THE ROYAL SOCIETY

doi 10.1098/rspb.2001.1605

Weeding and grooming of pathogens in agriculture by ants

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The ancient mutualism between fungus-growing ants and the fungi they cultivate for food is a textbook example of symbiosis. Fungus-growing ants' ability to cultivate fungi depends on protection of the garden from the aggressive microbes associated with the substrate added to the garden as well as from the specialized virulent garden parasite *Escovopsis*. We examined ants' ability to remove alien microbes physically by infecting *Atta colombica* gardens with the generalist pathogen *Trichoderma viride* and the specialist pathogen *Escovopsis*. The ants sanitized the garden using two main behaviours: grooming of alien spores from the garden (fungus grooming) and removal of infected garden substrate (weeding). Unlike previously described hygienic behaviours (e.g. licking and self-grooming), fungus-grooming and garden-removal behaviours are specific responses to the presence of fungal pathogens. In the presence of pathogens, they are the primary activities performed by workers, but they are uncommon in uninfected gardens. In fact, workers rapidly eliminate *Trichoderma* from their gardens by fungus grooming and weeding, suggesting that these behaviours are the primary method of garden defence against generalist pathogens. The same sanitary behaviours were performed in response to the presence of the specialist pathogen *Escovopsis*. However, the intensity and duration of these behaviours were much greater in this treatment. Despite the increased effort, the ants were unable to eliminate *Escovopsis* from their gardens, suggesting that this specialized pathogen has evolved counter-adaptations in order to overcome the sanitary defences of the ants.

Keywords: behaviour; *Escovopsis*; fungus-growing ants; mutualism; pathogens; symbiosis

1. INTRODUCTION

Fungus-growing ants' (Attini) ability to cultivate fungus for food is relatively unique, occurring in only a few other insects. The attine fungus, which belongs mostly to the family Lepiotaceae (Chapela *et al.* 1994; Mueller *et al.* 1998), serves as the main food source of the ants, while the ants provide the fungus with substrate on which to grow and a means of dispersal to new colonies by foundress queens. The success of this ancient mutualism depends on the ants' ability to protect their fungal cultivars from being overgrown by the competitively superior microbes associated with the vegetative material that the workers continuously add to the garden. In addition, the gardens of fungus-growing ants are host to a specialized and virulent fungal parasite in the genus *Escovopsis* (Ascomycota: anamorphic Hypocreales) (Currie *et al.* 1999*a*). This fungal pathogen can devastate gardens rapidly, even in the presence of the ants and its persistent presence significantly reduces the growth rate of colonies, both in terms of garden mass and number of workers (Currie *et al.* 1999*a*; Currie 2001*a*). Knowledge of how fungus-growing ants are able to suppress or eliminate the specialized pathogen *Escovopsis* and other potential microbial invaders of the garden is fundamental to understanding this fascinating mutualism.

Fungus-growing ants are able to suppress or partially eliminate *Escovopsis* through a mutualistic association with antibiotic-producing bacteria (Currie *et al.* 1999*b*, 2001). These filamentous bacteria are typically carried on the cuticle of fungus-growing ants (often in specialized locations), and they produce antibiotics that specifically target the growth of *Escovopsis* (Currie *et al.* 1999*b*). The removal of the bacterium from workers results in an increase in the abundance and impact of the pathogen within infected gardens (Currie *et al.* 2001). Although the bacterium is important in helping the ants suppress *Escovopsis*, it does not appear to be the only defence because gardens tended by workers with the bacterium removed are not completely overwhelmed by this parasite (Currie *et al.* 2001). Many additional defences have been proposed and examined, including the production of antibiotics in the ants' metapleural glands and the dissemination of proteolytic enzymes into critical locations by workers in order to increase the competitive advantage of the mutualistic fungus (Martin & Martin 1970; Schildknecht & Koob 1970, 1971; Boyd & Martin 1975). However, there is currently little empirical evidence demonstrating the role of other mechanisms in preventing the invasion of the garden by alien fungi or bacteria (see Weber 1972; Currie 2000, 2001*b*).

The physical removal of microbial contaminants by workers, which is generally referred to as 'weeding', has been assumed to be the main defence employed by fungus-growing ants in maintaining healthy gardens (e.g. Hölldobler & Wilson 1990; North *et al.* 1997). Considering the fundamental importance of defending the garden

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Received 20 October 2000 *Accepted* 2 February 2001

from microbes, it is somewhat surprising that this relatively old idea, which was first suggested by Möller (1893), has received only limited study and has not been well described or well defined. Weber (1957) reported observing weeding of alien fungi in a short published abstract. The behavioural details of these observations were subsequently never published and he later stated that weeding was of little importance in fungus cultivation by attine ants (see Weber 1972, pp. vii, 1 and 99). Fungus-growing ants minimize exposure of the garden to alien microbes by licking new substrate and all nest surfaces with their tongues (Stahel & Geijskes 1939; Autuori 1941; Quinlan & Cherrett 1977, 1979) and it has been suggested that this behaviour is important in eliminating alien microbes from within gardens (Bass & Cherrett 1994). In addition, it appears that ants collect spores in their infrabuccal pockets, which are filtering devices within their mouth parts (Eisner & Happ 1962), and subsequently discard the spores outside the nest in the form of pellets (Eisner & Happ 1962; Febvay & Kermarrec 1981). Nevertheless, the behaviours involved in physically removing alien fungi have received little study. It is also unclear whether the ants are able to detect and physically remove the specialized parasite *Escovopsis*.

We video recorded *Atta colombica* colonies that were experimentally infected with one of two different pathogenic fungi (*Trichoderma viride* and *Escovopsis*) in order to investigate whether fungus-growing ants are able to detect and physically remove alien fungi from their gardens. *Trichoderma* spp. are aggressive necrotrophic fungal parasites (Dix & Webster 1995) that rapidly overgrow the fungus cultivated by leaf-cutter ants in pure culture bioassays (C. R. Currie, unpublished data). Yet, *Atta* spp. workers are able to prevent *Trichoderma* spp. from proliferating within their fungal gardens (Currie *et al.* 1999*a*; Currie 2000; C. R. Currie, personal observation). Our experiment examined not only whether *A. colombica* workers are capable of removing general fungal contaminants (i.e. *Trichoderma*) and/or the specialized pathogen *Escovopsis*, but also how the removal is performed for both types of fungal invaders.

2. MATERIAL AND METHODS

We used 15 completely intact colonies of *A. colombica* in order to examine the response of workers to alien fungi. Four- to five-month-old colonies were collected in Gamboa, Republic of Panama, between the middle of September and late October. Colonies were kept at the University of Toronto in dual chamber systems with a single fungus garden in a plastic container (diameter of 8 cm and height of 4.5 cm) located within a larger foraging and dump chamber (height of 10 cm, length of 19.5 cm and width of 14 cm). In order to prevent between-colony movement of mites, which could serve as vectors for spores of *Escovopsis* and *Trichoderma*, colonies were kept on moats of heavy mineral oil and the inside edges of containers were coated with fluon (Northern Products, Inc., Woonsocket, RI, USA). Water was added as needed to small dishes with cotton pads in the outer chamber in order to maintain an adequate level of humidity within colonies. Colonies were provided *ad libitum* access to forage substrate, *Quercus* spp. and *Euonymus* spp., both before and during the experiment. All colonies used in this study were confirmed to be free of *Escovopsis* infection at the start of the experiment, following the methods of Currie *et al.* (1999*a*).

The colonies were placed in groups of three according to similarity in size (i.e. garden volume), with one colony from each block (group of three) randomly receiving one out of three spraying treatments: *Escovopsis* sp., *T. viride*, or water (as a control). The *T. viride* strain was collected from soil in the habitat occupied by *A. colombica* in the Republic of Panama. The strain of *Escovopsis* was isolated from the garden of an *A. colombica* colony collected from the same population as the colonies used in this study. *Escovopsis* and *T. viride* were grown on potato dextrose agar medium for ten days prior to the study. Each spore suspension was prepared by mixing fungal spores in sterile distilled water. A mist inoculator was used for applying *ca.* 30 000–50 000 spores in *ca.* 0.2–0.4 ml of water to the centre of the top surface of the garden. In order to evenly disperse the spores within the water dilution, 0.001 ml of Tween 20 (Fisher Scientific, Pittsburg, PA, USA) was added to each solution, including the water control treatment.

The responses of the colonies to the three treatments were video recorded using a Panasonic Omnimovie[1] video camera with a Sigma[1] VT-5 macro lens (Sigma Corporation of America, Ronkonkoma, NY, USA) mounted on a copy stand. A fibre-optic light source was used for illuminating the garden surface. Each colony was recorded just prior to spraying, immediately following spraying and at 3, 6, 12, 18, 24, 30, 48 and 72 h following the spraying treatment. Each video-recording session included an initial scan of the entire dorsal surface of the garden and the lens was then focused on the location with the most individuals for 3 min.

If workers remove alien fungi as hypothesized, we predicted that workers should move to the location sprayed with fungal spores, perform specific behaviours for removing the fungus and dump more or different garden material. We tested whether ants moved to the location of spraying by counting the number of ants on the top surface of the garden (where the spray was applied) at each recording interval (i.e. pre-spraying and 0, 3, 6, 12, 18, 24, 30, 48 and 72 h post-spraying). The ants were counted during the initial video scan of the top surface of the garden. The number of ants on top of the garden was then expressed as a percentage of the total number of ants in the garden (see below).

We examined the specific behaviours performed by fungus-growing ants by studying the videotapes as well as making direct observations with a stereomicroscope. We identified one behaviour, i.e. 'fungus grooming' (see § 3(b) for a description), that appeared to be common in the *Trichoderma* and *Escovopsis* treatments and uncommon in the control treatment. The frequency of fungus grooming was determined for each treatment by relating the number of workers exhibiting fungus grooming to the number of workers present in the field of view during the 3-min videotaped session. The number of workers was determined by counting all the workers present at the beginning of the taping period and all workers subsequently entering the field of view. When it was obvious that an individual had exited and immediately re-entered the field of view, that individual was counted only once.

The refuse material that accumulated between taping periods was collected at the end of each videotaping session from the foraging/dumping chamber, placed in 1.5-ml tubes and stored in a freezer for later examination. Additional collections were made at 100, 125 and 150 h after spraying. The refuse material was dried for 48 h at 70 °C and then weighed on an electrobalance. Since dump removal depends upon garden size, we

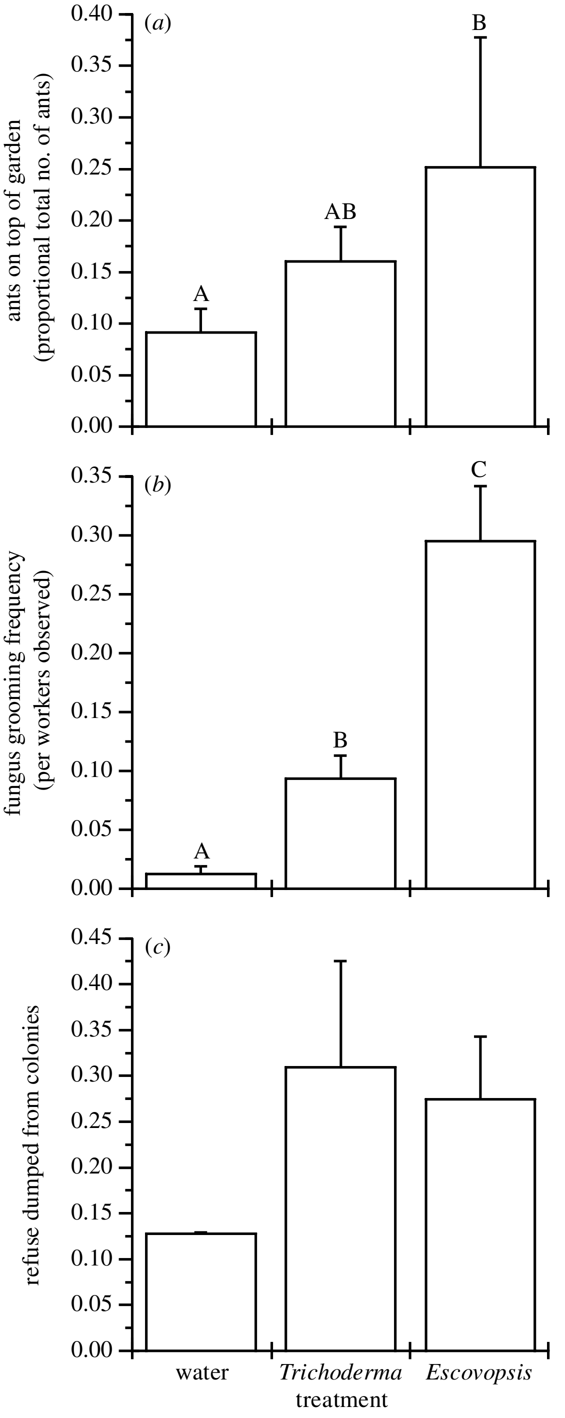

Figure 1. Frequency of different behavioural responses to the presence of water, *T. viride* and *Escovopsis* in intact colonies of *A. colombica*. (*a*) The average proportion of workers located on the top surface of the garden. (*b*) The average proportion of workers observed during the 3 min interval that engaged in fungus-grooming behaviour. (*c*) The average total dry mass of refuse material placed in the dump during the 150 h following treatment (proportional to the garden mass) ($n = 5$). Bars sharing letters are not significantly different and error bars represent standard errors.

calculated the total amount of refuse removed as a proportion of the total garden mass (see below). The composition of the dump material was assessed with a stereomicroscope prior to drying. Specifically, we noted whether the refuse had a normal appearance (i.e. dried decomposed vegetative material with no visible signs of the fungal mutualist) or whether the fungal mutualist and fresh (green) leaf material were present in the refuse (and, thus, prematurely dumped). Although a few pellets from the workers' infrabuccal pockets were noted in all dumps, the refuse piles with large numbers of pellets were noted.

Each garden was sampled for the presence of *Trichoderma* and *Escovopsis* at the completion of the experiment, 150 h after garden treatment. Twenty pieces of garden material were collected from throughout the garden and placed on nutrient agar in Petri dishes (following Currie *et al.* 1999*a*). After the garden was sampled, all workers were removed, grouped into size classes (castes) and counted. The garden material was dried and weighed.

Our experiments produced three data sets: (i) the number of ants on top of the garden relative to the total number of ants in the garden, (ii) the number of ants performing fungus grooming relative to the number of ants in view during the 3-min trial, and (iii) the dump weight per hour relative to the garden mass. The data were arcsine transformed in order to produce normality. Pre-spraying and time 0 were used as controls in order to ensure that there was no difference between the treatments before or at the time of spraying. Statistical differences between all three treatments (i.e. control, *Trichoderma* and *Escovopsis*) at all remaining time intervals were analysed using a repeated-measures ANOVA on Data Desk v. 4.1 (Velleman 1993). Each pair of treatments at all remaining time intervals was also analysed with a repeated-measures ANOVA (i.e. control versus *Escovopsis*, control versus *Trichoderma* and *Trichoderma* versus *Escovopsis*). In addition, each time interval was analysed separately using an ANOVA.

3. RESULTS

(a) *Workers on the top surface of the garden*

Atta colombica workers responded to the presence of alien fungi by moving into the infected locations from other regions of the garden (repeated-measures ANOVA, d.f. = 2 and $p = 0.045$). On average, a larger proportion of the workers were on the top surface of the garden in colonies treated with *Escovopsis* than in colonies sprayed with water (figure 1*a*) (repeated-measures ANOVA, d.f. = 1 and $p = 0.035$). There was also a trend towards a higher proportion of workers being on the top surface of the garden in *Trichoderma*-infected colonies as compared to the control group (repeated-measures ANOVA, d.f. = 1 and $p = 0.08$). The movement of workers to infected locations was rapid in both the *Escovopsis* and *Trichoderma* treatments, occurring within 3–6 h of spraying (figure 2*a*). The number of ants on the top surface of the garden in the *Escovopsis* treatment remained elevated throughout the 72 h of the experiment, either significantly different from the control or approaching significance (p-values ranging from 0.033 to 0.074 for 3–72 h post-spraying). However, the number of ants on the top surface of the garden in the *Trichoderma* treatment was only significantly different from the control at 6 h post-spraying ($p = 0.0047$).

(b) *Fungus grooming*

We found that workers groomed their fungal mutualist in the presence of *Escovopsis* and *Trichoderma* spores. During fungus grooming, the antennae were used to search the nearby vicinity of the garden. When the ant stopped searching, the maxillae and labium opened,

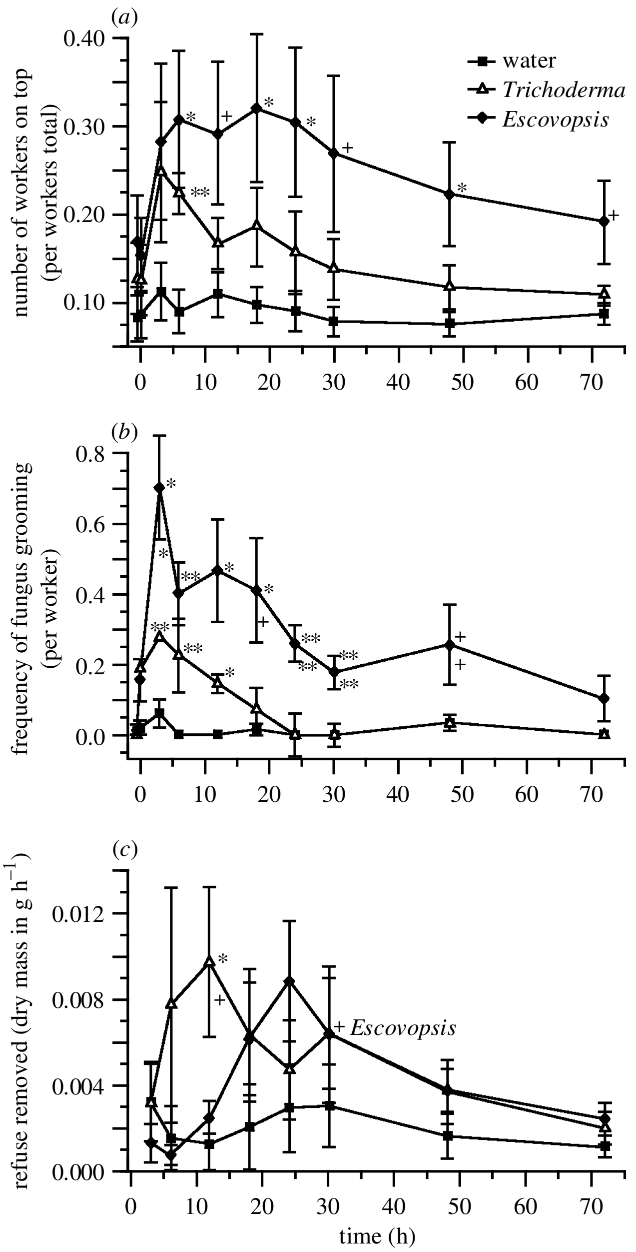

Figure 2. Worker behavioural responses over the 72 h following treatment with water, *T. viride* and *Escovopsis* in intact colonies of *A. colombica*. (*a*) The average proportion of workers located on the top surface of the garden. (*b*) The average proportion of workers observed that engaged in fungus-grooming behaviour. (*c*) The average dry mass of refuse material placed in the dump (proportional to garden mass) ($n = 5$). Symbols above the line represent significant differences between the corresponding treatment and control, while those below the line represent significant differences between *Trichoderma* and *Escovopsis*: plus symbols, less than 0.10 level of significance; single asterisks, less than 0.05 level of significance; double asterisks, less than 0.01 level of significance. Error bars represent standard errors.

grasped some fungus, closed and retracted, thus raising the fungus off the substrate and pulling it through their mouth parts. This pattern was repeated between two and ten times, during which the antennae were orientated parallel to their respective mandibles and held stationary. The ant then moved slightly and resumed grooming. Parallel, motionless antennae were the most obvious indication that ants were fungus grooming. Even when searching, the orientation of the antennae remained unchanged. Generally, the body of the ant remained very still for long periods during fungus grooming. Based on our observations, fungus grooming was performed exclusively by minima workers.

Fungus grooming is a behavioural response to the presence of alien fungi. We found a significant difference in the frequency of fungus grooming between treatments (figure 1*b*) (repeated-measures ANOVA, d.f. = 2 and $p = 0.0001$), with a significantly higher frequency of fungus grooming within both the *Escovopsis*- and *Trichoderma*-treated gardens as compared to the controls (figure 1*b*) (repeated-measures ANOVA, compared to *Escovopsis* d.f. = 1 and $p = 0.0005$ and compared to *T. viride* d.f. = 1 and $p = 0.0012$). In addition, fungus grooming was almost three times more frequent in colonies sprayed with *Escovopsis* than colonies sprayed with *T. viride* ($p = 0.0025$). Fungus grooming appeared to be a relatively rare behaviour in the absence of alien fungi, with an average of less than 2% of workers performing fungus grooming in the colonies sprayed with water.

The number of workers performing fungus-grooming behaviours increased immediately following the spraying treatment in both the *Trichoderma* and *Escovopsis* treatments (figure 2*b*). Interestingly, the response in the *Escovopsis*-treated colonies was more dramatic and more sustained than the response in the *Trichoderma*-treated colonies (figure 2*b*). The *Escovopsis*-treated colonies groomed the fungus significantly more than the control colonies for 3–30 h post-spraying (p-values ranging from 0.001 to 0.05). Conversely, fungus grooming in the *Trichoderma*-treated colonies was only significantly greater than the controls 3–12 h post-spraying. The response in the *Escovopsis*-treated colonies was significantly greater than that in the *Trichoderma*-treated colonies at 3, 24 and 30 h post-spraying.

(c) *Weeding*

Workers in colonies sprayed with *Trichoderma* and *Escovopsis* frequently removed fresh, young, green leaf material covered with the hyphae of their cultivar (young garden material) from the top surface of the garden. Removal of this young garden material involved several steps and multiple workers. First, minima workers loosened the piece by chewing at the edges where it was connected to the rest of the garden by fungal mycelium. Once the garden piece was loose, the minima worker (or sometimes a larger worker depending on the size of the piece to be removed) detached it from the garden matrix by holding onto the piece with its mandibles and rocking laterally, side to side on its legs. Once the piece was detached from the garden, a medium or major worker picked it up and carried it to the dump. We refer to the physical removal of garden pieces, particularly abnormal pieces, as 'weeding' (see § 4).

The presence of both *Trichoderma* and *Escovopsis* within gardens resulted in an increased rate of refuse removal by leaf-cutting ants. In our experiment, at least one worker removed a piece of garden during 52.5% (21 out of 40) and 75.0% (30 out of 40) of the observation periods for the *Trichoderma* and *Escovopsis* treatments, respectively.

Table 1. *Presence of the fungal mutualist and green leaf material in the refuse material removed from the garden and placed in the dump by the leaf-cutter* A. colombica *in colonies infected with* Trichoderma *or* Escovopsis

(Symbols: minus, no pieces; single plus, one or two pieces; double plus, approximately half the dump pieces; triple plus, greater than 85% of the dump pieces composed of mutualist and green leaf material.)

time post-spraying (h)	water	*Trichoderma*	*Escovopsis*
0–3	–	–	–
3–6	–	+ +	–
6–12	–	+ + +	+ +
12–24	–	+ +	+ + +
24–30	–	+	+ + +
30–72	–	–	+ + +
72–150	–	–	+ +

Table 2. *The presence or absence (proportion of colonies infected) between colonies and prevalence (proportion of pieces infected) within gardens of* T. viride *and* Escovopsis *after the experimental period*

(The values given are percentages.)

	Trichoderma		*Escovopsis*	
treatment	presence	prevalence	presence	prevalence
control	0	0	0	0
Trichoderma	20	1	0	0
Escovopsis	0	0	100	41

Weeding was observed in only 5% (2 out of 40) of the control colonies.

The average amount of total refuse removed by colonies in the *Escovopsis* and *Trichoderma* treatments was almost double that of the control treatment (figure 1*c*). However, this difference was not statistically significant. There was a significant difference in the pattern of refuse removal over time (figure 2*c*) (repeated-measures ANOVA, d.f. $= 2$ and $p = 0.0325$), with a peak rate of refuse removal in both the *Escovopsis* and *Trichoderma* treatments. However, refuse removal of the *Trichoderma*-treated colony peaked at 12 h post-spraying (material deposited in the dump between 6 and 12 h post-spraying), whereas the refuse removal in the *Escovopsis*-treated colony peaked at 24 h (material deposited between 18 and 24 h post-spraying).

The composition of the refuse material removed also differed between treatments (table 1). The control colonies only dumped normal refuse (old decomposed leaf material with no fungus present) whereas the *Trichoderma*- and *Escovopsis*-treated colonies dumped both normal and abnormal refuse (i.e. pieces of green leaves along with the fungal mutualist). The dumping of normal refuse material by workers is apparently largely abandoned in colonies infected with the pathogens (table 1); this may explain why an overall statistically significant difference was not obtained in our study. Colonies infected with different fungi initiated abnormal refuse removal at different times (*Trichoderma* at 3 h and *Escovopsis* at 6 h post-spraying). The refuse removal was also sustained for different lengths of time. The *Trichoderma*-treated colonies completed abnormal refuse removal within almost 24 h post-spraying, whereas the *Escovopsis*-treated colonies were still removing some abnormal particles at 150 h post-spraying (table 1). The *Trichoderma*- and *Escovopsis*-infected colonies also dumped a substantially greater number of infrabuccal pellets (i.e. approximately five to ten times more) during their peak fungus-grooming activity.

(d) *Removal of infection*

Isolations from the garden at the end of the experiment revealed that the ants were effective at eliminating *T. viride*, as the presence of this fungus could only be detected from one garden piece in one colony (table 2). However, *Escovopsis* was detected within all colonies treated with the specialized pathogen and, in fact, was common within gardens (41% of pieces). The control colonies were confirmed to be free of both *T. viride* and *Escovopsis*.

4. DISCUSSION

Our study indicated that the rare ability of fungus-growing ants to culture fungus for food is possible because workers actively remove potentially devastating microbial pathogens from their gardens. When colonies are sprayed with spores of the fungal pathogens, workers quickly move to the infected areas from other locations (figure 2*a*). These individuals engage in a behaviour that we term 'fungus grooming', i.e. removing alien spores by cleaning the garden using their mouth parts (figure 2*b*), collecting the invading spores in their infrabuccal pockets and later expelling pellets of spores into the dump. In addition, the ants engage in the removal of unhealthy pieces of garden (including fresh leaf material covered in their fungal cultivar) (table 1 and figure 2*c*). These hygienic behaviours are specific to the presence of fungal pathogens as they are the primary behaviours workers engage in in the presence of pathogens, while they are uncommon in uninfected colonies. The relative absence of workers performing these behaviours in uninfected colonies may help explain why the importance and, in fact, occurrence of these behaviours has mostly been overlooked.

Our experiment also revealed that *A. colombica* workers engage in the same behaviours in attempting to remove both general fungal contaminants (i.e. *Trichoderma*) and the specialist pathogen *Escovopsis*. However, the intensity and duration of the colony response to *Escovopsis* was much higher. The number of workers on the top surface of the garden and the rate of fungus grooming was always greater in treatments with *Escovopsis* than with *Trichoderma*, although this was not always statistically significant. In addition, the *Escovopsis*-treated colonies had on average three times the proportion of ants engaging in fungus grooming than the *Trichoderma* treatments (29.4 versus 9.3%, respectively). In fact, this response occurred almost immediately after spraying (3 h post-spraying) (70.1 versus 27.5%, respectively) ($p = 0.037$), suggesting that the ants can distinguish between the mycelium and/

or spores of the generalist and specialist fungal pathogens. In addition, all three behavioural responses (relocation to the top surface of the garden, fungus grooming and weeding) were maintained for a longer time in the *Escovopsis*- than in the *Trichoderma*-treated colonies. The decline in these behaviours in the *Trichoderma*-treated colonies appeared to be because the ants were successful at removing *Trichoderma*, whereas they were unsuccessful at completely removing the *Escovopsis* contamination (table 2) and, therefore, these behaviours were continued for a longer amount of time in the *Escovopsis*-treated colonies.

As mentioned previously, we suggest that the behaviour of using their mouth parts for cleaning the fungal cultivars (as described in § 3(b)) be termed fungus grooming. This terminology is appropriate because the removal of parasites and pathogens among ants, as well as among other social animals, is generally referred to as 'grooming', although it is important to differentiate this behaviour from other forms of grooming. Superficially, fungus grooming may appear to be the same behaviour as licking of the fungus; however, these are distinct behaviours (C. R. Currie and A. E. Stuart, unpublished data). We observed licking of the fungus immediately following the spraying of gardens in our study, with workers actually lapping up the water droplets and spores applied to the garden, and licking of the garden occurred at approximately the same frequency in all three treatments. Within 3 h following treatment, licking was not a common behaviour in any treatment (see also Bass & Cherrett 1994). Discovering that fungus-growing ants groom their garden is not surprising, since they are obligately dependent upon their cultivars and grooming as a defence against parasites and pathogens is widespread among social animals.

Workers also responded to the presence of alien fungi by removing chunks of garden. This is a complex behaviour, typically involving several minor workers loosening a piece from the matrix of the garden and then medium- and major-sized workers removing and carrying it to the dump. It is clear that the material removed by the ants included recently cut leaves and the mycelium of their fungal cultivars (table 1); thus, this is not the normal process of removing vegetative substrate depleted by the fungus. Instead, this behaviour is involved in the removal of garden regions infected by alien fungi. We suggest that the specific behaviour of discarding garden substrate with the mycelium of the mutualist fungus be referred to as 'weeding'. Mature *A. colombica* colonies in the field frequently discard fresh garden pieces composed of fresh vegetative material and fungus and this material is frequently overgrown by the pathogen *Escovopsis* (C. R. Currie, personal observation), indicating that weeding is very common in the field. This also explains why *Escovopsis* is commonly associated with the dump material discarded by leaf-cutting ants (Bot *et al.* 2001).

Fungus-growing ants, particularly leaf-cutters such as *A. colombica*, are very effective at protecting their gardens from generalist fungal pathogens such as *Trichoderma* (Currie *et al.* 1999*a*; Currie 2000; this study). Our findings indicated that fungus gardens are primarily protected by the physical removal of these fungi by fungus grooming and weeding. The importance of these behaviours is obvious, as they are the primary tasks performed by minima workers in colonies treated with the two fungal contaminants. We suggest that three physical methods are used in defending fungus gardens from alien microbes. First, ants attempt to prevent their gardens from being exposed to microbial contaminants. This is apparently achieved by licking both the nest surfaces upon which the fungus gardens are placed and the substrate added to the garden (Stahel & Geijskes 1939; Autuori 1941; Quinlan & Cherrett 1977), thereby reducing the abundance and diversity of microbes that may come into contact with the garden. Second, colonies attempt to prevent inoculated microbes from growing and establishing an infection; prevention is apparently achieved predominantly by fungus grooming. Finally, once infections are established, workers attempt to suppress and remove the pathogen. Removal of infected garden pieces (weeding) is clearly an important step in treating infected regions of the garden. Obviously, other mechanisms, some identified and some as yet undiscovered, are employed by the ants in dealing with alien microbes in some or all of these three lines of defence. Experimental work is needed in order to establish the importance of other mechanisms in defending the gardens of fungus-growing ants from alien microbes and the specialized pathogen *Escovopsis*. It is interesting that, despite all of these lines of defence, the specialized parasite *Escovopsis* is still able to establish infections and spread through the garden. An examination of how *Escovopsis* circumvents the defences of the ants and their cultivated fungi would provide interesting insights into the dynamics of this unique symbiosis.

C.R.C. was supported by a Smithsonian predoctoral award and by Natural Sciences and Engineering Research Council of Canada (NSERC) postgraduate and postdoctoral awards. A.E.S. was supported by an NSERC postgraduate award. We thank the Smithsonian Tropical Research Institute and Autoridad Nactional del Ambiente of the Republic of Panama for facilitating the research and granting collecting permits. We acknowledge J. Burford, D. Currie, A. Herre, F. Hunter, M. Leone, D. Malloch, S. Margaritescu, S. Rehner, J. Scott, R. Thompson and B. Wcislo for valuable logistic support. We thank R. Adams, K. Boomsma, G. Currie, U. Mueller and T. Murakami for valuable comments on this manuscript. Additional research support was provided by an NSERC operating grant awarded to D. Malloch and NSF CAREER grant DEB-9983879 awarded to U. Mueller.

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