ruidum</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Solenopsis geminata</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crematogaster abstinens</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Pheidole sp. 1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wasmannia auropunctata</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomyrmex gr. pallens</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiocondyla gr. minutior</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C, Cachimbalito; J, Josefina; LM, Limonar; LT, Lituania; M, Marañón; S, Sachamate.

The temperature cooled in the afternoons and usually foraged individually. It was responsible for most removal events and also for carrying seeds over the longest distances, while the smaller S. geminata and Pheidole sp. 1 transported fewer seeds. W. auropunctata, C. abstinens, Cardiocondyla gr. minutior, and Pseudomyrmex gr. pallens moved seeds for short distances and removed the honey, oil, or parts of the aril from the paper disk.

Field Trials With the Artificial Aril

Ants moved 340 (70.8%) out of 480 seeds (120 seeds per treatment) in the six pastures. An overall 80% of seeds with an artificial aril were moved, as opposed to 32% of seeds without arils. The trend was consistent in the six pastures and the means of the proportions of removed seeds were statistically different ($F_{3,15} = 9.14; p = 0.001$) (Fig. 2). Least squares means showed that the three treatments significantly increased the chances of removal by ants with respect to the controls ($p < 0.05$). However, no statistical differences were found between them (artificial aril, tuna oil, and honey).

A total of 106 out of 480 offered seeds were followed visually. The longest distances were registered for the artificial aril (28 cm), and in some events the ants carried the seeds + arils to their nests (Table 3).

The temporal pattern of seed removal was similar for the three treatments, with the exception of tuna oil, in which most of the removal events occurred within the first 2 hours (Fig. 3). For the remaining treatments, the activity increased during the evening and continued throughout the first night and into the second day, but at a lower rate. For the control (untreated seeds), the removal of seeds occurred only after all the treated seed resources had been removed, toward the end of the day. Ant activity decreased after 4 hours of observation (i.e. 11:30 AM), a time at which the solar incidence increases until 2:00 PM.

Table 3. Maximum and minimum removal distances for $S$. spectabilis seeds that could be followed after removal by ants in field trials for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distance from the Paper Disk (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Untreated seeds</td>
<td>2.1 (3.8)</td>
</tr>
<tr>
<td>Seed + honey</td>
<td>2.7 (3.3)</td>
</tr>
<tr>
<td>Seed + tuna oil</td>
<td>4.1 (6.5)</td>
</tr>
<tr>
<td>Artificial aril</td>
<td>4.7 (7.5)</td>
</tr>
</tbody>
</table>

Discussion

The presence of an artificial aril enhanced the attractiveness of $S$. spectabilis seeds, doubling their chance of being transported by ants. In contrast, ants usually consumed the sticky honey cover in situ, without transporting the seeds. As expected, tuna oil acted as a powerful phagostimulant or bait for ants, even more than the artificial aril. However, at this point it is necessary to emphasize that seed removal is not equivalent to dispersal (Philpott et al. 2010). In 73% of the positive tuna

![Figure 2](image1.png)

Figure 2. Percentage of seeds removed by ants per treatment during 2 days ($N = 6$ cattle pastures). Uppercase letters describe groups formed after a Tukey test ($\alpha = 0.05\%$).

![Figure 3](image2.png)

Figure 3. Seed removal (percentage) during 48 hours in field trials. $N = 6$. 

Restoration Ecology
oil interactions, seeds showed evidence of predation, whereas none was observed in the aril-baited seeds. Hence, ants appeared to be more interested in removing fatty acids from the tuna-baited seeds than in haul ing them. We claim that the artificial aril did not induce seed predation, but instead offered a nutritious reward, as occurs in nature. For instance, Bottcher and Oliveira (2010) found that Odontomachus chelifer ant workers would feed their larvae with Cabralea canjerana (Meliaceae) arils, leaving the seed intact and viable. The aril-fed larvae were heavier than the control ant larvae. However, since tuna oil is one of the most successful baits, it is important to highlight its role in attracting generalist ants (e.g. Risch & Carroll 1982; Perfecto 1990; Armbrecht & Ulloa-Chacón 2003) that play important roles in moving seeds in degraded agroecosystems (Escarob et al. 2007) and lands under restoration programs (Majer & Nichols 1998; Domínguez-Haydar & Armbrecht 2011).

E. ruidum was the most active ant-moving seeds, which is consistent with Horvitz and Schemske’s (1986) study, where large solitary hunters such as Pachycondyla harpax and P. apicalis moved Calathea seeds more frequently and up to 925 cm, while small S. geminata were moved seeds no more than 20 cm. In a simultaneous experiment in the same plots, it was noted that small Crematogaster abstinens (1.58 ± 0.2 mm) preferred to move larger arillated seeds of Pithecellobium dulce (0.307 ± 0.10 g) than smaller non-arillated S. spectabilis seeds (0.026 ± 0.001 g) or Leucaena leucocephala seeds (0.062 ± 0.015) (unpublished data). These results are similar to those obtained by Pizo and Oliveira (2001) in a lowland forest of Brazil, where lipid content and size of arils and seeds were determinant factors for removal by ants.

The artificial aril was successful, as it facilitated the objective of attracting ants and inducing them to move the seeds toward the nest, as opposed to consuming the aril on the paper disk. Artificial aril seeds were moved over longer distances than tuna oil seeds because the protrusions allowed the seeds to be grabbed by ants. However, in the context and the projections of this study, the distance at which a seed is displaced is less important than the destination of the seed, that is the ant-nest. For instance, a hypothetical rehabilitation practice would provide a “rain” of “artificially arillated” seeds for restoration/rehabilitation purposes, and soil-nesting ants would place them in the enriched microhabitats of their nests or refuse piles with high organic matter from ant corpses and excretions (Beattie & Culver 1983; Hanzawa et al. 1985; Horvitz & Schemske 1986). As has been shown elsewhere, ants may play an important role by redirecting seeds to suitable microhabitats (see Rico-Gray & Oliveira 2007 and references therein), and creating small regenerating vegetation patches in degraded cattle pastures. Such patches formed by soil nesting ants might accelerate the formation of resource islands attractive to primary seed dispersers.

Regardless of the ecological or behavioral mechanisms that induce seed removal by ants, an artificial aril might be useful for enhancing the dispersal of larger seeds by facilitating grabbing by ant mandibles; however, in the case of small seeds it would not improve the efficiency of simply adding tuna oil, given that ants tend to bite them. Further, the artificial aril showed no effect on germination of these small seeds. Casual observations in a preliminary trial, in which the seeds were sown directly in a pot, detected no differences in seed germination between treated (with an artificial aril) and untreated seeds.

In summary, an artificial aril developed from natural and cheap ingredients complies with desirable attributes such as being relatively easy to prepare, of low-cost, and attractive to ants. In conclusion, artificially adding arils to seeds without a natural aril (such as S. spectabilis) can increase their probability of being transported to ant nests. These nests provide safe sites, with loose, fertile, and moist soil (Hanzawa et al. 1988) and relaxed competition with grasses. Nevertheless the viability of seeds moved by ants remains to be tested. Ants may direct movements of shrub and tree seeds thereby benefiting agroecosystems in the process of rehabilitation, such as silvopastoral systems with fodder and shade trees studied here.

Implications for Conservation

- Soil dwelling ants may enhance seed dispersal by removing seeds from deposits deliberately placed close to nests.
- The use of an artificial aril may enhance seed hauling by soil ants, thus providing a tool for intentionally directing secondary dispersal by ants in degraded pastures.
- The artificial aril is not only a bait but also a tool with useful properties such as easier grabbing by ant mandibles, hard consistency, attractiveness, and decay resistance.

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LITERATURE CITED


