Insect. Soc. (2012) 59:285–288
DOI 10.1007/s00040-012-0219-9

Insectes Sociaux

RESEARCH ARTICLE

Observation of leaf-cutting ants foraging on wild mushrooms

B. E. Lechner · R. Josens

Received: 15 November 2011 / Revised: 24 December 2011 / Accepted: 4 January 2012 / Published online: 25 January 2012

Abstract This study reports on the observation of an unusual behavior in leaf-cutting ants: foraging on wild mushrooms. A colony of *Acromyrmex lundi* in Buenos Aires (Argentina) was observed intensively harvesting basidiomes (mushroom fructifications) of wild *Agrocybe* fungus developing on a tree bark. Another colony maintained for a month in laboratory conditions also accepted *Agrocybe* mushroom and incorporated the cut bits into the fungus garden in the same way as they do with leaves. We recorded these events confident that they open a new perspective on the study of the feeding habits of leaf-cutting ants as well as on the relationship between their fungus garden and other organisms.

Keywords Leaf-cutting ants · Foraging · Fungi · *Acromyrmex* · Wild mushrooms

The monophyletic tribe Attini is an ant group limited to the New World that lives in obligate symbiosis with a basidiomycete fungus that provides the ants with nutrients (Martin and Weber, 1969), while the ants provide the fungus with adequate substrates (Weber, 1972). The Attini comprises 16 genera that use a variety of different substrates to nourish the symbiotic fungus they cultivate inside the nest (Mehdiabadi et al., 2010). Only two genera (*Acromyrmex* and *Atta*) are called leaf-cutting ants because they primarily use freshly cut plant material (leaves, flowers, fruits, and also seeds). All the fungus gardens cultivated by leaf-cutting ants recorded correspond to *Leucoagaricus gongylophorus* (Kermarrec et al., 1986; Bass and Cherrett, 1995). The fungal mycelium (particularly the gongylidia: hyphal swellings) has been proposed as the main food source of the brood, queen and new reproductors, and only as a secondary food source for the adult workers of leaf-cutting ants (Quinlan and Cherrett, 1979).

In Argentina, *Acromyrmex* ants are distributed from the northeast to higher latitudes in temperate and even cold areas. In the city of Buenos Aires, *Acromyrmex lundi* is one of the most common species. On the campus of the University of Buenos Aires, in mid-September 2010, something unexpected drew our attention: a colony of *A. lundi* was actively foraging on wild mushrooms. We observed hundreds of ants on a few mushrooms. This colony, which we named fungi-forager colony (FF colony), nested at the foot of a poplar tree in a parking lot on the campus (34°32′42.2″S; 58°26′22.2″W). To our knowledge, only *Euprenolepis procera* ants, a species that belongs to the sub-family Formicinae, have been reported to be specialized in harvesting a broad spectrum of naturally growing mushrooms (Witte and Maschwitz, 2008).

The basidiomes (mushroom fructifications) on which the ants were foraging belonged to the species *Agrocybe cylindracea* (V. Brig.) Singer (Syn. = *A. aegerita* (V. Brig.) Singer); (Agaricales, Bolbitiaceae). The basidiomes were developing at three spots on the poplar bark (A, B, and C respectively in supplementary online material 1). *Agrocybe cylindracea* is a lignocellulolytic mushroom, which implies

Electronic supplementary material The online version of this article (doi:10.1007/s00040-012-0219-9) contains supplementary material, which is available to authorized users.

B. E. Lechner
Departamento de Biodiversidad y Biología Experimental,
Facultad de Ciencias Exactas y Naturales,
PROPLAME-PRHIDEB (CONICET),
Universidad de Buenos Aires, Buenos Aires, Argentina

R. Josens (✉)
Grupo de Estudio de Insectos Sociales, Facultad de Ciencias Exactas y Naturales, IFIBYNE-CONICET,
Universidad de Buenos Aires, Buenos Aires, Argentina
e-mail: roxy@bg.fcen.uba.ar

that it produces enzymes to hydrolyze lignin and cellulose and it grows on wood.

A photographic record was performed from the day of our discovery and for the following 10 days while there was foraging activity (camera Canon Eos Digital Rebel XTi with a Canon Compact-Macro lens EF 50 mm 1:2.5). The ants foraged on the mushrooms until they were completely removed. All cut fragments were brought into the nest and none were left on the trail or by its side. In all cases, the ants foraged not only on the caps but also on the stems (supplementary online material 1 and Fig. 1). Simultaneously, the colony also foraged on plant sources (supplementary online material 1). On the eighth day, a new spot of basidiomes of *A. cylindracea* appeared at approximately 1.10 cm from the ground (spot D). They were immediately visited by the ants, cut in small pieces and transported to the nest (supplementary online material 1). After a few days, nothing remained of these new mushrooms. We recorded foraging activity on all the basidiomes over 10 days until all traces of the fungi were transported to the nest (photos were taken daily between 12:30 p.m. and 6:30 p.m.).

Once the fungi were totally consumed, we conducted a simple assay to evaluate the acceptance of other varieties of mushrooms by the FF colony and by another one of the same species located 140 m away. We simultaneously offered one small piece of a mushroom (approximately 0.2 cm per side) and one piece of sugared oat (1 cm away from the mushroom) on an active trail (Saverschek et al., 2010). In each 30-minute trial we offered only one out of 15 different options of dry or fresh mushrooms. When an ant took one of the pieces offered, it was immediately replaced by another one. We recorded the number of oat and fungus pieces that were taken to the nest (accepted pieces) and the number of pieces that were removed from the trail per trial.

The FF colony picked up several pieces (min: 2, max: 35) of mushroom from 10 of the 15 different options offered. While *Pleurotus ostreatus*, *Lentinula edodes*, and *Agaricus bisporus* were offered both as fresh or dry, only the dry form was accepted; *Suillus granulatus* was accepted dry and fresh. *Agrocybe cylindracea*, *Panellus pusillus*, *Cantharellus* sp., *Pycnoporus sanguineus*, and *Hericium erinaceum* were only offered dry and were all accepted. The dry *S. granulatus* was the most accepted mushroom, which was picked up in similar quantity as the oat pieces (35 and 45, respectively, with a ratio of 0.78 between them). *Hericium erinaceum* was the second most accepted mushroom (with a ratio of 0.61); only this fungus was tested again 1 week later obtaining the same result. The FF colony removed from the trail few pieces of only two fungi offered. Conversely, the other colony evaluated in the same way picked up very few pieces of mushroom (min: 1 max: 3; maximum ratio mushroom/oat 0.09) from five different options and they cleared the trail of most of them.

At the end of the hot season, March to April 2011, new *Agrocybe* basidiomes appeared in the base of the same tree. Foraging on these new basidiomes was as active as on the first ones (supplementary online material 2). It is worth mentioning that 15 m away from this tree there was another tree with a nest of the same species of ant where a basidiome of *Agrocybe* had developed at the same time. This basidiome remained almost intact with very few ant bites on its surface until it dried.

We collected a small nest of *A. lundi* from the area and maintained it in laboratory conditions for a month with a supply of water, corn flakes, fruit, and fresh leaves. In April 2011, when the basidiomes appeared on the poplar trees, we took one of them and offered a piece to the laboratory nest. Ants were foraging on a piece of apple and fresh leaves at the moment we offered the *Agrocybe* basidiome. In a few minutes (10 min approx.) the ants were foraging actively on the basidiome and slowly stopped foraging on the apple, which remained there for several days. Each forager cut off a piece or a lamina of the mushroom and went directly to the fungus garden. The ants put the pieces inside it in the same way that others were doing with leaf fragments (supplementary online material 2).

Before offering the *Agrocybe* mushroom to the laboratory colony, we isolated a piece of their fungus garden. It only contained *L. gongylophorus.* Afterward, we inoculated cultures of this fungus and *A. cylindracea* in 90 mm Petri dishes using Nobles' (1948) medium, and incubated them in the dark at 25°C. After 16 days, *Agrocybe* showed a higher growth rate than *Leucoagaricus*, while the former grew at 0.56 cm per day, the latter only grew 0.1–0.3 cm per week (Fig. 2).

Discussion

We found a colony of the leaf-cutting ant *A. lundi* actively foraging on basidiomes of wild *A. cylindracea*. The foraging on wild fungi lasted for 10 days and probably more, since it had started before being discovered. Furthermore, 6 months later active foraging of new basidiomes was again recorded. Signs of this activity were also recorded 1 and 12 months after the first recording.

Many questions arise from these observations. Does the foraged fungus develop mycelium inside the nest? Do the ants feed the larvae with wild fungi basidiome or with its mycelium? Do the alien fungi contain nutrients that cannot be supplied by the resident garden fungus? At the moment these questions remain unanswered. In the laboratory colony, workers that cut *Agrocybe* basidiome fragments added them directly to the fungus garden near to the leaf fragments. In the next 5 days, no evidence of *Agrocybe* growth was detected. The growth rate of the mycelium of

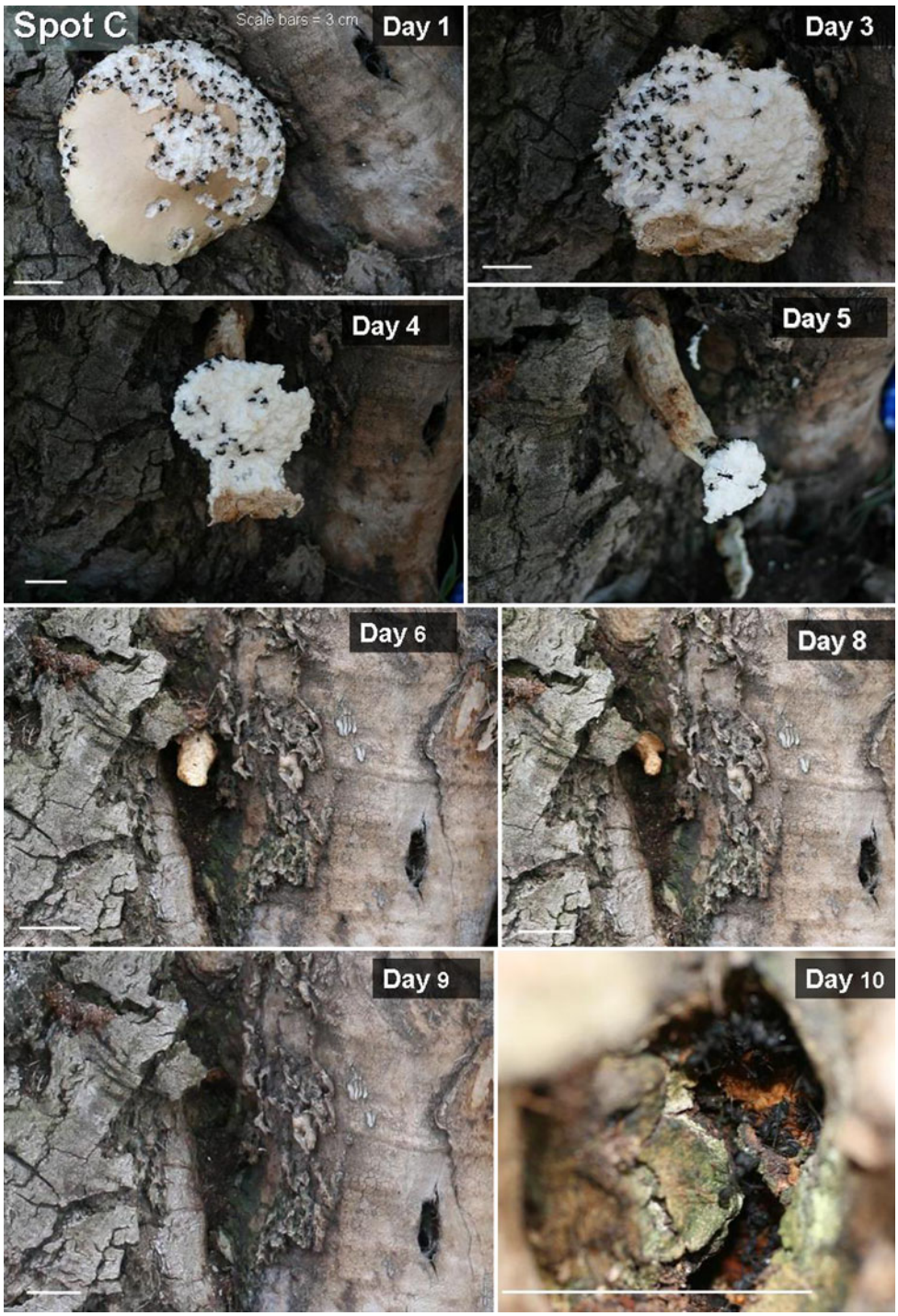

Fig. 1 Foraging activity on basidiome of *Agrocybe cylindracea* (designated as spot C), during several days. Horizontal white scale bar = 3 cm (colour figure online)

A. cylindracea on Nobles' medium was 10 times higher than that of *Leucoagaricus* (isolated from the same laboratory colony). The growth rate of *A. cylindracea* was similar to others reported for the same fungus (Uhart, 2007; Sarker et al., 2008). In spite of the fact that *Agrocybe* grows faster, the previous growth of *Leucoagaricus* mycelium on the lignocellulosic substrate of the ant garden might prevent *Agrocybe* mycelia from growing on this material.

Considering that workers can acquire a delayed rejection to vegetal material when it has detrimental effects on the fungus garden (Herz et al., 2008; Saverschek et al., 2010), we might suppose that the wild mushrooms had no detrimental effect on the fungus garden, because workers did not stop foraging on them. Five days after the incorporation of the *Agrocybe* to the fungus garden, it seemed to be in similar conditions as before.

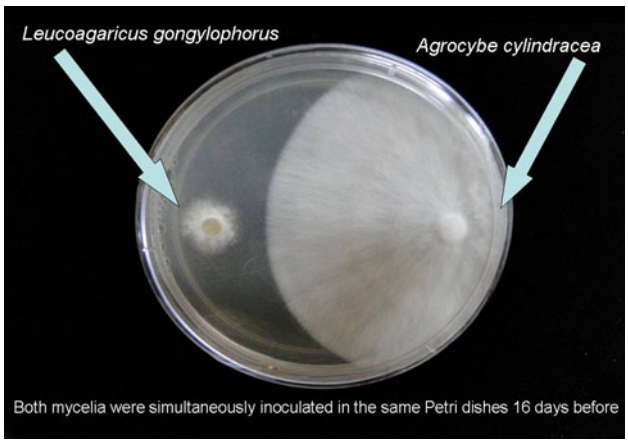

Fig. 2 *Agrocybe cylindracea* (*right side*) and *Leucoagaricus gongylophorus* (*left side*) mycelia were simultaneously inoculated on Nobles' medium

Foragers might consume part of the basidioma and/or feed larva with this material as a complement to their diet. The protein content of fresh mushrooms is about twice that of vegetables and contains all the essential amino acids. Mushrooms are especially rich in lysine and leucine (Chang, 1991), and many minerals and vitamins (Zhanxi and Zhanhua, 2001).

Even if there are few photographic records online of Attini ants foraging on wild mushroom (Holley, 2005; Wild, 2010), our observations revealed that an *A. lundi* colony foraged on mushroom (1) regularly for several days, (2) at the same time it foraged on plant material, and (3) every time mushrooms were available. This colony was prone to accept a wide spectrum of fungi, while other colonies in the same area rejected them. Another colony maintained for a month in laboratory conditions also accepted *Agrocybe* mushroom and incorporated the cut bits into the fungus garden in the same way and simultaneously with leaves. We hope that this report opens new questions about the evolution of this symbiotic system and its specificity, the fungus garden as a complex multi-organisms system and the feeding habits of leaf-cutting ants, among many others.

Acknowledgments The authors thank Prof. B. Hölldobler for his advice and suggestions. This work was supported by the National Agency for Scientific and Technological Promotion (PICT 01319) and the National Council for Scientific and Technological Research from Argentina.

References

Bass M. and Cherrett J.M. 1995. The role of leaf-cutting ant workers (Hymenoptera: Formicidae) in fungus garden maintenance. *Ecol. Entomol.* **19**: 215-220

Chang S.T. 1991. Cultivated mushrooms. In: *Handbook of Applied Mycology III* (Arora D.K., Mukerji K.G. and Elmer H.M., Eds), Marcel Dekker inc. New York. pp 221-240

Herz H., Hölldobler B. and Roces F. 2008. Delayed rejection in a leaf-cutting ant after foraging on plants unsuitable for the symbiotic fungus. *Behav. Ecol.* **19**: 575-582

Holley J.A. 2005. Notes from Underground: Page 25. http://www.notesfromunderground.org/alwaysneeded/photos/holley/holleypictures.html

Kermarrec A., Febvay G. and Decharme M. 1986. Protection of leaf cutting ants from biohazards: Is there a future for microbiological control? In: *Fire Ants and Leaf-cutting Ants: Biology and Management* (Lofgren S. and Vander Meer R.K., Eds). Westview Press, Boulder and London. pp 339-355

Martin M.M. and Weber N.A. 1969. The cellulose-utilizing capability of the fungus cultured by the Attine ant *Atta colombica tonsipes*. *Ann. Entomol. Soc. Am.* **62**: 1386-1387

Mehdiabadi N.J. and Schultz T.R. 2010. Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myrmecol. News* **13**: 37-55

Nobles M.K. 1948. Studies in forest pathology VI. Identification of cultures of wood rotting fungi. *Can. J. Res.* **26**: 281-431

Quinlan R.J. and Cherrett J.M. 1979. The role of the fungus in the diet of the leaf-cutting ant *Atta cephalotes* (L.). *Ecol. Entomol.* **4**: 151-160

Sarker N.C., Shaheen M., Amin S.M.R. and Khan A.S. 2008. Domestication and cultivation of *Agrocybe aegerita* on straw and sawdust substrate. *Bangladesh J. Mushroom* **2**: 73-79

Saverschek N., Herz H., Wagner M. and Roces F. 2010. Avoiding plants unsuitable for the symbiotic fungus: learning and long-term memory in leaf-cutting ants. *Anim. Behav.* **79**: 689-698

Uhart M. 2007. *Diversidad de especies del género Agrocybe (Basidiomycetes, Agaricales) en la República Argentina. Estudios de compatibilidad sexual y cultivo intensivo de Agrocybe cylindrica de Europa, América y Asia*. Universidad de Buenos Aires, Buenos Aires. Thesis

Weber N.A. 1972. *Gardening Ants, the Attines.* American Philosophical Society, Philadelphia

Wild A. 2010. Myrmecos: http://myrmecos.net/2010/08/05/do-attine-ants-forage-for-fungi

Witte V. and Maschwitz U. 2008. Mushroom harvesting ants in the tropical rain forest. *Naturwissenschaften* **95**: 1049-1054

Zhanxi L. and Zhanhua L. 2001. *Juncao Technology.* China Agricultural Scientech press, Beijing