

Weaver Ants Increase Premature Loss of Leaves Used for Nest Construction in *Rhizophora* Trees¹

Joachim Offenberg², Mogens Gissel Nielsen

The Institute of Biological Sciences, University of Aarhus, Universitetsparken, 8000 Aarhus C, Denmark

Donald J. Macintosh

Centre for Tropical Ecosystems Research, The Institute of Biological Sciences, University of Aarhus, Universitetsparken, 8000 Aarhus C, Denmark

Sanit Aksornkoe

Faculty of Forestry, Kasetsart University, Paholyothin Road, Chatuchak, Bangkok 10900, Thailand

and

Sopon Havanon

Mangrove Research and Development Group, Department of Marine and Coastal Resources, Paholyothin Road, Payatai, Bangkok 10400, Thailand

ABSTRACT

Oecophylla ants utilize living leaves when they construct their nests. We investigated how *Oecophylla smaragdina* nests in southern Thailand affected leaf performance on the mangrove tree *Rhizophora mucronata*. Leaves used in nests and neighboring leaves showed a higher rate of premature leaf loss compared to control leaves farther from the nests. However, a tree's total cost due to the higher premature leaf loss was estimated to be approximately between 3- and 20-fold lower than the benefit derived from ant protection, detected in a previous study (Offenberg *et al.* 2004).

Key words: ant leaf nests; ant–plant interaction; cost/benefit; mangrove forest; *Oecophylla smaragdina*; *Rhizophora mucronata*; scale insects; Thailand.

ANT–PLANT PROTECTION MUTUALISMS, where ants protect their host plants against herbivores, involve, in some cases, plants that produce ant attractants in order to attract and facilitate their ant partners (reviewed by Agrawal & Rutter 1998, Bronstein 1998). In these specialized interactions, plants produce food rewards for their ants and/or provide them with specialized structures (domatia) for nesting (Beattie 1985). Costs and benefits of these ant–plant interactions have been studied intensively to understand the evolution and maintenance of these mutualisms (Bronstein 1994, 1998). In contrast, nonspecialized ant–plant interactions, *i.e.*, interactions where plants do not produce ant attractants but nevertheless are visited by or host ants, have received less attention (but see review by Way & Khoo 1992). These interactions are more widespread than specialized interactions and may also confer benefits to the plants involved (Way & Khoo 1992 and references therein, Peng *et al.* 1995, Offenberg *et al.* 2004, Peng & Christian 2004). Contrary to the specialized ant–plant interactions, the nonspecialized interactions are characterized by plant partners who pay no costs to the production of ant attractants.

According to plant protection theory, plant defenses with high maintenance costs should be more likely to evolve in resource-rich habitats compared to habitats with fewer resources available to the

plants (Coley *et al.* 1985). Since the maintenance of a continuous supply of ant attractants in specialized ant–plant interactions may be costly to plants (Davidson & Fisher 1991), it has been proposed that these interactions are most likely to be found in resource-rich habitats such as forest gaps (Davidson & Fisher 1991, Schupp & Feener 1991, Feener & Schupp 1998). Accordingly, the nonspecialized interactions where plants do not spend resources on ant attractants should characterize more harsh environments. Mangroves living in intertidal habitats experience very stressful conditions due to high salinities and anoxic sediments (Hogarth 1999, Sherman *et al.* 2003) and may therefore have limited resources available to invest in ant attractants. Only a few true mangrove species produce ant attractants, and the two species we are aware of (*Pelliciera rhizophorae* and *Laguncularia racemosa*) both produce extrafloral nectar (Biebl & Kienzel 1965, Collins *et al.* 1977). Yet mangroves may associate with ants in nonspecialized interactions and gain protection despite the lack of ant rewards (Nielsen 2000, Cogni *et al.* 2003, Offenberg *et al.* 2004, Offenberg *et al.* 2005). Such plants, however, may still pay indirect costs even though they do not supply ants directly.

Oecophylla ants are effective plant protectors and are opportunistic in their choice of host plants (Way & Khoo 1992). They build their nests by weaving leaves together and feed on a diet of arthropod prey and honeydew obtained from attended homopterans (Way 1954a, 1954b; Hölldobler 1979, 1983; Dejean 1991; Van Mele & Cuc 2003; Offenberg *et al.* 2004). Consequently, they are

¹ Received 31 December 2004; revision accepted 4 January 2006.

² Corresponding author; e-mail: offenberg@biology.au.dk

independent of plant-produced domatia and food rewards and are often associated with nonspecialized host plants. Nest building by *Oecophylla* however, may, incur an indirect cost to the leaves utilized for nest construction for at least three reasons. First, when leaves are woven together to form the nest, ants bend the leaves out of their original position resulting in increased tension on the leaf petiole and potentially causing a premature detachment of the leaf from the shoot. Second, the leaf may be forced into a position where the axial side of the leaf blade will be hidden in shade inside the leaf nest and the abaxial side exposed to direct sunlight, which is most likely a stressful condition to a leaf evolved to the opposite arrangement. Third, inside their leaf nest the ants may attend sap-sucking homopterans, which may increase leaf stress by depleting the leaf sap (Dillwith *et al.* 1991, Riedell & Blackmer 1999).

The objective of this study was to assess whether leaf performance on a nonspecialized mangrove tree was affected by the presence of weaver ant nests. Specifically, we tested if nest leaves differed in size, homopteran infection level, and in the risk of premature death, compared to neighboring control leaves not used for ant nest building.

The study was conducted in January 2003 within the Ranong Biosphere Reserve, a coastal protected area covering about 30,000 ha of mangrove forest and waterways in southern Thailand. A detailed description of the area and climate is provided by Macintosh *et al.* (2002). A rehabilitated forest site with 8-yr-old *Rhizophora mucronata* trees was selected along the main estuary, Klong Ngao. All the trees had been replanted at the same time (with a spacing of 1.5×1.5 m) and thus were of equal size and appearance. The *R. mucronata* stand was searched randomly for trees belonging to weaver ant (*Oecophylla smaragdina*) territories until 15 ant trees were found. All shoots harboring an ant nest (nest shoots) were collected from each of these trees. The nearest shoot to each nest shoot was also collected, to serve as a control group (control shoots). All shoots were placed individually in plastic bags and frozen until further examination. The number of leaves, individual leaf positions on the shoot (including lost leaves), individual leaf sizes, leaf condition (green leaves vs. yellow withered leaves), and the number of homopterans on the leaves were recorded. Leaves from nest shoots were further divided in two groups: nest leaves that had been part of an ant nest and controls that had not been used for ant nest construction. All the leaves on control shoots were treated as control leaves. Leaf positions were registered by allocating a number to each leaf representing the order of the leaf from the apical end of the shoot, with opposite leaves assigned equal numbers. Thus, in the youngest leaf pair both leaves were assigned number one. Leaves that had been lost above the position of the two oldest leaves on the shoot (premature leaf loss) were also registered. Additionally, one of the examined trees was selected randomly to determine the total number of shoots present.

The effect of shoot type (nest shoots vs. control shoots) and/or leaf type (nest leaves vs. control leaves) was analyzed for four response variables. (1) Leaf size was measured as leaf area in cm^2 and means were compared between nest leaves and control leaves. Furthermore, the association between nest leaf category and leaf position (first position vs. higher positions) was tested with Fisher's

exact test. (2) Leaf condition was assigned by dividing leaves into one of two groups: fresh green leaves or yellow withered leaves. The distribution of leaf condition was compared between groups and analyzed with Fisher's exact test. (3) The rate of premature leaf loss (RPLL) was calculated by subtracting the number of lost (or withered) leaves from the mean number of the two oldest leaves and then summing these numbers for each lost leaf on a particular shoot ($\text{RPLL} = \sum (v_1 + v_2/2) - v_i$; where v_1 = the number of the oldest leaf, v_2 = the number of the second oldest leaf, and v_i = the number of a particular lost leaf). This rate is therefore a measure of how long the leaf could have lived additionally if it had not died prematurely. The loss of young leaves contributes more to the RPLL than the loss of older leaves. RPLL was compared between nest shoots and control shoots and analyzed with a Kruskal–Wallis test since data could not be transformed to obtain normality. Also, the distribution of shoots that lost at least one leaf prematurely was compared between groups using Fisher's exact test. (4) The distribution of leaves with and without homopterans and the number of homopterans/leaf was calculated and group comparisons were analyzed with Fisher's exact test and the Kruskal–Wallis test, respectively.

The total number of leaves on a tree was estimated by multiplying the total number of shoots counted on one of the trees by the mean number of leaves per shoot, calculated from all the shoots sampled from the 15 ant trees. From this estimate the proportion of nest leaves (using the average number of nest leaves per tree) to the total number of leaves was calculated.

On nest shoots, the control leaves were 9.5 percent larger than the nest leaves. However, this size difference was caused by a strong preference by the ants to use first position leaves for nest building; these leaves are still expanding and therefore smaller than the older leaves on the shoot. Forty-one percent of the nest leaves were placed on the first leaf position whereas first-position leaves only accounted for 11 percent within the control leaves (Fisher's exact test: $P < 0.0001$). Thus, nest leaves were smaller because the ants preferred to place the nest at the apical end of the shoot.

No withered leaves were found on the control shoots, whereas 11 percent (32 leaves) on nest shoots were withered. Most of these leaves had been used for nest construction; 26 of them belonged to the nest leaf group and only 6 to the control group. Thus, when all leaves were considered there were significantly more withered leaves within the nest leaves (23.9%) than within the control leaves (1.4%; Fisher's exact test: $P < 0.0001$). After the exclusion of nest leaves, there were still significantly more withered leaves on nest shoots (3%) compared to control shoots (0%; Fisher's Exact test: $P < 0.0008$). The latter result should be considered with caution since the analysis is based on only six withered leaves. Thus, withering was exclusively associated with nest shoots, and on these shoots both leaves used for nest construction and control leaves were affected. Premature leaf loss was also affected by nest building. Significantly more nest shoots (12 of 30) had lost at least one leaf prematurely compared to the control shoots (3 of 30; Fisher's exact test: $P = 0.007$). Likewise, mean RPLL was more than five times higher on nest shoots compared to the controls (Kruskal–Wallis: $= 7.15$, $df = 1$, $P = 0.0075$). The mean numbers of lost leaf positions were 2.0 and 0.37 on nest shoots and control shoots, respectively.

Ant-attended coccids of the species *Coccus hesperidum* Linnaeus (Coccidae) were found in high numbers on nest leaves, whereas, they were almost absent outside ant nests. Eighty-six percent (94 of 109) of the nest leaves were infected with these scales but only 4 percent (16 of 433) of the control leaves (Fisher's exact test: $P < 0.0001$) were infected. Moreover, the mean number of scales/leaf was 108.7 (SE = 12.02, $N = 109$) on nest leaves and only 0.08 (SE = 0.035, $N = 433$) on controls (Kruskal–Wallis: $\chi^2 = 385.7$, $df = 1$, $P < 0.0001$).

The total number of shoots on the sampled tree was 339. The average number of leaves per shoot on the 15 ant trees was 8.36 ± 0.27 (SE). This leads to an estimate of 2858 ± 92 (SE) leaves per tree assuming that all trees had approximately an equal number of shoots. Thirty trees were examined before we obtained 15 ant trees. Thirteen of the fifteen ant trees contained nests and the mean number of nest leaves per ant tree was 7.26 ± 1.8 (SE). This means that the average percentage of leaves used for nest construction on ant trees was 0.25%. Regarding RPLL, the nest shoots lost 1.63 (2.0–0.37) more leaf positions than the control shoots on average. This difference and the average time a leaf occupied each leaf position (=1.9 mo; J. Offenberg, pers. obs.) means that nest shoots lost 3.1 more leaf month compared to control shoots. With an average of two nest shoots per ant tree, the cost to each ant tree was 6.2 leaf months. An average tree with 2858 leaves will have 5430 leaf months available. The cost associated with the presence of ant nests will thus be 0.11 percent. This measure only accounts for leaves that were either lost or withered prematurely. If leaves incorporated into nests are assumed to be shaded to an extent where they no longer contribute to photosynthesis, they may be considered lost. Including this effect on leaf function, the mean RPLL on nest shoots will be 9.85 instead of 2.0 and thus the difference between nest shoots and control shoots will be 9.48 lost leaf positions per nest shoot. This analysis will result in a cost of 0.66 percent due to the presence of ants.

That most of the withered leaves were nest leaves could partly have been because the nest leaves remained attached to the nest after natural withering and petiole detachment since they were weaved together with other fresh leaves. In that case, leaves that are part of a nest will spend more post-withering time on the shoot compared to other leaves. On the other hand, six of the withered leaves were not directly involved in the nest constructions and we did not find any withered leaves on shoots without nests. This may indicate a negative impact of ant nests on neighboring leaves that are not directly incorporated in a nest but are borne on the same shoot. A reduced performance among leaves from nest shoots was supported further by the higher mean RPLL (measured as the number of lost leaf positions) and the higher probability of losing at least one leaf prematurely on nest shoots compared to the control shoots. Also, nest leaves were partially (and in some cases totally) imbedded in the nests and were therefore shaded or in some cases their abaxial side was exposed to sunlight while the axial side was shaded; the opposite arrangement compared to natural conditions. This kind of shading may further reduce the performance of nest leaves.

These results show that although *R. mucronata* trees do not produce ant attractants, they still incur indirect costs by the presence

of weaver ants in the form of an increased RPLL and a higher standing crop of ant-attended homopterans. Nonetheless, the cost to plants in this ant–plant association seems to be minor in relation to the benefit conveyed by the ants. A previous study at the same field site showed that *R. mucronata* trees with *O. smaragdina* ants on average had 0.57 percent of their leaf area removed against 2.7 percent on trees without ants (Offenberg *et al.* 2004). Consequently, the benefit due to the ants in this case was 2.13 percent, which is approximately 20-fold higher than the costs (0.11%) captured by the present study. However, if nest leaves are considered lost (not contributing to photosynthesis) resulting in a cost of 0.66 percent, the benefit will only be approximately three times higher than the costs. Since photosynthesis in nest leaves probably does not cease completely, the true cost due to ant nests will more likely be somewhere between 0.11 and 0.66 percent according to the amount of shading. The benefit of ants will thus be between 3 and 20 times higher than the costs. On top of the costs captured by this study there will also be the additional cost associated with the depletion of plant resources by coccids, which may reduce the net benefit of ant presence further. These assumptions can be validated by further research using the *Oecophylla–Rhizophora* association.

ACKNOWLEDGMENTS

The Danish International Development Assistance (DANIDA) financed this research, project no.: 91060. We are grateful to Professor P. Gullan, University of California, for the identification of the coccids. We would also like to thank the National Research Council of Thailand and the Royal Forest Department for their permission to conduct research in the Ranong Biosphere Reserve and for valuable help during the study. Finally we are grateful to the staff at the Ranong Mangrove Forest Research Centre and other local people who assisted with the fieldwork.

LITERATURE CITED

- AGRAWAL, A. A., AND M. T. RUTTER. 1998. Dynamic anti-herbivore defence in ant–plants: The role of induced responses. *Oikos* 83: 227–236.
- BEATTIE, A. J. 1985. The evolutionary ecology of ant–plant mutualisms. Cambridge University Press, Cambridge, England.
- BIEBL, R., AND H. KINZEL. 1965. Blattban und Salzhaushalt von *Laguncularia racemosa* (L.). Gaertn. und anderer mangrovenbaume auf Puerto Rico Oest. Bot. Z. 112: 56–93.
- BRONSTEIN, J. L. 1994. Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.* 9: 214–217.
- . 1998. The contribution of ant–plant protection studies to our understanding of mutualism. *Biotropica* 30: 150–161.
- COGNI, R., A. V. L. FREITAS, AND P. S. OLIVEIRA. 2003. Interhabitat differences in ant activity on plant foliage: Ants at extrafloral nectaries of *Hibiscus pernambucensis* in sandy and mangrove forests. *Entomol. Exp. Appl.* 107: 125–131.
- COLEY, P. D., J. P. BRYANT, AND F. S. CHAPIN. 1985. Resource availability and plant antiherbivore defence. *Science* 230: 895–899.
- COLLINS, J. P., R. C. BERKELHAMER, AND M. MESLER. 1977. Notes on the natural history of the mangrove *Pelliciera rhizophorae* Tr. & Pl., (Theaceae). *Brenesia*, no. 10/11, pp. 17–29.

- DAVIDSON, D. W., AND B. L. FISHER. 1991. Symbiosis of ants with *Cecropia* as a function of light regime. In C. R. Huxley & D. F. Cutler (Eds.). Ant-plant interactions, pp. 289–309. Oxford University Press, Oxford.
- DEJEAN, A. 1991. Adaptation of *Oecophylla longinoda* to spatiotemporal variations in prey density. *Entomophaga* 36: 29–54.
- DILLWITH, J. W., R. C. BERBERET, D. K. BERGMAN, P. A. NEESE, R. M. EDWARDS, AND R. W. MCNEW. 1991. Plant biochemistry and aphid populations—Studies on the spotted alfalfa aphid, *Therioaphis maculata*. *Arch. Insect Biochem. Physiol.* 17: 235–251.
- FEENER, D. H., AND E. W. SCHUPP. 1998. Effect of treefall gaps on the patchiness and species richness of Neotropical ant assemblages. *Oecologia* 116: 191–201.
- HOGARTH, P. J. 1999. The biology of mangroves. Oxford University Press, Oxford.
- HÖLDOBLER, B. 1979. Territories of the African weaver ant (*Oecophylla longinoda* Latreille)—A field study. *Zeitschrift Für Tierpsychologie-J. Comp. Ethol.* 51: 201–213.
- . 1983. Territorial behavior in the green tree ant (*Oecophylla smaragdina*). *Biotropica* 15: 24.
- MACINTOSH, D. J., E. C. ASHTON, AND S. HAVANON. 2002. Mangrove rehabilitation and intertidal biodiversity: A study in the Ranong mangrove ecosystem, Thailand. *Estuar. Coast. Shelf Sci.* 55: 331–345.
- NIELSEN, M. G. 2000. Distribution of the ant (Hymenoptera : Formicidae) fauna in the canopy of the mangrove tree *Sonneratia alba* J. Smith in northern Australia. *Aust. J. Entomol.* 39: 275–279.
- OFFENBERG, J., S. HAVANON, S. AKSORNKOAE, D. MACINTOSH, AND M. G. NIELSEN. 2004. Observations on the ecology of weaver ants (*Oecophylla smaragdina* Fabricius) in a Thai mangrove ecosystem and their effect on herbivory of *Rhizophora mucronata* Lam. *Biotropica* 36: 344–351.
- , M. G. NIELSEN, D. MACINTOSH, S. HAVANON, AND S. AKSORNKOAE. 2005. Lack of ant attendance may induce compensatory plant growth. *Oikos* 111: 170–178.
- PENG, R. K., AND K. CHRISTIAN. 2004. The weaver ant, *Oecophylla smaragdina* (Hymenoptera : Formicidae), an effective biological control agent of the red-banded thrips, *Selenothrips rubrocinctus* (Thysanoptera : Thripidae) in mango crops in the Northern Territory of Australia. *Int. J. Pest Manag.* 50: 107–114.
- , K. CHRISTIAN, AND K. GIBB. 1995. The effect of the green ant, *Oecophylla smaragdina* (Hymenoptera, Formicidae), on insect pests of cashew trees in Australia. *Bull. Entomol. Res.* 85: 279–284.
- RIEDEL, W. E., AND T. M. BLACKMER. 1999. Leaf reflectance spectra of cereal aphid-damaged wheat. *Crop Sci.* 39: 1835–1840.
- SCHUPP, E. W., AND D. H. FEENER. 1991. Phylogeny, lifeform, and habitat dependence of ant-defended plants in Panamanian forest. In C. R. Huxley and D. F. Cutler (Eds.). Ant-plant interactions, pp. 175–197. Oxford University Press, Oxford.
- SHERMAN, R. E., T. J. FAHEY, AND P. MARTINEZ. 2003. Spatial patterns of biomass and aboveground net primary productivity in a mangrove ecosystem in the Dominican Republic. *Ecosystems* 6: 384–398.
- VAN MELE, P., AND N. T. T. CUC. 2003. Ants as friends: Improving your tree crops with weaver ants. CABI Bioscience, Cali, Columbia.
- WAY, M. J. 1954a. Studies of the life history and ecology of the ant *Oecophylla longinoda* Latreille. *Bull. Entomol. Res.* 45: 93–112.
- . 1954b. Studies on the association of the ant *Oecophylla longinoda* (Latr) (Formicidae) with the scale insect *Saissetia zanzibarensis* Williams (Coccidae). *Bull. Entomol. Res.* 45: 113–134.
- , AND K. C. KHOO. 1992. Role of ants in pest-management. *Annu. Rev. Entomol.* 37: 479–503.