Sociobiology 65(2): 208-214 (June, 2018) DOI: 10.13102/sociobiology.v65i2.1017

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Weaver ant *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) performance in mango and cashew trees under different management regimes

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Article History

Edited by
Evandro N. Silva, UEFS, Brazil
Received 03 March 2016
Initial acceptance 07 August 2016
Final acceptance 07 December 2016
Publication date 09 July 2018

Keywords
Oecophylla ants, density index, nest number, mango, cashew.

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Abstract

Weaver ants are used for biological control of insect pests in plantation crops. To obtain proper pest control, ant densities need to be high. Food availability and nesting facilities on host plants and management practices may affect ant performance. In the present study, we tested the effect of two host plant species (mango and cashew) and three different management practices (ants only, ants fed with sugar and ants combined with the soft chemical insecticide Spinosad) on weaver ant performance. Performance was assessed over a 22 month period, as an index value based on the number of ant trails per tree and as the number of ant nests per tree. A total of 216 trees (72 per treatment) were observed in each crop. In all treatments, the ants performed better in mango compared to cashew. Using the index based performance measure, ants also performed better in the sugar treatment as compared to the two other treatments, whereas this was not the case in cashew. We conclude that sugar feeding can be used to increase ant populations in mango. We also found that the treatment with Spinosad in combination with ants showed performance equal to the treatments with only ants, suggesting that Spinosad did not negatively affect ant populations. We therefore conclude that Spinosad is compatible with the use of weaver ants in integrated pest management programs.

Introduction

Ants represent one of the most abundant and ubiquitous arthropod groups on earth and play a major role in regulating the environment (Hölldobler & Wilson, 1994). In tropical regions, weaver ants, *Oecophylla* spp (Hymenoptera: Formicidae) are dominant ants (Lim, 2007) in a wide range of host plant species such as citrus, mango, cashew, coconut, cocoa, among others. They build their nests by weaving living leaves on their host plants together with silk from their larvae. The host plants, thus, provide a habitat for the ants and they also provide the ants with food via floral and extra-floral nectaries and honeydew excreted from associated hemipteran trophobionts (Way, 1963; Kenne et al., 2003). Although *Oecophylla* ants are opportunistic in their choice of host plants (Way & Khoo, 1992), the choice of nest sites is not random (Djiéto-Lordon & Dejean, 1999). Nest site selection depends on innate selective attraction and on environmental factors (Djiéto-Lordon & Dejean, 1999). For example, leaf flexibility, i.e. the degree to which leaves bends when pulled by the ants, determines nest site selection (Hölldobler, 1983). Therefore, weaver ants may have preference toward certain host plant species (Dejean et al., 2008). For instance, they preferred the leaves of citrus and mango rather than the leaves of cocoa and guava (Dejean et al., 2007) and Lim (2007) observed that *Oecophylla smaragdina* (Hymenoptera: Formicidae) showed

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

a strong preference for *Morinda citrifolia* L. (Rubiaceae) because its leaves were larger and appear more pliable than the leaves of other hosts plants assessed. Moreover, according to Offenberg et al. (2006) weaver ants has a preference to use first position leaves for nest building because these leaves are still expanding and therefore smaller and more flexible than the older leaves and also more likely to host trophobionts. The characteristics of host plant leaves are therefore an important factor in the choice of nesting site.

In return for a nesting site and food, weaver ants may protect their hosts against insect pests (Hölldobler & Wilson, 1990; Majer, 1993; Djiéto-Lordon & Dejean, 1999). As biocontrol agents weaver ants have proven efficient in controlling several pests in plantations of mango (Peng & Christian, 2004; Peng & Christian, 2005a; Peng & Christian, 2005b ; Peng & Christian, 2006; Peng & Christian, 2007; Peng & Christian, 2008; Davidson et al., 2007; Adandonon et al., 2009; Van Mele et al., 2007), citrus (Huang & Yang, 1987; Van Mele & Cuc, 2000; Van Mele et al., 2002; Van Mele, 2008), cashew (Peng et al., 1995; Peng et al., 1997; Peng et al., 2008; Dwomoh et al., 2009, Olotu et al., 2013), coconut (Way, 1954; Vanderplank, 1960; Varela, 1992; Sporleder & Rapp, 1998) and cocoa (Ayenor et al., 2004; Ayenor et al., 2007). For reviews see Way and Khoo (1992) and Offenberg (2015).

For the ants to be effective control agents they need to be present at high densities (Peng et al., 2008), therefore in some cases they need to be managed by e.g. providing artificial food (Abdullah et al., 2015; Peng et al., 2008). In other cases the ants need to be supplemented with complementary control methods in integrated pest management (IPM) programs in order to achieve adequate host plant protection (Peng & Christian, 2006). However, this type of plant management action may have negative effect on ants. In Northern Benin, mango and cashew are the most important tree crops with high economic importance (Vayssières et al., 2008; Balogoun et al., 2014). Recently, pest control by weaver ants has been observed in mango and cashew (Adandonon et al., 2009; Van Mele et al., 2007; Anato et al., 2015) and here the fruit fly bait GF-120 has been identified as an effective supplementary IPM component that in combination with weaver ants may lead to better control of pests such as fruit flies and thrips (Vayssières et al., 2009; Anato et al., 2015). At the same time GF-120 is an environmentally safe insecticide to use. Therefore weaver ant combined with this insecticide may be used in an IPM approach for the biological control of pests in Benin.

To test the effect of host plant species, ant feeding, and the application if IPM components on ant performance we compared ant performance in cashew and mango trees where weavers ant were managed as either not fed, fed sugar or combined with GF 120. Performance was assessed by counting ant trails and nest numbers in the trees.

Materials and Methods

Study site and experimental design

The study was conducted in one mango and one cashew orchards in the Parakou area (09° 22’ 13”N / 02° 40’16”E) of Benin from 2012 (August) to 2014 (May). Both plantations are located in the same area with approximately 20 m distance between them. Mango trees were between 30 and 32 years old and cashew trees between 20 and 25 years old. Each orchard had an average of 100 trees/ha at 10 m × 10 m spacing and trees were homogeneous in height. In each orchard 216 trees were randomly divided into three treatments: (1) trees with weaver ants (ants) used as control, (2) trees with weaver ants where the ants were fed with sugar (ants feeding), and (3) trees where weaver ants were combined with the conventional insecticide GF-120 (ants + GF-120). In each crop there was a total of 72 trees per treatment and two rows of trees were used as a buffer zone between two consecutive treatments. Buffer zones of this size have been used in previous studies to protect neighboring treatments against the effect of insecticides (Peng & Christian, 2005b; Offenberg et al., 2013).

Treatments

In all treatments, an artificial bridge made of twisted polystrings of approximately 3 mm diameter was used to connect all the trees belonging to the same weaver ant colony in order to facilitate ant communication (Peng et al., 2008; Vayssières, 2012), and interlocking tree branches between neighboring trees with different ant colonies were removed by pruning, to avoid ant fighting between colonies.

In the ant + GF-120 treatment, a conventional insecticide GF-120 (NF Naturalyte fruit fly bait, Dow Agro- Sciences LLC, Indianapolis, IN) with 0.02% spinosad (AI) and 98.8% inert ingredients (water, sugars, and attractants) was used to spray the trees. Spraying was done weekly from February to March in 2013 and again in 2014 in the cashew orchard and from February to April in 2013 and 2014 in the mango orchard. One square meter of each tree was sprayed as recommended at head height in an area without fruits. The recommended dose (1.5 l of GF-120/ha) was used for treatment (Vayssières et al., 2009) at the ratio of 1 liter of GF-120 for 5 liters of water (Dow Agrosciences, 2001). The insecticide was applied as a foliar spot spray, using a manual sprayer (Berthoud Apollo 16-AF) with a conventional conical nozzle (1–2 mm aperture to deliver droplets of 2–6 mm). During the weekly applications, rotation around the tree was used to avoid phytotoxicity on previously treated surfaces (Vayssières et al., 2009).

In the ant feeding treatment, weaver ants were fed with a 30% sucrose solution provided in 60 ml plastic bottles plugged with cotton with one bottle per tree. The sugar feeders were placed upside down on a tree trunk with a busy ant trail and refilled once per week. Also, water was provided *ad libitum* in a bottle on each of the trees in the ant feeding treatment. Sugar and water were provided at the same time in mango and cashew plantations.

Weaver ant density on mango and cashew trees

Weaver ant density was assessed fortnightly in each tree from August 2012 to May 2014 (50 times during the study period) in both the mango and the cashew orchard. Density was assessed using the "branch method" presented in Offenberg and Wiwatwitaya (2010). With this method the number of ant trails in a tree is used as a measure of ant density, resulting in an index value ranging between 0 and 100 %. The counting of ant trails on the trees was conducted between 9:30 and 13:30 which is within the most active period of *O. longinoda* in Benin (Vayssières et al., 2011). Weaver ant colonization level was also assessed by counting the number of weaver ant nests per tree in each tree once a month (22 times during the study period). Nest numbers only included naturally occurring nests as artificial movement of nests between trees was not used in the management of ants in these plantations.

Data analysis

Weaver ant performance for each particular tree was expressed as the mean value of (1) the weaver ant density indexes recorded over the seasons and, (2) as the mean number of weaver ant nests counted over the two seasons. Log10 (x + 1) and arcsin $\sqrt{x}$ transformations were used, respectively, on nest number and index values to stabilize variance and normalize the data before analysis. Values obtained were then compared with two way ANOVAs and Tukey pairwise comparisons to test the effect of crops (mango and cashew) and treatments (ants, ants feeding and ants + GF-120) on weaver ant performance. The number of main branches on each tree was also assessed and compared between crops and treatments with a Poisson distribution, as trunk numbers may in some cases affect the index values (Wargui et al., 2015). All the statistical analyses were done with JMP 10.0.0. (SAS, 1995).

Results

Weaver ant abundances were above 50 % on the two crops during the entire study period (Fig 1A) and both index values and nest numbers differed significantly between crops and among treatments (Fig 1, Tables 1 and 2).

Using either of the two ant density measures, the ants performed significantly better on mango compared to cashew in all the three treatments (Tables 1 and 2, Fig 1). The weaver ant density index in mango was on average 15 % higher than in cashew (8 %, 25 % and 12 % in the ants, ants feeding and IPM treatments, respectively), whereas, nest numbers were on average 95 % higher in mango (79 %, 107 % and 98 % in ants, ants feeding and IPM treatments respectively).

In mango, ants performed better in the ants feeding treatment compared to both the ant (index: $t_{ratio} = 10.80$, $P < 0.0001$; nest numbers: $t_{ratio} = 5.09$, $P < 0.0001$) and the ant + GF-120 treatments (index: $t_{ratio} = 9.35$, $P < 0.0001$; nest number: $t_{ratio} = 2.95$, $P = 0.039$). On the other hand, there was no significant difference between the ant and the ant + GF-120 treatments (index: $t_{ratio} = -1.45$, $P = 0.70$; nest number: $t_{ratio} = -2.14$, $P = 0.27$).

When comparing the ant feeding treatment with the ant and ant GF-120 in cashew, there were only significant (or nearly significant) differences when comparing the index values ($t_{ratio} = 2.78$, $P = 0.063$; $t_{ratio} = 2.98$, $P = 0.036$, respectively), not when using nest numbers ($t_{ratio} = 1.99$, $P = 0.34$; $t_{ratio} = 1.58$, $P = 0.61$, respectively). Also, in cashew, there were no differences between the ant and the ant + GF-120 treatments,

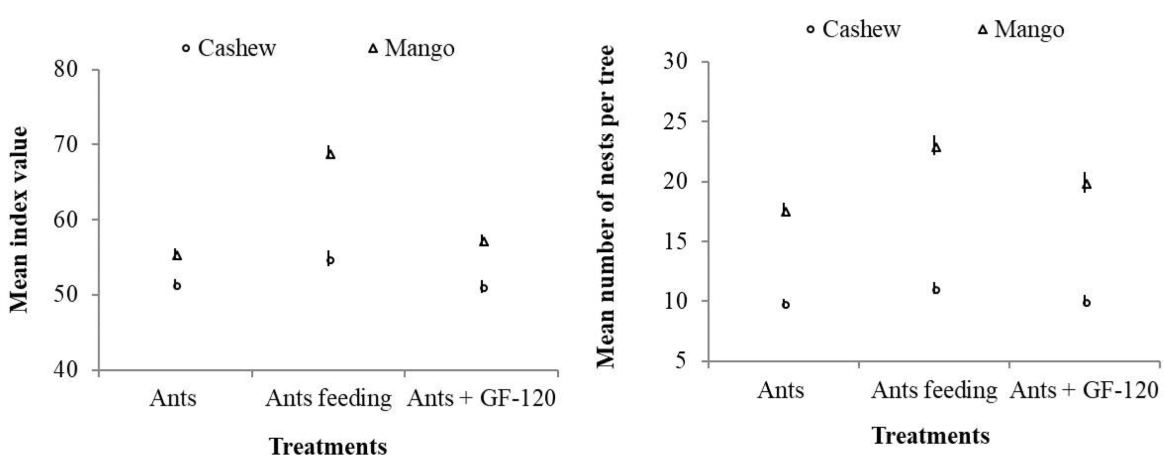

Fig 1. Average density of weaver ant tree-1(± SE) (A) and average number of weaver ants nests tree-1 (± SE) (B) on cashew and mango crops. N= 72 trees per treatment in each crop.

Table 1. Two way ANOVA test for comparison of weaver ant mean density index per tree between crops (mango and cashew) and treatments (ants, ants feeding and ants + GF-120). N = 432 trees.

	DF	Sum of Squares	F Ratio	P
Crops	1	0.76	121.4	< 0.0001
Treatments	2	0,70	56.3	< 0.0001
Crop* Treatment	2	0.22	17.9	< 0.0001

neither with the index (t_{ratio} = 0.20, P = 0.99) nor with nest numbers (t_{ratio} = -0.41, P = 0.998).

The number of main trunks on mango trees was significantly higher compared to cashew (Chi-square = 52.7, df = 1, P < 0.0001), whereas no difference was observed between treatments within each crop (Table 3). The number of main trunks on mango ranged from three to eight with a median of five, whereas they ranged from two to seven with a median of four, in cashew.

Discussion

This study showed that weaver ants performed better on mango than on cashew, based on ant trail activity and nest numbers. It should be noted, though, that also the numbers of main trunks on trees, which may affect the index values, were significantly different between the crops. According to Wargui et al. (2015) a higher number of trunks may reduce the ant trail index value on trees. In the present case that would mean that index values are underestimated in mango and therefore the difference from cashew is conservative. Moreover, the fact that nest numbers, which are unaffected by the number of main trunks, were also higher in mango, further support the conclusion that *O. longinoda* perform better in this crop. Nest numbers may also not be a precise measure of ant abundance, especially when comparing different crops, since nest sizes may differ between host plant species due to different leaf morphology. Ouagoussounon et al., (Personal communication) demonstrated that *O. longinoda* build smaller nest in cashew trees compared to mango trees, and therefore each nest probably hold fewer ants. But again, this supports our conclusion as we may then have overestimated ant performance in cashew and yet found performance to be higher in mango.

That ants prefer or perform better on some plants compared to others is also supported by other studies. For instance, according to Kenne et al. (2003), *O. longinoda* was more frequent on both citrus and mango trees compared to guava trees and Dejan et al. (2007) mentioned that *Oecophylla* spp founding queens preferred citrus and mango leaves rather than the leaves of cocoa and guava trees. Also the ants *Camponotus acvapimensis* (Mayr) and *Paratrechina longicornis* (Latreille) have been recorded more frequent on citrus than on mango trees (Kenne et al., 2003) in Cameroon, and the same authors (Kenne et al., 2009) demonstrated that the arboreal ant species *Atopomyrmex mocquerysi* (E. André), was frequently found on safoo trees and rarely on cocoa, avocado, guava, mango and citrus.

Table 2. Two ways ANOVA comparing the mean number of weaver ant nests per tree between crops (mango and cashew) and treatments (ants, ants feeding and ants + GF-120). N = 432 trees.

	DF	Sum of Squares	F Ratio	P
Crops	1	7.8	514.7	< 0.0001
Treatments	2	0.4	12.9	< 0.0001
Crop* Treatment	2	0.07	2.4	0.09

Table 3. Poisson test for comparison of number of main trunk per tree between crops (mango and cashew) and treatments (ants, ants feeding and ants + GF-120). N = 432 trees.

	DF	L-R Chi Squares	P
Crops	1	52.7	< 0.0001
Treatments	2	1.6	0.46
Crops x Treatment	2	2.0	0.37

Thus, for several ant species, there is a preference of host plant species, probably influenced by plants characteristics. According to Lim (2007), characteristics such as leaf size, leaf flexibility and food resources on the plants may be important. *O. smaragdina* has been reported to prefer host plant species with leaf sizes ranging from 5 x 8 cm and up to 20 x 20 cm and avoiding tough-leaved plant species (Blüthgen & Fiedler, 2002). As mango leaves are smaller and more pliable than the rather tough cashew leaves (Rosine Wargui, personal observations) this may explain the difference found in the present study. Further, secretions of extra-floral and floral nectars by host plants and the presence of hemipteran trophobionts on these host plant may favor the development of the ants (Kenne et al., 2003). Mango trees in the Sudanian area of Benin were reported to harbor large numbers of scale insects (12 species) particularly on mango fruit petioles and on the fruits (Germain et al., 2010). In contrast, Vayssières (2012) recorded only two Hemiptera species on cashew in northern Benin. These factors together, may explain why *O. longinoda* was more abundant in mango. However, further studies are needed to clarify mango and cashew characteristics allowing better knowledge of weaver ant preference and performance.

The implications of the different performance in mango and cashew is, for example, that when using weaver ant as biological control agents the ants may require more management attention in cashew than in mango plantations. Indeed, management measures such as baiting competitive ants species, connecting trees within the same ant colony, removing interlocking branches between trees belonging to different colonies and feeding ants, may facilitate weaver ants and in this way contribute to boost ant densities in less favorable crops. Such measures are required in plantations when weaver ant densities are below 50 % using the index developed by Peng and Christian (2004). It is important to examine different crop species effect on ants and to monitor their abundance in order to gain profit from their presence in plantations trees. A varying performance on different hosts also suggests that intercropping inferior host with more ant profitable crops may increase biocontrol effectiveness. This is especially the case when crops have different phenologies. In that case the ants can continuously migrate to more actively growing hosts as the season change. This would benefit ants, as food is more abundant on trees with growing plant tissues, and it will benefit crops as ants will aggregate on developing plant tissue (new leaves, flowers and fruits) which is most in need of protection.

Our study also showed that weaver ants were more abundant on trees with sugar feeders compared to the two other treatments, suggesting that ant feeding promoted the ant population and can be used as a management tool to facilitate biological control. This was the case in both crops if the index was used as a population measure. However, nest numbers were not significantly higher in cashew, only in mango. As mentioned in a previous study (Offenberg & Wiwatwitaya, 2010), sugar feeders may themselves increase the index value, as they are placed on the main trunks where also ant trails are assessed. Thus, if the sugar feeders attract more ants to the trunk, than would otherwise be the case, the ant trail based index may become inflated. Therefore, the result based on the index should be interpreted with caution. As a result we cannot conclude with certainty that sugar feeding improved ants in cashew, though it is likely. However in mango we observed higher nest numbers as well as higher index values in the ant feeding treatment, indicating that sugar feeding did increase the populations in this crop. We therefore suggest sugar feeding as a possible measure to increase weaver ant population in plantations.

Lastly we found not difference in population measures between treatments with only ants and the treatments where ants were combined with GF-120. This was the case for both crops and using both population measures. We therefore find it safe to conclude that Spinosad in the form of GF-120 and applied as described here and in Anato et al. (2015), is compatible with the use of weaver ant. This is of importance as spinosad have proven effective against fruit flies in mango (Prokopy et al., 2003; Wang et al., 2005; Vayssières et al., 2009) and against thrips in cashew (Anato et al., 2015) and since these species are important pest in West Africa (Vayssières et al., 2008; Anato et al., 2015), the efficiency of pest control may be advanced by combining weaver ants with GF-120. This is in concordance with previous studies demonstrating that GF-120 had no detectable effect on beneficial insects such as the parasitoids *Aphytis* spp (Thomas & Mangan, 2005) and *Fopius arisanus* (Sonan) (Vargas et al., 2002). According to Stark et al. (2004) parasitoids are less susceptible than fruit flies to spinosad (Provesta protein bait spray). Spinosad in the form of Gf-120 can therefore be included in IPM programs with *O. longinoda* as a major component for the control of both mango and cashew major pests.

Acknowledgments

We would like to thank M. Zoumarou-Wallis, the owner of the two orchards where this study was conducted at Korobororou village for providing field facilities. We thank also Gbetable Gabin, Jacques Sogbossi and Crépin Aniwanou, for their technical assistance. Grateful thanks to the project "Increasing value of African Mango and Cashew production". DANIDA (DFC No. 10 – 025AU) for financial support.

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