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# Growth and Conidiation Response of *Escovopsis weberi* (Ascomycota: Hypocreales) Against the Fungal Cultivar of *Acromyrmex lundii* (Hymenoptera: Formicidae)

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**ABSTRACT** Leaf-cutter ants (*Acromyrmex* and *Atta* spp.) exhibit ancient and complex interactions with the symbiotic fungus *Leucoagaricus* (Basidiomycetes: Agaricales) from which they feed, and with the virulent and specific fungus *Escovopsis weberi* J.J. Muchovej & Della Lucia (Ascomycetes: Hypocreales) that attacks the ants' fungal gardens. This system offers a unique opportunity to study possible avenues for replacing polluting pesticides with a biological control agent against the ants. We isolated both *Leucoagaricus* sp. and *E. weberi* from the gardens of *Acromyrmex lundii* Guérin-Méneville colonies and confronted them with each other by growing *Leucoagaricus* on petri dishes and placing inoculated pieces of agar inoculated with *E. weberi* at the edges. Here we present growth curves of *Leucoagaricus* sp. and *E. weberi* in the absence of each other, as a baseline to which we compare the effect of the fungi on each other. As expected, we found a negative effect of *E. weberi* on the ant cultivar from different colonies of *A. lundii*. *E. weberi* increased its growth rate, as well as the levels of conidiation, in the presence of the ant cultivar. We determined that a soluble and diffusible compound, released by the cultivar, triggered, was responsible for, or did both for the increased levels of conidiation in *E. weberi*, and that this response was reversible. We discuss why our results are encouraging from a biological control perspective.

**KEY WORDS** ant pests, biological control, fungi-fungi interactions, induction of conidiation, leaf-cutter ants

Leaf-cutter ants (*Acromyrmex* and *Atta* spp.) are among the most interesting social insects because of their complex organization, their sophisticated level of agriculture, as well as their fascinating interactions with other organisms (Hölldobler and Wilson 1990). They are extremely important from an ecological perspective, with their multiple and amplified effects on other organisms per se and in their roles as ecosystem engineers (Folgarait 1998). From an applied perspective, their effects on the plants are profound as well, as these ants are considered to be the main herbivores of the Neotropics (Cherrett 1986).

Leaf-cutter ants have ancient symbiotic relationships with fungi in the genus *Leucoagaricus* (Basidiomycetes: Agaricales), which the ants cultivate within their nests for food, as well as with specific pathogenic fungi in the genus *Escovopsis* (Ascomycetes: Hypocreales) that target the cultivar (Currie et al. 1999, 2003, Reynolds and Currie 2004). In addition, these ants maintain relationships with Actinobacteria that produce chemical compounds (Haeder et al. 2009, Oh et al. 2009) against the pathogen (Currie et al. 1999, Cafaro and Currie 2005, Poulsen et al. 2010, Cafaro et al. 2010). Among this constant net of interactions there is a myriad of other bacteria (Pinto-Tomás et al. 2009, Suen et al. 2010); yeasts (Little and Currie 2008, Pagnocca et al. 2008); and filamentous fungi (Rodrigues et al. 2005) with predominantly unknown roles in the association.

The leaf-cutter ant symbiosis offers a unique opportunity to exploit specific natural enemies as potential agents for biological control of these ants. Among them, the pathogen *Escovopsis* is specific and virulent and can result in the abandonment of nests or their destruction (Currie 2001). Among the two species of *Atta* and six species of *Acromyrmex* designated as pests in Argentina (Elizalde 2009), *Acromyrmex lundii* Guérin-Méneville is one of the most damaging and has a broad geographical distribution (Farji Brener and Ruggiero 1994, Elizalde and Folgarait 2010). To develop a biological control strategy against *A. lundii,* using the pathogen *Escovopsis* to destroy its fungal food source, it is necessary to better understand the interaction between the cultivar and the selected pathogen. If *Escovopsis* is to be used as a biological control agent, understanding factors affecting growth rate and conidiation levels of the pathogen is war-

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ranted because ideal conditions for their use commercially can be analyzed and refined.

Fungi typically show a growth phase called trophophase and a second phase, idiophase, where most of the secondary compounds are produced (Aziz and Smyk 2002). However, these two phases can overlap causing the induction of chemical compounds at different times. Most of the literature dealing with induction of secondary metabolites relates to mycotoxin production in food contaminants, gene regulation, or stress conditions that trigger secondary metabolism (Aziz and Smyk 2002, Schmidt-Heydt et al. 2008). Factors, such as carbon sources, $NH_4^+$ availability, or presence of particular metabolic precursors, seem to be involved in inducing chemical compounds in fungi (Demain 1986). In many cases, production of secondary metabolites and induction of conidiation can result from fungal–fungal interactions (White and Boddy 1992, Savoie et al. 1998, Whipps 2001, Tsujiyama and Minami 2005). Understanding how fungi, as biological control agents, respond to other microorganisms, including the target to be controlled (i.e., in this case fungus cultivar of the ants), is a prerequisite for any strategy to be used.

We tested the effect of one strain of *E. weberi* isolated from *A. lundii* against cultivars obtained from different colonies. We challenged *Leucoagaricus* isolates with *E. weberi* (or grown alone as controls) to observe the effects of the *Leucoagaricus* and *E. weberi* isolates on each other. We measured the growth and conidiation levels of *E. weberi* in the presence and absence of *Leucoagaricus.* Our prediction was that *E. weberi* would be an efficient pathogen against all the cultivar isolates tested based on results obtained previously by others (Gerardo et al. 2004, Gerardo and Caldera 2007, Taerum et al. 2007, Poulsen et al. 2010. We also expected that *E. weberi,* as an efficient pathogen, would be able to respond accordingly by maximizing its growth and conidiation when detecting the presence of its host, *Leucoagaricus* sp. Furthermore, we measured if *E. weberi* growth and conidiation responses were induced by the presence of a soluble chemical produced by the cultivar.

## Materials and Methods

**Sample Collection.** We collected three colonies of *A. lundii* (01, 02, and 04) from Mercedes (29° 11′05″ S, 58° 05′26″ W), Corrientes province in Argentina. The landscape was natural grasslands used for cattle ranching. We collected fungus gardens after excavating the nest as delicately as possible. We used sterilized spoons to transfer garden material into a sterilized container with as many ants as we were able to collect and allowed the colony to stabilize. In the laboratory, we isolated *Leucoagaricus* sp. from the garden of three colonies by taking a piece of the cultivar and growing it on potato dextrose agar (PDA). Isolations for *E. weberi* were done on PDA after procedures from Currie et al. (1999), but isolates only were obtained from one colony (04).

**Growth Curves and Challenges.** We placed small pieces of each isolated *Leucoagaricus* sp. in petri dishes with PDA ($N_{L01} = 6$, $N_{L02} = 13$, $N_{L04} = 14$). We allowed them to grow until reaching an area equivalent to 6.5 $cm^2$ before starting the experiments. We built challenges by adding a piece of agar of 0.28 $cm^2$ with conidiated *E. weberi* at the edge of the petri dish. We did not quantify the amount of conidia, but we made sure that the surface of each piece was similarly covered by brown (mature) conidia. We made controls for each of the *Leucoagaricus* sp. isolates ($N = 6$) as well as for *E. weberi* ($N = 6$) by allowing growth alone in the middle of the petri dish. We took photographs of each petri dish every 12 h for 6 d, and subsequently analyzed the area of mycelium covered by each fungus with the software ImageJ 1.4 (Wayne Rasband, National Institutes of Health, Bethesda, MD). We also recorded whether *E. weberi* response included conidiation or not. When conidia were present, we determined the degree of conidiation (defined as the percentage of the petri dish, irrespectively of its density, that was covered with conidia). We terminated the experiment at the day when *E. weberi,* challenged and control, completely covered the petri dish.

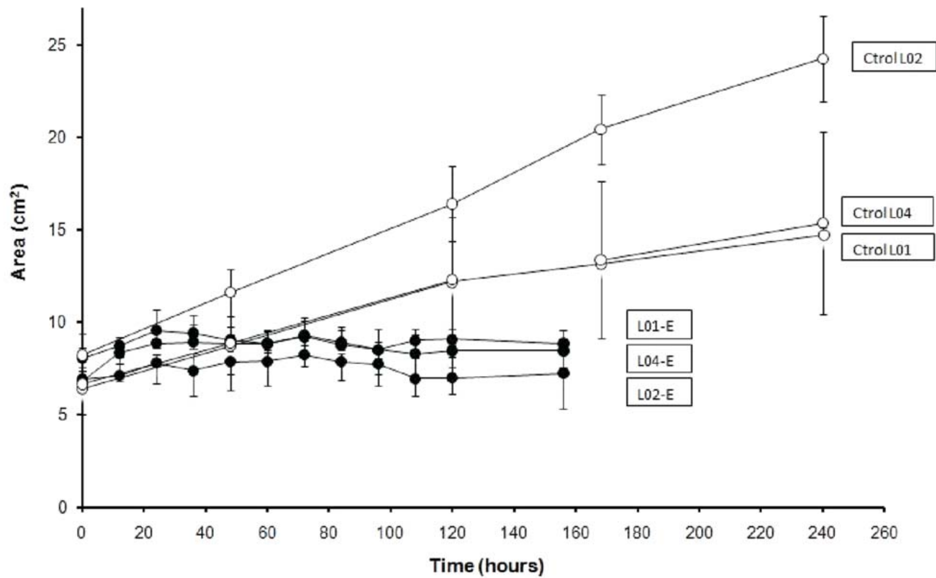
With the information of the initial and final area of *Leucoagaricus* sp. we calculated the following index:

$$\frac{\text{final area (challenge)} - \text{initial area (challenge)}}{\text{final area (control)} - \text{initial area (control)}}$$

If the index was not significantly different from a *t* distribution with a mean lower than 1, we concluded that there were no effects of *E. weberi* on the cultivar. However, our expectation was to find a value significantly lower than one if *E. weberi* negatively affected *Leucoagaricus* sp.

We statistically analyzed growth curves with repeated measures analysis of variance (ANOVA) as each petri dish was followed from the beginning to the end of the experiment. We transformed the data to ln to fulfill the parametric tests assumptions. In addition, for *E. weberi* we compared (for each *Leucoagaricus* sp. challenge) the final area with that of the control by using nonparametric Mann–Whitney tests. We also compared the percentage of conidiation per petri dish using a nonparametric Kruskal–Wallis test using a posteriori contrasts adjusted by Bonferroni correction to keep an alpha level of 0.05. Finally, we measured the proportion of plates with conidiation by a two sample proportion test (Siegel 1976).

**Induction of Conidiation by a Soluble Chemical.** From petri dishes where *Leucoagaricus* isolate L02 covered >75% of the petri dish, and after growth for at least 45 d, we took 1 $cm^2$ mycelium free pieces of agar close to the fungal cultivar. We placed the agar piece in the middle of another petri dish with PDA where we added a 1-$cm^2$ piece of agar with *E. weberi* without conidia (experimental group) at the edge. As a positive control we used a 1 $cm^2$ piece of agar with *Leucoagaricus* sp. mycelium plus the *E. weberi* without conidia, whereas we made a negative control only with a 1 $cm^2$ piece of agar with *E. weberi* without conidia growing alone on PDA. We performed 12 challenges per treatment.



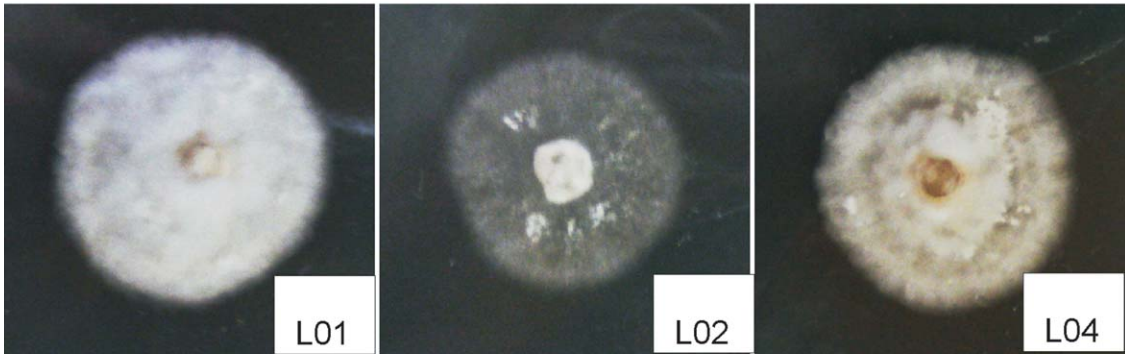




**Fig. 1.** Top section. Growth curves (means and standard deviations of area) of each of the *Leucoagaricus* isolates challenged with *E. weberi* on PDA, plus cultivar controls. Bottom section. Morphology of *Leucoagaricus* isolates L01, L02, and L04 on PDA. (Online figure in color.)

Because the experimental group induced conidiation of *E. weberi*, we repeated the experiment mentioned above, but instead used conidiated *E. weberi.* We did this altered experiment not only to corroborate the existence of a chemical compound that could be detected by the pathogen, but also to determine if the induction effect was transitory or prolonged. If transitory we did not expect a difference in conidiation between both induction experiments for each treatment. We compared, by pairs of treatments, the proportion of cultures that conidiated using the proportion test, whereas we used the Kruskal–Wallis test with a priori contrasts considering adjusted alpha levels (Siegel 1976) to compare the levels of conidiation. We used the STATISTIX for Windows program (Analytic Software, Tallahassee, FL) to performed statistical analyses.

## Results

**Growth Curves and Challenges.** The indices calculated for each challenged *Leucoagaricus* isolate showed a significantly smaller value than one (in each case $t > 18.59$; $P < 0.00001$), demonstrating the negative effect of *E. weberi* on the cultivar, with all the means were close to zero (mean and standard error for L01: $0.3390 \pm 0.0338$, for L02: $6.87\text{E-}03 \pm 0.0456$, and for L04: $0.0638 \pm 0.0473$). There were also significant differences in the area (final area−initial area) of each of the challenged cultivars versus its own control (each $P < 0.001$).

There was no evidence for differences in growth area among the three *Leucoagaricus* isolates challenged with *E. weberi* ($F_2 = 2.089$; $P < 0.0757$), but there was a significant effect of time ($F_{11} = 8.239$; $P < 0.0001$) as well as in the interaction ($F_{22} = 2.183$; $P < 0.0018$), and the contrasts showed that *Leucoagaricus* isolate L02 differed significantly from the other two (each $P < 0.0001$), but L01 and L04 were similar to each other ($P > 0.0001$) (Fig. 1).

There was no evidence for differences in growth area among the three *Leucoagaricus* isolates grown alone as controls ($F_2 = 0.101$; $P < 0.9043$), and a

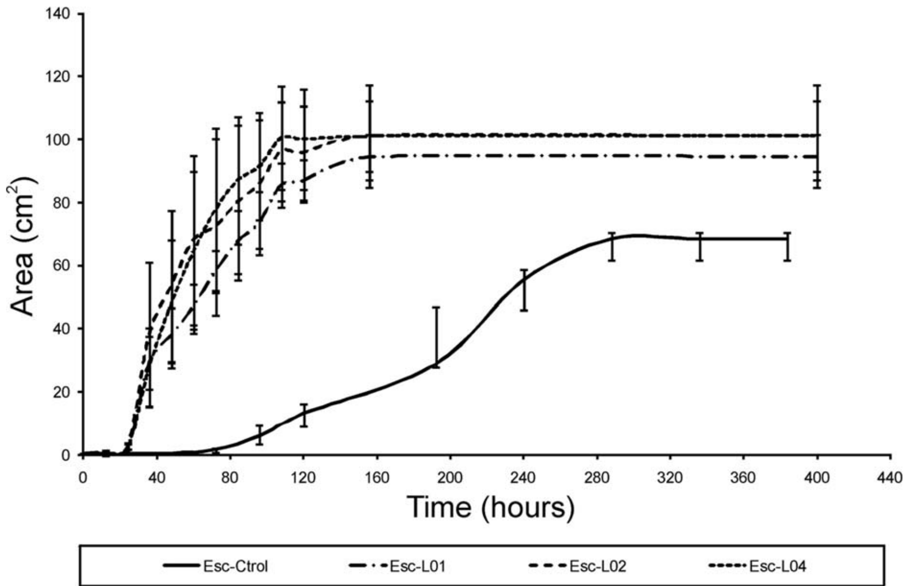



**Fig. 2.** *E. weberi* growth curves (means and standard deviations of area). Esc-L01, Esc-L02, Esc-L04: Same *E. weberi* strain challenged with each of the three cultivar isolates L01, L02, and L04, respectively. Esc-Ctrol: *E. weberi* control growing alone.

significant effect of time was found ($F_5 = 393.037$; $P < 0.0001$) as well as the interaction ($F_{10} = 2.19$; $P < 0.0273$), although the contrasts were nonsignificant after the correction by Bonferroni ($P > 0.0167$) (Fig. 1). The cultivar isolate L02 was slower-growing than the other two cultivar isolates in the challenges with *Escovopsis*, faster-growing than the other two cultivar isolates in the controls, and exhibited slightly different morphology after 35 d of growth (Fig. 1).

The growth area of *E. weberi* was not significant between challenges with the different *Leucoagaricus* isolates ($F_2 = 1.67$; $P < 0.2048$), but there was a significant effect of time ($F_6 = 1563.14$; $P < 0.0001$), although the interaction was not significant ($F_{12} = 0.681$; $P < 0.7684$) (Fig 2). Five days after starting the challenge, all the cultures of *E. weberi* conidiated and by the ninth day 92% of the petri dish area was covered by conidia (Fig. 3). Each of the *E. weberi* challenged reached its stationary phase significantly faster (each case $P_{L01} = 0.0051$, $P_{L02} = 0.0034$, $P_{L04} = 0.0034$) and with a final greater area than the control (each case $P < 0.001$) (Fig. 2).

Challenged *E. weberi* did not differ statistically among each other in the percentage of petri dish covered by mature conidia ($P > 0.016$). However, each challenged *E. weberi* exhibited a significantly greater percentage of area covered with mature conidia in comparison to the control (each $P < 0.0076$). By the fourth day, none of the controls had mature spores, and by the end of the experiment, on the fourteenth day after inoculation, all the controls had <10% of the area covered with mature conidia (Fig. 3).

**Induction of Conidiation.** When the proportion of petri dishes with mature conidia of *Escovopsis* was compared across treatments, there were significant differences between the two controls ($z = 2.46$, $P < 0.014$). No differences were found between the positive control (*E. weberi* without conidia exposed to mycelium of *Leucoagaricus* L02 culture) and the experimental group (*E. weberi* without conidia exposed to mycelium-free agar piece from *Leucoagaricus* L02 culture) ($z = 1.69$, $P > 0.0917$), or between the negative control (*E. weberi* without conidia growing alone) and the experimental group ($z = -0.043$, $P > 0.6650$) (Fig. 4). There were significant differences among the three treatments in the percentage of mature spores covering the plates ($KW = 10.36$; $P < 0.0056$), and the adjusted contrasts ($P < 0.016$) exhibited the same pattern as above: no differences between each control and the experimental group but significant differences between the two controls.

When the induction test was repeated with the conidiated *E. weberi* instead, the differences in the proportion of petri dishes with conidiation were apparent between the two controls ($z = -3.03$, $P < 0.0024$), but there was no differences between the positive control and the experimental group ($z = 1.64$, $P > 0.1003$), nor between the negative control and the experimental group ($z = 1.22$, $P > 0.2207$) (Fig. 4). There were significant differences among the three treatments in the percentage of mature conidia covering the plates ($KW = 18.52$; $P < 0.0001$), although the adjusted contrasts ($P < 0.016$) exhibited the same pattern as above: no differences between each control and the experimental group, but significant differences between the two controls.

Conidiation levels tended to be greater in the second induction experiment in comparison to the first one for each of the three treatments; however, neither the proportion of cultures with conidiation, nor the comparison of the percentage of petri dish covered

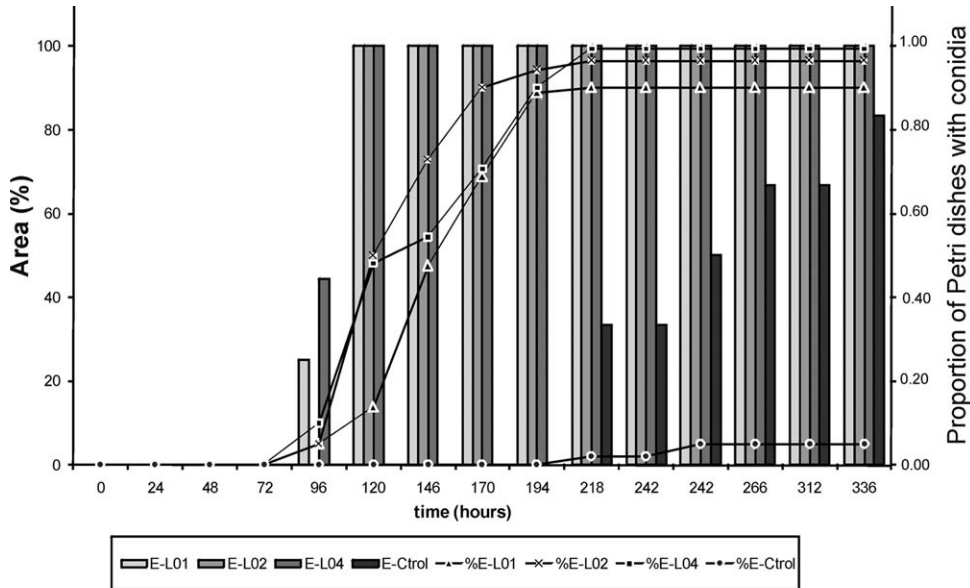



**Fig. 3.** Conidiation of *E. weberi* challenged with each of the three *Leucoagaricus* isolates. Results shown as proportion of petri dishes with conidia (bars) and average percentage of the area of each petri dish covered with conidia (lines).

with conidia, exhibited significant differences (all $P > 0.05$), except between the positive controls that showed significantly greater percentages of each petri dish covered by conidia in the second experiment ($U = 26.5$, $P < 0.009$).

## Discussion

Our results show that *E. weberi* was able to negatively affect isolates of *Leucoagaricus* sp. obtained from different colonies of *A. lundii.* Despite the fact that our *Leucoagaricus* isolates did not decrease their original growth area after exposure to the pathogen, we observed how cultivar hyphae suffered cellular breakdown as evidenced by cytoplasm vacuolization and loss of opacity. This evidence suggests that *E. weberi* was negatively affecting the ant cultivar. This is important because we found that one of the cultivar isolates (L02) presented a slightly different morphology and growth rate (Fig. 1) than the others. Although there is evidence of horizontal transfers (Chapela et al. 1994, Mueller et al. 1998, Mikheyev et al. 2010), *Leucoagaricus* sp. is transferred across generations through virgin queens that carry cultivar fragments from their original nest to found new colonies (Autuori 1941, Mueller et al. 2001). This mode of vertical dissemination of *Leucoagaricus* species has favored the selection of clones across the leaf-cutter ants.

*E. weberi* growth rate increased significantly in presence of the cultivar. In fact, we showed that the idiophase of *E. weberi* was accelerated in the presence of *Leucoagaricus* sp. (Fig. 2). Gerardo et al. (2006) found that *Escovopsis* isolated from *Apterostigma* ants (lower Attini) presented specific chemotaxis toward cultivar isolates. In their experiments, they established that *Escovopsis* is attracted to the cultivar, but infection depended on the cultivar defenses and the genotype of the *Escovopsis*–cultivar interaction. Folgarait et al. (2008) showed that *E. weberi* was able to change growth strategies, from vegetative growth to dispersing conidia, in the presence of an antagonist Actinobacterium (*Streptomyces* sp.), which was not in direct contact with the fungus. Therefore, *E. weberi* seems to be able to detect the presence of other microorganisms from a distance (Reynolds and Currie 2004) and to respond accordingly by changing growth strategies. This characteristic is desirable from a biological control perspective because such organisms are able to reach the target without the need of direct contact with it; it is enough to sense a chemical compound from a distance. It remains to be tested if sensing from a distance works in a soil made nest.

Our induction experiments support the idea that *E. weberi* can detect a soluble and agar-diffusible compound produced by *Leucoagaricus* sp. in *A. lundii*, as has been shown for other attines (Gerardo et al. 2006). We have shown that after being in contact with the cultivar, *E. weberi* produced more conidia than when we maintained it alone. Also, we could possibly explain an increase in conidiation in the presence of the cultivar by *Escovopsis* using cultivar resources (i.e., nutrients derived from hyphae destruction, Reynolds and Currie 2004). Many fungi produce secondary metabolites that are used for their own defense or attack (Demain 1986), and some of these have been coopted by other organisms to their own advantage (Sonnenbichler et al. 1994), mediate fungal–fungal interactions, or both (White and Boddy 1992, Savoie et al. 1998, Tsujiyama and Minami 2005). We believe this could be the case in the studied system. The piece of agar without *Leucoagaricus* sp., but with some diffusible compound produced by it did not differ from the

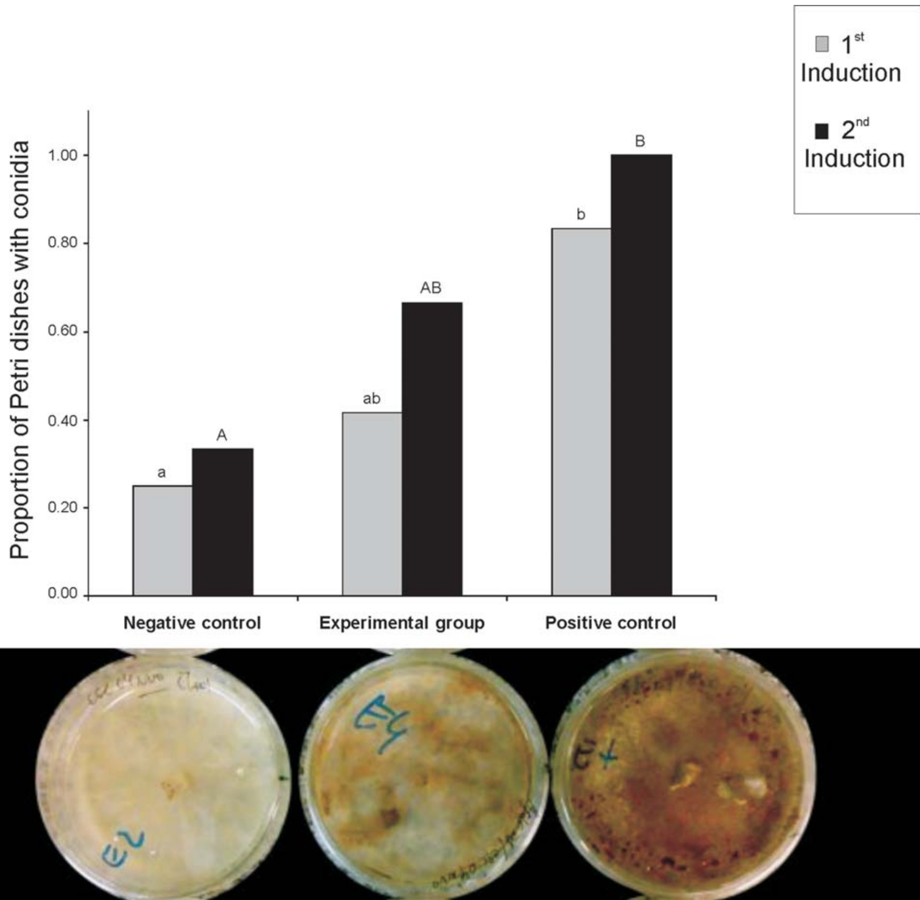



**Fig. 4.** Top section. Proportion of petri dishes exhibiting mature conidia after the first and second induction. Different letters refer to significant differences, same letters refer to lack of statistical differences. Small letters refer to statistical comparisons made within the first induction experiment whereas capital letters refer to the second one. Bottom section. From left to right, negative control (only *Escovopsis*), the experimental group (*Escovopsis* without conidia plus mycelium-free agar block from *Leucoagaricus* isolate L02 growing alone), positive control (*Escovopsis* and *Leucoagaricus* growing on the same plate). (Online figure in color.)

negative and positive controls, but both controls statistically differed from each other. This information suggests that a chemical is being detected with a response that may be concentration dependent.

Another explanation for the lack of differences in conidiation by *Escovopsis* between the first and second experiment could be that the induction is transitory. Several subcultures of *E. weberi* alone stopped producing conidia, suggesting that the higher cost in the absence of a target may be responsible, although it could also be related to lower nutrient availability. In addition, we showed that the presence of the cultivar produced an accumulated effect over time in conidiation levels, as the positive control of the second experiment was significantly greater than that of the first one. This is a very exciting characteristic of the pathogen because once *Escovopsis* finds the cultivar (Reynolds and Currie 2004), a positive feedback mechanism of conidia production appears, which potentially could enhance its efficiency as a biocontrol agent.

The variability observed in *E. weberi* conidia production could be related to several causes linked to the concentration of a compound produced by the cultivar. It is highly probable that different hyphae of the cultivar have different ages and are at different physiological stages that correspond to the production of secondary metabolites. We found slightly different morphologies and growth rates for different cultivar isolates, and they could have different physiological characteristics and production of chemical compounds. From another point of view, fungi commonly show complex signaling pathways involved in conidiation (Hicks et al. 1997, Shimizu and Keller 2000). Sometimes physical contact between hyphae is needed to produce or amplify a physiological response (Sonnenbichler et al. 1994). In this study, we only looked at one possible inducer and enhancer of conidiation in *E. weberi.* However, conidiation response may involve other factors (i.e., light, $Na^{+}$ concentration, pH, intermediate metabolites; Mandels and Reese

1960, Mani and Swamy 1983) besides the interaction with the ant cultivar. Hence, an attenuated induction could be expected in vitro, which could explain why each of the controls did not differ from the experimental group.

Besides the mechanism of recognition involved, which deserves further study, our results are encouraging from a biological control perspective because of three reasons: 1) a single strain of *E. weberi* is able to kill cultivars from different colonies of the same ant species, corroborating previous findings (Gerardo et al. 2004, Taerum et al. 2007, Poulsen et al. 2010); 2) *E. weberi* growth rate can be accelerated and levels of conidiation enhanced by the presence of *Leucoagaricus* sp. (i.e., target species); 3) in the absence of its target, *E. weberi* in the laboratory stops producing conidia, which should limit its dispersion in the field and the possible elimination of other target cultivars from sympatric, nonpest, leaf-cutter ants.

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## References Cited

**Autuori, M. 1941.** Contribuiçao para o conhecimiento da saúva (*Atta* spp. Hymenoptera: Formicidae). Evoluçao do sauverio (*Atta sexdens rubropilosa* Forel 1908). Arch. Inst. Biol. Andina 12: 197–228.

**Aziz, N. H., and B. Smyk. 2002.** Influence of UV radiation and nitrosamines on the induction of mycotoxins synthesis by nontoxigenic moulds isolated from feed samples. Nahrung 46: 118–121.

**Cafaro, M. J., and C. R. Currie. 2005.** Phylogenetic analysis of mutualistic filamentous bacteria associated with fungus-growing ants. Can. J. Microbiol. 51: 441–446.

**Cafaro, M. J., M. Poulsen, A.E.F. Little, S. L. Price, N. M. Gerardo, B. Wong, A. E. Stuart, B. Larget, P. Abbot, and C. R. Currie. 2010.** Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria. Proc. R. Soc. Lond., Ser. B: Biol. Sci. (in press) (doi:10.1098/rspb. 2010.2118).

**Chapela, I. H., S. A. Rehner, T. R. Schultz, and U. G. Mueller. 1994.** Evolutionary history of the symbiosis between fungus-growing ants and their fungi. Science 266: 1691–1694.

**Cherrett, J. M. 1986.** The economic importance and control of leaf-cutting ants, 1665–1192. *In* S. B. Vinson (ed.), Economic impact and control of social insects. Praeger Press, New York.

**Currie, C. R. 2001.** Prevalence and impact of a virulent parasite on a tripartite mutualism. Oecologia 128: 99–106.

**Currie, C. R., U. G. Mueller, and D. Malloch. 1999.** The agricultural pathology of ant fungus gardens. Proc. Natl. Acad. Sci. U.S.A. 96: 7998–8002.

**Currie, C. R., B. Wong, A. E. Stuart, T. R. Schultz, S. A. Rehner, U. G. Mueller, G.-H. Sung, J. W. Spatafora, and N. A. Straus. 2003.** Ancient tripartite coevolution in the attine ant–microbe symbiosis. Science 299: 386–388.

**Demain, A. L. 1986.** Regulation of secondary metabolism in fungi. Pure Appl. Chem. 58: 219–226.

**Elizalde, L. 2009.** Biogeografía y comunidades de fóridos parasitoides de hormigas cortadoras de hojas: diversidad del sistema y partición del recurso hospedador. Ph.D. dissertation, Universidad Nacional de Quilmes, Buenos Aires, Argentina.

**Elizalde, L., and P. J. Folgarait. 2010.** Host diversity and environmental variables as determinants of the species richness of the parasitoids of leaf-cutting ants. J. Biogeogr. 37: 2305–2316.

**Farji Brener, A., and A. Ruggiero. 1994.** Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range sizes. J. Biogeogr. 21: 391–399.

**Folgarait, P. J. 1998.** Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers. Conserv. 7: 1221–1244.

**Folgarait, P. J., N. B. Gorosito, M. Poulsen, and C. R. Currie. 2008.** Interacciones entre microorganismos simbiontes de hormigas cortadoras de hojas: importancia para su control biológico. VI Congreso Latinoamericano de Micología, Mar del Plata, Argentina.

**Gerardo, N. M., and E. J. Caldera. 2007.** Labile associations between fungus-growing ant cultivars and their garden pathogens. The ISME J. 1: 373–384.

**Gerardo, N. M., U. G. Mueller, S. L. Price, and C. R. Currie. 2004.** Exploiting a mutualism: parasite specialization on cultivars within the fungus-growing ant symbiosis. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 271: 1791–1798.

**Gerardo, N. M., S. R. Jacobs, C. R. Currie, and U. G. Mueller. 2006.** Ancient host-pathogen associations maintained by specificity of chemotaxis and antibiosis. PloS Biol. 4: 1358–1363.

**Haeder, S., R. Wirth, H. Hertz, and D. Spiteller. 2009.** Candicidin-producing *Streptomyces* support leaf-cutting ants to protect their fungus garden against the pathogenic fungus *Escovopsis.* Proc. Natl. Acad. Sci. U.S.A. 106: 4742–4746.

**Hicks, J. K., J. Yu, N. P. Keller, and T. H. Adams. 1997.** *Aspergillus* sporulation and mycotoxin production both require inactivation of the FadA G$\alpha$ protein-dependent signaling pathway. The EMBO J. 16: 4916–4923.

**Hölldobler, B., and E. O. Wilson. 1990.** The ants. Harvard University Press, Cambridge, Massachusetts.

**Little, A.E.F., and C. R. Currie. 2008.** Black yeast symbionts compromise the efficiency of antibiotic defenses in fungus-growing ants. Ecology 89: 1216–1222.

**Mandels, M., and E. T. Reese. 1960.** Induction of cellulase in fungi by cellobiose. J. Bacteriol. 79: 816–826.

**Mani, K., and R. N. Swamy. 1983.** Induction of sporulation in *Pestalotiopsis palmarum* by sodium chloride. Trans. Br. Mycol. Soc. 80: 151–156.

**Mikheyev, A. S., U. G. Mueller, and P. Abbot. 2010.** Comparative dating of attine ant and lepiotaceous cultivar phylogenies reveals coevolutionary synchrony and discord. Am. Nat. 175: E126–E133.

**Mueller, U. G., S. A. Rehner, and T. D. Schultz. 1998.** The evolution of agriculture in ants. Science 281: 2034–2038.

**Mueller, U. G., T. R. Schultz, C. Currie, R. Adams, and D. Malloch. 2001.** The origin of the attine ant-fungus symbiosis. Q. Rev. Biol. 76: 169–197.

**Oh, D. C., M. Poulsen, C. R. Currie, and J. Clardy. 2009.** Dentigerumycin: a bacterial mediator of an ant-fungus symbiosis. Nat. Chem. Biol. 5: 391–393.

**Pagnocca, F. C., A. Rodrigues, N. S. Nagamoto, and M. Bacci Jr. 2008.** Yeasts and filamentous fungi carried by the gynes of leaf-cutting ants. Antonie van Leeuwenhoek 94: 517–526.

**Pinto-Tomás, A. A., M. A. Anderson, G. Suen, D. M. Stevenson, F.S.T. Chu, W. W. Cleland, P. J. Weimer, and C. R. Currie. 2009.** Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. Science 326: 1120–1123.

**Poulsen, M., M. J. Cafaro, D. P. Erhardt, A.E.F. Little, N. M. Gerardo, B. Tebbets, B. S. Klein, and C. R. Currie. 2010.** Variation in *Pseudonocardia* antibiotic defence helps govern parasite-induced morbidity in *Acromyrmex* leaf-cutting ants. Environ. Microbiol. 2: 534–540.

**Reynolds, H. T., and C. R. Currie. 2004.** Pathogenicity of *Escovopsis weberi:* the parasite of the attine ant-microbe symbiosis directly consume the ant-cultivated fungus. Mycologia 96: 955–959.

**Rodrigues, A., F. C. Pagnocca, M. Bacci Jr., M.J.A. Hebling, O. C. Bueno, and L. H. Pfenning. 2005.** Variability of non-mutualistic filamentous fungi associated with *Atta sexdens rubropilosa* nests. Folia Microbiol. (Praha), 50: 421–425.

**Savoie, J. M., G. Mata, and C. Billette. 1998.** Extracellular laccase production during hyphal interactions between *Trichoderma* sp. and Shiitake, *Lentinula edodes.* Appl. Microbiol. Biotechnol. 49: 589–593.

**Schmidt-Heydt, M., N. Magan, and R. Geisen. 2008.** Stress induction of mycotoxin biosynthesis genes by abiotic factors. FEMS Microbiol. Lett. 284: 142–149.

**Shimizu, K., and N. P. Keller. 2000.** Genetic involvement of a cAMP-dependent protein kinase in a G protein signaling pathway regulating morphological and chemical transitions in *Aspergillus nidulans.* Genetics 157: 591–600.

**Siegel, S. 1976.** Estadística no paramétrica aplicada a las ciencias de la conducta. Trillas, México.

**Sonnenbichler, J. A., J. Dietrich, and H. Peipp. 1994.** Secondary fungal metabolites and their biological activities, V. Investigations concerning the induction of the biosynthesis of toxic secondary metabolites in Basidiomycetes. Biol. Chem. 375: 71–80.

**STATISTIX for Windows. 1998.** User's Manual. Analytical Software, Tallahassee, FL.

**Suen, G., J. J. Scott, F. O. Aylward, S. M. Adams, S. G. Tringe, A. A. Pinto-Tomás, C. E. Foster, M. Pauly, P. J. Weimer, K. W. Barry, et al. 2010.** An insect herbivore microbiome with high plant biomass-degrading capacity. PLoS Genet. 6: e1001129.

**Taerum, S. J., M. J. Cafaro, A.E.F. Little, T. R. Schultz, and C. R. Currie. 2007.** Low host-pathogen specificity in the leaf-cutting ant-microbe symbiosis. Proc. R. Soc. Lond., B, Biol. Sci. 271: 1971–1978.

**Tsujiyama, S. I., and M. Minami. 2005.** Production of phenol-oxidizing enzymes in the interaction between white-rot fungi. Mycoscience 46: 268–271.

**Whipps, J. M. 2001.** Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. 52: 487–511.

**White, N. A., and L. Boddy. 1992.** Extracellular enzyme localization during interspecific fungal interactions. FEMS Microbiol. Lett. 98: 75–7.

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