



Leaf-Cutting Ants (Formicidae, Attini) Prune Their Fungus to Increase and Direct Its Productivity

Author(s): M. Bass and J. M. Cherrett

Source: *Functional Ecology*, Vol. 10, No. 1, (Feb., 1996), pp. 55-61

Published by: British Ecological Society

Stable URL: <http://www.jstor.org/stable/2390262>

Accessed: 09/08/2008 11:47

---

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/action/showPublisher?publisherCode=briteco>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

---

JSTOR is a not-for-profit organization founded in 1995 to build trusted digital archives for scholarship. We work with the scholarly community to preserve their work and the materials they rely upon, and to build a common research platform that promotes the discovery and use of these resources. For more information about JSTOR, please contact [support@jstor.org](mailto:support@jstor.org).

# Leaf-cutting ants (Formicidae, Attini) prune their fungus to increase and direct its productivity

M. BASS and J. M. CHERRETT\*

Trinity College, Carmarthen, Dyfed, South Wales SA31 3EP and \*School of Biological Sciences, University of Wales, Bangor LL57 2UW, UK

## Summary

1. The mutualistic fungus of leaf-cutting ants produces ant rewards in the form of nutritive bundles of hyphae called staphylae. We studied how worker activities affect staphyla production by the fungus garden of *Atta sexdens*.
2. Ant-free fungus garden that was exposed to workers for 3 h produced 1.3 times more staphylae 2 days later than garden that remained ant-free.
3. Simulating the possible mechanical and chemical reasons for this effect showed that damaging hyphae with a mounted needle increased the subsequent standing crop of staphylae after 3 days by 1.2 times. Removing staphylae had no effect on subsequent standing crops, but total crops (which included the staphylae previously removed) were greater than on control garden. Crude head extracts and worker faeces had no visible effect on staphyla production.
4. Surfaces of the fungus garden that were easily accessible to workers produced significantly more staphylae than inaccessible surfaces.
5. The pruning activity of the ants and the response of the fungus can be interpreted both as an evolved behavioural adaptation by the ants to maximize the production of the staphylae they eat, and as a physiological adaptation by the fungus to produce nutritive staphylae for the ants only in those areas where and when the ants are active.

*Key-words:* *Atta sexdens*, fungus garden, pruning, staphylae

*Functional Ecology* (1996) **10**, 55–61

## Introduction

Pruning is a common horticultural strategy for diverting resource allocation in plants from general growth into the production of useful parts such as fruits. Similarly, the myrmecophytic plant *Cecropia* controls the production of its ant rewards, Müllerian bodies, in response to the presence or absence of its symbiotic ants, which harvest these bodies (Folgarait, Johnson & Davidson 1994). The myrmecophyte *Piper cenocladum* produces its ant rewards only in the presence of its guest ants (Risch & Rickson 1981). Pruning can also affect fungi; fungal hyphae respire, branch and grow at a faster rate when lightly grazed by Collembola (Van der Drift & Jansen 1977; Hanlon 1981).

Leaf-cutting ants of the Tribe Attini, which are confined to the New World, cultivate fungi on freshly harvested vegetable material. These fungi do not usually produce reproductive structures and were therefore placed in the Mycelia Sterilia by Kriesel (1972). Powell (1984) has since shown that the fungi are Basidiomycetes. These fungi produce swollen hyphae (gongylidia), bunches of which are known as staphylae (Fig. 1). These provide food for the ants and their larvae (Moeller 1893; Weber 1972).

Staphylae appear to have no other purpose and are derived from vegetative hyphae, although their evolutionary origin is obscure (Cherrett, Powell & Stradling 1989).

In fungi, branches arise at a distance behind the hyphal apex, suggesting some apical dominance (Deacon 1980). Trinci & Collinge (1974) found that when hyphae of *Neurospora crassa* were damaged so that their apices were lost, the hyphae regenerated by forming branches. We hypothesized that trimming or pruning the ant fungus might also stimulate branching of hyphae, which in turn might produce more staphylae. A possible pruning mechanism would be provided by the large numbers of workers that lick the fungus garden surface continually (Quinlan & Cherrett 1979). These workers break off and ingest fragments of hyphae as they lick the fungus (Bass 1993). In this paper, we investigate the possibility that workers licking the fungus garden are actually pruning the mycelium to encourage the production of more staphylae.

## Materials and methods

Laboratory colonies of *Atta sexdens* (L.) were maintained at 27 °C and 80% relative humidity. Fresh

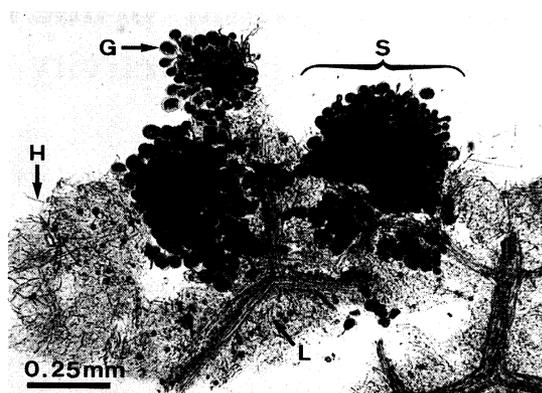


Fig. 1. Staphylae on the surface of the fungus garden cultivated by *Atta sexdens*. S, staphyla; G, gongylidium; H, hyphae; L, substrate leaf fragment.

leaves were supplied daily and fungus gardens were built in clear plastic domes.

#### METHOD USED TO COUNT STAPHYLA NUMBERS IN PETRI DISHES OF FUNGUS GARDEN

The numbers of staphylae present on the garden surface in Petri dishes were assessed by drawing grids on the dish lids and counting staphylae within them, using a binocular microscope. The grid ensured that different areas of the Petri dish were not missed or counted twice. When more than one dish was being assessed, the dishes were mixed up and their labels covered to prevent unconscious bias.

#### ASSESSING THE EFFECT OF WORKER ACCESS ON STAPHYLA PRODUCTION

Twenty Petri dishes (5 cm diameter) were filled with ant-free fungus garden, obtained by anaesthetizing the workers present with carbon dioxide gas, then removing them with forceps. The numbers of staphylae present per dish were recorded. Replicates were maintained in humid chambers for 2 days. Staphyla numbers per dish were then recounted and the lids of 10 randomly selected dishes were replaced by lids with 0.5 cm entrance holes. These were exposed to the parent nest for 3 h and workers allowed to enter them. The dishes were then removed and the workers present anaesthetized with carbon dioxide and removed with forceps. The original lids were replaced and staphyla numbers per dish were recounted immediately, and also 2 and 4 days after treatment. By adding the numbers of staphylae lost during the exposure period, total crops could be calculated for each dish. The 10 control dishes to which ants were not given access were otherwise treated similarly, although they were not exposed to carbon dioxide. The results of an experiment using fungus garden from an *Atta*

*cephalotes* nest suggested that the carbon dioxide used to anaesthetize and remove workers did not affect subsequent staphyla production (M. Bass, unpublished data).

#### ASSESSING DIFFERENCES BETWEEN STAPHYLAE ON GARDEN EXPOSED OR NOT EXPOSED TO WORKERS

Staphylae from several sources were compared, namely non-isolated mature garden, garden isolated from workers for 7 days, garden isolated from workers for 4 days with a period of 3 h exposure to workers after 2 days, and garden similarly isolated but not exposed to workers.

Staphylae were mounted in cotton-blue in lactophenol for microscopic examination. Diameters were measured using an eyepiece graticule mounted in a microscope. Gongylidium numbers per staphyla were assessed by counting the numbers visible in the outer circumference of each staphyla; counting total numbers of gongylidia was unpractical. The ratio of diameter to gongylidium numbers was used as a measure of compactness. Staphyla biomasses were not compared because collecting staphylae from the garden surface is a slow process and many staphylae may be lost or missed. About 30 staphylae were picked at random and examined for each garden type.

#### SIMULATING THE EFFECTS OF ANT ACCESS TO FUNGUS GARDENS ON STAPHYLA YIELD

Two main factors could affect staphyla production; pruning (the mechanical removal of staphylae or of hyphae) and chemical depositions by the ants. Several glands are associated with the ant mouthparts and these might produce growth-promoting factors, although there is no evidence in the literature for any such action. In contrast, the deposition of worker faeces may be vital for the establishment and growth of the mycelium on newly-added leaf fragments, supplying the hyphae with nitrogenous material (Martin & Martin 1970). We attempted to simulate both the physical and chemical factors which might stimulate staphyla production. Fifteen Petri dishes (5 cm) were filled with ant-free garden and divided into quarters by placing two 1 mm wide plastic strips onto the garden surface at 90° to each other. Each quarter (4.9 cm<sup>2</sup>) acted as a single treatment area. There were therefore 60 treatment areas in total. After 2 days isolation, staphyla numbers were counted in each treatment area and the following five treatments were applied, distributed at random. Twelve replicates were used for each treatment, so that the 60 available treatment areas were all used.

1. Staphylae were removed using a mounted needle.
2. Hyphae were broken off by gently stroking the garden surface with the side of a mounted needle (this also removed some staphylae).

3. Faecal droplets were applied (obtained by squeezing the gasters of living workers, of head-widths 1.2–1.6 mm) at rates of five droplets per cm<sup>2</sup>.
4. Worker heads (head-widths 1.4–1.6 mm) were crushed in 0.1 ml distilled water. This suspension was applied with a fine syringe, at the rate of one head per cm<sup>2</sup>.
5. No treatment; control.

In treatments 1 and 2, where staphylae were lost during treatment, staphyla numbers were recounted immediately after treatment. Numbers in all treatment areas were then recounted after a further 2 days.

The effect of worker head extracts was not further investigated. However, because of the stimulatory effect of faecal material on hyphal growth on newly added substrate (Martin & Martin 1970), the possible effect of faeces on staphyla production was investigated more fully. When a worker is anaesthetized with carbon dioxide and held carefully by the thorax with forceps, gently squeezing the gaster with flexible forceps expels the rectal contents without injury. Workers treated in this way cannot immediately produce more rectal fluid and at least 6 h are required for the rectum to refill (Bass 1993). Ten groups of 50 workers (head-widths 1.0–1.6 mm) with emptied (evacuated) recta, were introduced into Petri dishes of ant-free garden. These dishes had been maintained under humid conditions for 2 days. Groups of 50 intact, anaesthetized workers of similar size were placed in another 10 dishes, while a further 10 control dishes were left ant-free. After 4 h the workers were removed from the Petri dishes. Numbers of staphylae present in each dish were recorded before, immediately after and 2 days after treatment.

#### ASSESSING NUMBERS OF STAPHYLAE PRESENT ON ACCESSIBLE AND 'HIDDEN' SURFACES OF THE FUNGUS GARDEN

Workers are denied access to fungus growing between substrate particles of the fungus garden and this fungus remains unpruned (personal observations). The numbers of staphylae produced by such 'hidden' surfaces were examined and compared with staphyla production on the surface of the garden. Staphylae present on the outer surface of the garden were counted on 0.25 cm<sup>2</sup> areas (this was the largest area of homogeneous fungus garden that could be examined in the honeycomb structure of the garden). 'Hidden' surfaces were then obtained by peeling away surface substrate fragments with a scalpel. The numbers of staphylae present on 0.25 cm<sup>2</sup> areas of this 'hidden' fungus garden were then counted.

#### STUDIES ON A DECLINING NEST

Observations were made on a queenless *Atta sexdens* nest to see how the breakdown of social organization

affected staphyla production. This nest was 11 years old with 22 gardens, and it declined and died over 11 months after losing its queen. After 2 months of decline, staphyla numbers were counted by dissecting randomly chosen 0.1 g samples of fungus garden. This was the amount of fungus garden that could be assessed in a 15 min period. Numbers of workers present on the garden surface were assessed on randomly chosen 16 cm<sup>2</sup> areas, using a binocular microscope and a cold, fibre-optic light source.

#### STATISTICAL ANALYSIS

Normally distributed data with similar variances were subjected to analysis of variance (ANOVA). Significant effects were subjected to Tukey's multiple comparison to distinguish significantly different treatments. Data that failed to meet the conditions for ANOVA were analysed using non-parametric methods.

### Results

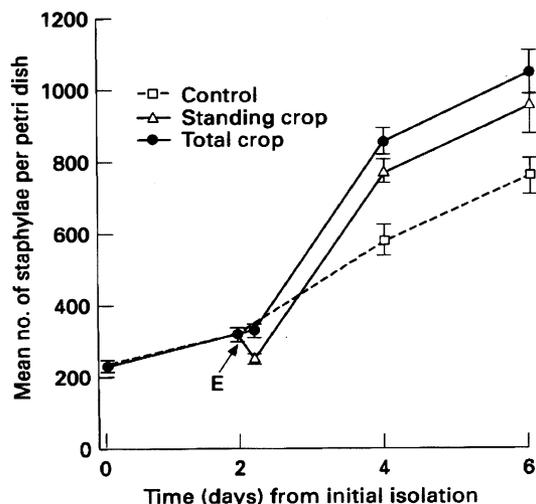
#### THE EFFECT OF WORKER ACCESS ON STAPHYLAE PRODUCTION

There were no significant differences between the numbers of staphylae present in test and ant-free replicates before treatment ( $P > 0.7$ , ANOVA), but exposure to workers for 3 h significantly reduced the numbers of staphylae present ( $P < 0.01$ , ANOVA). Two days after treatment, standing crops were significantly higher in test dishes ( $P < 0.01$ , ANOVA), despite the number previously eaten by the workers. This difference was no longer significant 4 days after treatment, although total crop (which included the staphylae previously harvested during treatment) was still significantly higher on exposed replicates than on the controls ( $P = 0.05$ , ANOVA with Tukey's multiple comparison). Total crop can be considered as a valid measure because although staphylae on ant-free garden have a median persistence of 2 days, they can persist for up to 8 days (Bass 1993).

Exposing fungus garden to workers therefore led to a subsequent increase in the number of staphylae present, and 2 days after treatment there was a 1.3-fold increase in the standing crop of staphylae compared to the controls (Fig. 2).

#### DIFFERENCES BETWEEN STAPHYLAE ON GARDEN EXPOSED OR NOT EXPOSED TO WORKERS

Staphyla diameters were larger on garden isolated from workers for 7 days than on garden isolated for 4 days (with or without 3 h ant access) or on non-isolated mature garden (Table 1). The numbers of gongylidia in the circumference of each staphyla showed similar relationships (Table 1). Variances for both



**Fig. 2.** The effects of *Atta sexdens* workers on the production of staphyiae by their fungus. Petri dishes (5 cm diameter) containing ant-free fungus garden were maintained for 2 days. Test replicates were then exposed to workers for 3 h (E). Mean numbers of staphyiae per dish ( $\pm$ SE) are shown for exposed test and non-exposed control dishes ( $n=10$ ). Numbers of staphyiae are expressed as standing crops and as total crops (which include staphyiae lost during the exposure period).

measures used were greater after 7 days, indicating that there was now a wide range of staphyia sizes present, including small young ones and old staphyiae, which had grown beyond the size at which the ants normally harvest them.

Exposing garden isolated for 4 days to workers for 3 h had no significant effect on the staphyiae present, in terms of their diameters or mean numbers of gongylidia per circumference.

#### SIMULATING THE EFFECTS OF ANT ACCESS TO GARDENS ON STAPHYLA YIELD

Some replicates became contaminated by alien fungi but at least 10 of each treatment remained. There were no significant differences in staphyia numbers before treatment ( $P>0.8$ , ANOVA) and each area started with a mean of 36.8 ( $SE\pm 3.9$ ) staphyiae that increased to

134.1 ( $SE\pm 85.8$ ) staphyiae after 2 days. Treatment 1 (removing staphyiae) significantly reduced their numbers ( $P<0.001$ , Mann-Whitney  $U$ -test) to 7.0 ( $SE\pm 0.9$ ). Treatment 2 (breaking hyphae) also significantly reduced the numbers of staphyiae present ( $P<0.001$ , Mann-Whitney  $U$ -test), to 26.6 ( $SE\pm 1.7$ ), although this was not its object. It proved difficult however to break off the hyphae without staphyiae also becoming detached.

Three days after treatment, there were significant differences between standing crops and between total crops [ $P=0.05$ , Dunn's non-parametric multiple comparison (as described by Zar 1984), Table 2]. Breaking hyphae had the greatest effect for both standing and total crops. No other treatment had any significant effect on standing crops. However, removing staphyiae did result in a larger total crop than the control.

The standing crops 3 days after breaking hyphae showed a 1.2-fold increase over the control yield. Mechanically pruning hyphae may cause more damage than ant pruning and could initially retard rather than stimulate growth. The 1.2-fold increase shown after mechanical pruning may therefore account for the whole of the effect shown when workers are given access to fungus (a 1.3-fold increase in standing crop).

#### THE EFFECT OF WORKER FAECES ON STAPHYLA PRODUCTION

There were no significant differences between staphyia numbers in the three sets of replicates before workers were introduced ( $P>0.2$ , ANOVA). Exposure to both intact and evacuated workers for 4 h caused a significant decrease in staphyia numbers per dish, although there was a significantly greater reduction with intact ants than with evacuated ones ( $P=0.05$ , ANOVA with Tukey's multiple comparison). This may have reflected trauma and possible injury in the latter. Two days after treatment, mean standing crops were significantly higher in replicates which had been exposed to workers, compared to controls. Mean standing crops did not differ between replicates exposed to intact and evacuated workers ( $P=0.05$ ,

**Table 1.** Mean diameters and numbers of gongylidia present in the other circumference of staphyiae removed from four samples of differently treated fungus garden from an *Atta sexdens* nest

Garden status	Mean staphyia diameter (mm) $\pm$ SE	Mean nos of gongylidia per circumference $\pm$ SE
Mature garden (not isolated)	0.45 $\pm$ 0.01 <sup>b</sup>	37.7 $\pm$ 1.1 <sup>b</sup>
Garden isolated for 4 days	0.44 $\pm$ 0.02 <sup>bc</sup>	28.5 $\pm$ 1.7 <sup>c</sup>
Garden isolated for 4 days, with 3 h ant access after 2 days	0.39 $\pm$ 0.02 <sup>c</sup>	33.2 $\pm$ 1.8 <sup>bc</sup>
Garden isolated for 7 days	0.79 $\pm$ 0.04 <sup>a</sup>	61.9 $\pm$ 2.8 <sup>a</sup>

Means bearing the same letter, when compared down each column, were not significantly different ( $P>0.05$ , Dunn's non-parametric multiple comparison,  $n=30-35$ ).

**Table 2.** Mean standing and total crops of staphylae per 4.9 cm<sup>2</sup> of *Atta sexdens* fungus garden, 3 days after the application of five treatments. Total crops included those staphylae which were removed or lost during treatment. Consequently, in treatments 3 to 5, standing and total crops are the same because no staphylae were lost as a result of treatment

Treatment	Mean standing crop ± SE	Mean total crop ± SE
1. Removal of staphylae	172.0 ± 5.9 <sup>ab</sup>	212.3 ± 6.3 <sup>ab</sup>
2. Hyphae cut	201.4 ± 8.6 <sup>a</sup>	222.8 ± 8.5 <sup>a</sup>
3. Faecal droplets, five per cm <sup>2</sup>	179.4 ± 4.2 <sup>ab</sup>	179.4 ± 4.2 <sup>bc</sup>
4. Head extract, one head per cm <sup>2</sup>	163.3 ± 7.0 <sup>b</sup>	163.0 ± 7.0 <sup>c</sup>
5. No treatment; control	169.0 ± 6.3 <sup>b</sup>	169.0 ± 6.3 <sup>c</sup>

Means bearing the same letter, when compared down the five treatments, were not significantly different ( $P > 0.05$ , Dunn's non-parametric comparison,  $n = 10-12$ ).

ANOVA with Tukey's multiple comparison). Temporarily re-exposing garden to workers therefore led to a 1.3-fold increase over controls in the standing crop of staphylae subsequently produced, irrespective of whether or not the ants could defecate on the fungus (Fig. 3).

#### NUMBERS OF STAPHYLAE PRESENT ON ACCESSIBLE AND 'HIDDEN' SURFACES OF THE FUNGUS GARDEN

Outer surfaces of the fungus garden, which were available to workers, had a mean of 9.9 staphylae per 0.25 cm<sup>2</sup> (SE ± 0.7), while inaccessible 'hidden' surfaces had only 2.7 staphylae per 0.25 cm<sup>2</sup> (SE ± 0.4). This difference was significant ( $P < 0.001$ , Mann-Whitney *U*-test,  $n = 25$ ).

#### STUDIES ON A DECLINING NEST

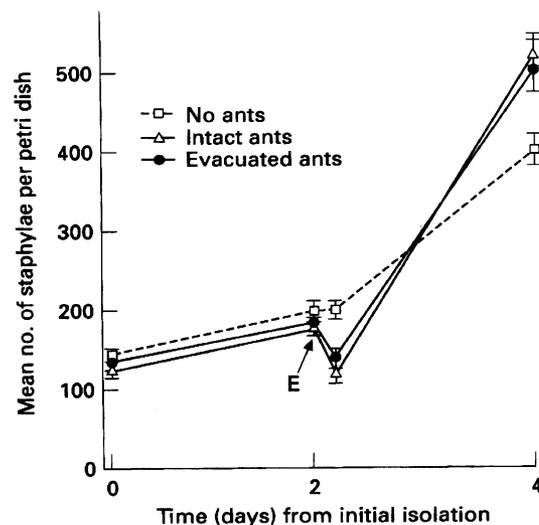
Some areas of fungus garden in the declining nest grew 'out of control', developing white rounded growths which sometimes reached diameters of several cm. Microscopic examination showed that these growths consisted of tightly packed hyphae with a few gongylidia. Fragments of these growths were attractive to workers from healthy nests, confirming that they were ant fungus.

The remainder of the fungus garden was paler than normal and had many staphylae. After 2 months decline, there were significantly fewer staphylae per 0.1 g of garden in areas with white growths (84.8 staphylae, SE ± 9.6) than in remaining areas (203.2 staphylae, SE ± 6.6;  $P < 0.001$ , Mann-Whitney *U*-test,  $n = 10$ ). In contrast, samples of garden from a healthy nest had 74.6 (SE ± 2.5) staphylae. Staphylae probably tended to build up on declining garden because there were no larvae to eat them and possibly because continued ant pruning would stimulate their production.

Significantly more workers were present on areas without white growths (46.2 per 16 cm<sup>2</sup>, SE ± 4.2) than on areas with them (10.2 per 16 cm<sup>2</sup>, SE ± 1.6;  $P < 0.001$ , Mann-Whitney *U*-test).

## Discussion

The presence of workers on the fungus garden appeared to stimulate the production of staphylae. This might have been owing to faecal deposition, because worker faeces contain nitrogenous materials (Boyd & Martin 1975), which might stimulate fungal growth. However, using workers unable to defecate showed that this was not so (Fig. 3). Workers frequently defecate onto substrate they have just added to the garden (Quinlan & Cherrett 1977). The ant fungus lacks the enzymes necessary to degrade substrate proteins efficiently and the application of faecal material compensates for this metabolic deficiency. The faeces contain ammonia and a mixture of amino acids (Boyd & Martin 1975) which supply the short-term needs of newly planted hyphae. In



**Fig. 3.** The effect of faecal material on the production of staphylae by the fungus garden of *Atta sexdens*. Petri dishes (5 cm diameter) containing ant-free fungus garden were maintained for 2 days. Test replicates were then exposed for 4 h to groups of 50 intact workers or 50 workers with emptied (evacuated) recta (E). Mean numbers of staphylae per dish (± SE) are shown for exposed test and non-exposed control dishes ( $n = 10$ ).

this study however, adding faecal fluid had little effect on the total numbers of staphylae produced on mature garden, which is at least 5 days old (Bass 1993).

Several glands are associated with ant mouthparts and these might produce growth-promoting substances. Leaf-cutting ant labial glands produce chitinase (Febvay, Decharme & Kermarrec 1984), while their mandibular glands produce alarm substances such as 4-methyl-3-heptanone (Riley, Silverstein & Moser 1974). In many ant species, the postpharyngeal gland is the source of larval food and in *Atta sexdens* this gland is disproportionately large in the smallest workers. This suggests that these workers either feed larvae by regurgitation or produce something that they apply to fungus garden (Hölldobler & Wilson 1990). There is no evidence in the literature for any growth-promoting substances produced in Attine head glands and in this study, crude aqueous head extracts had no effect on the production of staphylae. However, many of the glandular products may have been volatile or insoluble in water. Further work is needed to evaluate the chemical effects of the products of individual glands on the ant fungus. Such glands are not confined to the head. For example, the metathoracic glands of leaf-cutting ants produce antiseptic substances like phenylacetic acid (Maschwitz, Koob & Schildknecht 1970; Maschwitz 1974).

Simulated pruning of hyphae with a mounted needle significantly increased the number of staphylae relative to the controls. This suggests that workers stimulate staphyla production mechanically rather than chemically. However, it must be stressed that chemical aspects of worker exposure to garden may still have important roles, such as protecting the garden against contamination by alien fungi.

Removal of staphylae had a significant effect on total crops, while breaking hyphae affected both total and standing crops. Damaging hyphae therefore had a greater effect. This was probably because removing staphylae affected only small areas of the fungus garden surface, while breaking hyphae affected the whole surface, thus having a greater impact on subsequent staphyla numbers.

The out-of-control areas of declining garden, with their large white growths, coinciding with low worker populations and small numbers of staphylae, suggested that a lack of hyphal pruning by workers allowed the mycelium to 'escape' worker control and that staphyla production was not being stimulated. The second type of area, with numerous workers, many staphylae and no white growths, suggested that continuous pruning by workers stimulated staphyla production and prevented the mycelium from proliferating. However, such an effect may also occur if the fungus garden senesces asynchronously and stops producing staphylae independently of ant presence. This alternative explana-

tion seems unlikely because of the vigorous growth of the fungus in staphyla-free regions. In addition, even workers in declining nests do not tolerate dying or decaying fungus garden, and remove it (personal observations).

If workers prune the mycelium to encourage staphyla production, there remains the problem of how or whether they recognise incipient staphylae and allow them to develop from the mycelium. There may be some stimulus that makes a worker avoid licking a tuft of hyphae that will, given time, develop into a staphyla. Or, pruning an incipient staphyla may give rise to more staphylae. Little is known about this problem. However, staphylae may simply develop rapidly and a turnover of staphylae in the fungus garden every 9–12 h has been reported (Quinlan & Cherrett 1979).

Larger staphylae were produced when the ants were absent, suggesting that they are usually harvested before they reach their maximum size. Productivity per day per unit area may be higher if staphylae are harvested at a small size. Alternatively, small newly-produced staphylae may be more attractive to workers than large older staphylae.

Staphylae do arise without any pruning in artificial culture on agar media and their presence is the most important identifying feature of the ant fungus. This production of staphylae by fungus that has never been exposed to workers may illustrate the high degree of coevolution that has occurred between the ants and their fungus. Most myrmecophyte plant genera also produce food bodies in the absence of their symbiotic ants (Hölldobler & Wilson 1990). The experiments described in this study were carried out on naturally produced fungus garden, to more closely reflect the true relationship between the ants and their fungus. However, it would also be useful to test the effect of hyphal pruning on ant fungus cultures grown on agar.

For the ants, encouraging the production of more staphylae for food is beneficial. The fungus however, must avoid being totally consumed while still providing enough rewards for the ants to continue tending it. The sole function of the staphylae appears to be as food for the ants (Cherrett *et al.* 1989). It may therefore be advantageous for the fungus to produce as few staphylae as possible, while putting most of its resources into mycelial growth. Some of the mycelium is eaten by the ants (Bass & Cherrett 1995). However, most hyphae are safe from being eaten because they are too intimately mixed up with the inedible fragments of vegetation. Hyphae become available to the ants only when they grow out of the substrate (presumably to cross to nearby substrate fragments) and when they swell to produce staphylae. In the fungus garden, there are many places between substrate fragments where ant access is denied and few staphylae are produced on these 'hidden' surfaces.

The response of the fungus to pruning therefore seems to be a mechanism that allows the scarce

resources put into the production of staphylae to be made available only where and when harvesting by the ants is likely. This is unlike the response of fruit trees to pruning, which switches resources from vegetative growth to reproduction.

### Acknowledgements

We thank Ann Pennell for the daily care of the ant colonies. Funding was provided by a Llewellyn and Mary Williams Scholarship from the University of Wales, Bangor.

### References

- Bass, M. (1993) *Studies on the ant-fungus mutualism in leaf-cutting ants (Formicidae, Attini)*. PhD thesis, University of Wales, UK.
- Bass, M. & Cherrett, J.M. (1995) Fungal hyphae as a source of nutrients for the leaf-cutting ant *Atta sexdens*. *Physiological Entomology* **20**, 1–6.
- Boyd, N.D. & Martin, M.M. (1975) Faecal proteinases of the fungus-growing ant *Atta texana*: their fungal origin and ecological significance. *Journal of Insect Physiology* **21**, 1815–1820.
- Cherrett, J.M., Powell, R. & Stradling, D.J. (1989) The mutualism between leaf-cutting ants and their fungus. *Insect-Fungus Interactions*, pp. 93–120. Royal Entomological Society of London 14th Symposium, 1987, Academic Press, London.
- Deacon, J.W. (1980) *Introduction to Modern Mycology*. Blackwell Scientific Publications, Oxford.
- Febvay, G., Decharme, M. & Kermarrec, A. (1984) Digestion of chitin by the labial glands of *Acromyrmex octospinosus* Reich (Hymenoptera: Formicidae). *Canadian Journal of Zoology* **62**, 229–234.
- Folgarait, P.J., Johnson, H.L. & Davidson, D.W. (1994) Responses of *Cecropia* to experimental removal of Müllerian bodies. *Functional Ecology* **8**, 22–28.
- Hanlon, R.D. (1981) Influence of grazing by Collembola on the activity of senescent fungal colonies grown on media of different nutrient concentration. *Oikos* **36**, 362–367.
- Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Springer-Verlag, Berlin.
- Kriesel, H. (1972) Pilze aus Pilzgarten von *Atta insularis* in Kuba. *Zeitschrift für Allgemeine Mikrobiologie* **12**, 643–654.
- Martin, M.M. & Martin, J.S. (1970) The biochemical basis for the symbiosis between the ant *Atta colombica tonsipes* and its food fungus. *Journal of Insect Physiology* **16**, 109–119.
- Maschwitz, U. (1974) Vergleichende Untersuchungen zur Funktion der Ameisen metathorakaldrüse. *Oecologia* **16**, 303–310.
- Maschwitz, U., Koob, K. & Schildknecht, H. (1970) Ein Beitrag zur Funktion der Metathorakaldrüse der Ameisen. *Journal of Insect Physiology* **16**, 387–404.
- Moeller, A. (1893) Die Pilzgarten einiger sudamerikanischer Ameisen. *Botanische Mitteilungen aus den Tropen* **6**, 1–127.
- Powell, R. (1984) *The influence of substrate quality on fungus cultivation by some Attine ants*. PhD thesis, University of Exeter, UK.
- Quinlan, R.J. & Cherrett, J.M. (1977) The role of substrate preparation in the symbiosis between the leafcutting ant *Acromyrmex octospinosus* (Reich) and its food fungus. *Ecological Entomology* **2**, 161–170.
- Quinlan, R.J. & Cherrett, J.M. (1979) The role of fungus in the diet of the leaf-cutting ant *Atta cephalotes* (L.). *Ecological Entomology* **4**, 151–160.
- Riley, R.G., Silverstein, R.M. & Moser, J.C. (1974) Isolation, identification, synthesis and biological activity of volatile compounds from the heads of *Atta* ants. *Journal of Insect Physiology* **20**, 1629–1637.
- Risch, S.J. & Rickson, F.R. (1981) Mutualism in which ants must be present before plants produce food bodies. *Nature* **291**, 149–150.
- Trinci, A.P.J. & Collinge, A.J. (1974) Occlusion of the septal pores of damaged hyphae of *Neurospora crassa* by hexagonal crystals. *Protoplasma* **80**, 57–67.
- Van der Drift, J. & Jansen, E. (1977) The grazing of spring-tails on hyphal mats and its influence on fungal growth and respiration. *Soil Organisms as Components of Ecosystems* (eds V. Lohm & T. Persson). *Ecological Bulletin, Stockholm* **25**, 302–309.
- Weber, N.A. (1972) *Gardening Ants, the Attines*. American Philosophical Society, Philadelphia.
- Zar, J.H. (1984) *Biostatistical Analysis*, 2nd edn. Prentice Hall International Editions, New Jersey.

Received 21 June 1994; revised 26 May 1995; accepted 2 June 1995