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**Ant-produced chemicals are not responsible for the specificity of their *Ophiocordyceps*
fungal pathogens**

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Abstract

In this study, we investigated the effects of exocrine glandular extracts (mandibular glands, post-pharyngeal glands) and cuticular extracts of two species of formicine ant *Polyrhachis furcata* and *Colobopsis saundersi* on spore germination of two species of host-specific, entomopathogenic fungi *Ophiocordyceps unilateralis* (*O. polyrhachis-furcata* and *O. camponoti-saundersi*). Extracts from the two ant species inhibited the germination of *O. polyrhachis-furcata*, but did not affect the germination of *O. camponoti-saundersi*, invalidating the hypothesis of specificity mediated by exocrine glands. We also showed that metapleural gland extracts—well known for their anti-microbial activities—from ants not targeted by *O. unilateralis*, inhibited spore germination. Finally, we tested the effect of the glands of the two formicine ants on *Beauveria bassiana*, a widespread pathogen of insects. Germination of *B. bassiana* was stimulated by most extracts, suggesting that insect chemicals are used as signals for germination by this fungus. The chemical characterisation of ant glandular extracts was also performed.

Keywords

host-pathogen specificity, chemical ecology, spore germination, mandibular gland, exploding ants, *Colobopsis*, *Polyrhachis*, *Ophiocordyceps unilateralis*, *Beauveria bassiana*, zombie ants

1. Introduction

The species complex *Ophiocordyceps unilateralis sensu lato* is a group of entomopathogenic fungi that is specialized on ants of the tribe Camponotini (Hymenoptera, Formicidae), in particular of the genera *Camponotus*, *Colobopsis*, *Dinomyrmex* and *Polyrhachis*, and constitutes the most common fungal pathogen of these insects. Infected ants leave their colony a few days after infection and fix themselves to the vegetation by biting a leaf or a twig, hanging upside down until death. The fungus then secures the ant's grip on the plant by growing mycelium at the tip of the legs that attaches to the plant. The fungus then produces a stalk from the back of the ant's head from which the reproductive structure (perithecium) develops and spores are then released and fall to the floor. Such manipulation of the host behaviour is believed to increase the developmental success of the stalk and the dispersal capacity of the spores (Andersen et al. 2009; Pontoppidan et al. 2009; Hughes et al. 2011).

Recent studies revealed that each species within the *O. unilateralis* complex is specific to one ant species, suggesting host-driven speciation (Kobmoo et al., 2012; 2015). Patterns of specificity in other associations between fungi and ants have mostly been investigated in fungus-growing attine ants (Poulsen & Boomsma 2005; Mikheyev et al. 2010; Mueller et al. 2010) and ant-plant symbioses (Blatrix et al. 2013; Nepel et al. 2016; Vasse et al. 2017). Previous studies on *O. unilateralis s.l.* focused on species diversity and taxonomy (Evans et al. 2011; Luangsa-ard et al. 2011; Kobmoo et al. 2012, 2015), the ecological factors of epidemics on ants (Pontoppidan et al. 2009; Mongkholsamrit et al. 2012) and the mechanisms of ant-behaviour manipulation (De Bekker et al. 2014, 2015) but the proximal mechanisms of the arms-race between *O. unilateralis* species and the ants are yet to be elucidated.

The first step in the infection of insects by entomopathogenic fungi is the germination of spores on the cuticle. Hydrolytic enzymes of entomopathogenic fungi necessary to the degradation of insect cuticle were shown to be virulence factors (Valero-Jiménez et al. 2016). The degradation of cuticle also brings an initial nutritive ration to the growth and virulence of entomopathogenic fungi (Shah et al. 2006). Ants develop social behaviour to protect themselves against pathogens including allogrooming (Ugelvig & Cremer 2007). Cuticle seems, therefore, to be the place where reciprocal

selection leading to the host-pathogen arms race takes place. Ants have numerous exocrine glands that are involved in various activities such as reproduction, social communication and defence against predators and pathogens (Hölldobler & Wilson, 1990; Billen & Morgan 1998; Morgan 2009, Yek & Mueller 2011). At least six exocrine glands, the mandibular, metapleural, pygidial, poison, Dufour's and sternal glands, are known to play a role in defence against pathogens (Hölldobler and Wilson, 1990; Fernández-Marin *et al.*, 2006). Thus, chemical interaction at the cuticle level is a likely mechanism for explaining fungus-ant specificity, but this still remains to be investigated.

The cuticle of ants harbours various aliphatic hydrocarbons that are used for nestmate discrimination (Lahav *et al.* 1999, Blomquist & Bagnères 2010). Those compounds, produced in the fat body, are concentrated in the postpharyngeal gland and spread on the cuticle (Soroker & Hefetz 2000) through grooming activities. Other compounds produced from other exocrine glands are also spread on the cuticle. The secretion from the metapleural gland, in particular, is spread over the body and confers protection against microbes (Yek & Mueller 2011). More anecdotally, the content of the mandibular gland can reach the cuticle and serve in nestmate discrimination (Sainz-Borgo *et al.* 2013). The postpharyngeal gland, the metapleural gland and the mandibular gland are the most likely source candidates for the chemical cocktail at the surface of the cuticle and, thus, should be targeted in priority when looking for potential effects of ant chemicals on spore germination of entomopathogenic fungi. Interestingly, most ant species of the tribe Camponotini lack a metapleural gland (Yek & Mueller 2011) and the question of how other glands of these ants contribute to the protection against generalist or specialist pathogenic fungi has rarely been investigated (Voegtle *et al.* 2008). The source of chemicals that might potentially inhibit growth of *O. unilateralis s.l.* fungi and other potential entomopathogenic fungi is reduced to the postpharyngeal and mandibular gland. Several ant species that are hosts of these fungi belong to the so-called "exploding ants" of the genus *Colobopsis*, having mandibular glands with a hypertrophied reservoir (Davidson *et al.* 2012) used in territorial fights against other ants. The explosion of the reservoir (Maschwitz & Maschwitz 1974), and subsequent release of noxious and sticky compounds from the gland (Jones *et al.* 2004) lead to the death of the

opponent and the ants that try to rescue it. An antifungal role of these compounds is not excluded (Voegtle et al. 2008, Davidson et al. 2009).

The aim of this study was to test the hypothesis that ant-produced chemicals are responsible for the host specificity of *O. unilateralis s.l.* fungi. The two fungal species *O. camponoti-saundersi* and *O. polyrhachis-furcata* are specific pathogens of the ant species *Colobopsis saundersi* (an exploding ant) and *Polyrhachis furcata* respectively (Kobmoo et al. 2012). Although the two fungi and their hosts can be found in the same environments, cross-infection has never been detected in the field. Cross infection through direct injection in the laboratory showed that *O. unilateralis* species can kill unspecific ants but never induce behavior manipulation (De Bekker et al. 2014). We tested the effect of cuticular extracts and extracts of mandibular and postpharyngeal glands of the two host ant species on the germination of spores of each of the two *Ophiocordyceps* species. Under the hypothesis of a specificity driven by ant chemicals, we expected that spore germination would be inhibited by chemicals of the non-host species, but not by chemicals of the host species. At a broader taxonomical scale, as only formicine ants are targeted by *O. unilateralis*, we tested whether the metapleural glands of *Dolichoderus* sp. and *Pheidole* sp., which can be found in the same habitat as both *O. unilateralis* species, could inhibit the germination of this fungus. Finally, we also tested the effect of the ant extracts on the germination of spores of *Beauveria bassiana*, with the expectation that they would have little or no inhibitory effect on this generalist entomopathogenic fungus. We identified the chemical content of the ant-extracts to strengthen our interpretation of the patterns of the spore germination experiments.

2. Materials and methods

2.1. Chemical extracts from ants

Colonies of *Polyrhachis furcata*, *Colobopsis saundersi*, *Pheidole* sp. and *Dolichoderus* sp. were collected in Khao Yai National Park, Thailand. Ants were killed by freezing and kept at -20 °C

until use. Glandular extracts were obtained by dissecting ants in distilled water under a stereomicroscope and placing the appropriate gland in solvent (10 glands in 500 μ l). To increase the range of compounds extracted, two solvents were used separately: pentane and a mix of dichloromethane + methanol (9:1 by volume). The mandibular gland and the postpharyngeal gland were dissected from *P. furcata* and *C. saundersi*. The metapleural gland was dissected from *Pheidole* sp. and *Dolichoderus* sp. The cuticular extracts of *P. furcata* and *C. saundersi* were obtained by soaking 10 individual ants into 500 μ l of solvent for 10 min. The extracts were kept at -20 °C.

2.2. Germination tests with spores of *Ophiocordyceps* spp.

Specimens of the two focal fungal species, *Ophiocordyceps camponoti-saundersi* and *O. polyrhachis-furcata*, at the fruiting stage were collected in Khao Yai National Park. Spore isolation was done within 2 - 6 h after collecting. Each specimen was fixed to the lid of a Petri dish using vaseline or tape. The lid was placed over the dish filled with Potatoes Dextrose Agar (PDA) so that released spores fell on the PDA. Plates were kept in a moist chamber at 20 °C for 2 – 3 d. The presence of spores on the PDA was checked every 6 h. The timing of spore discharge is very narrow (6 – 12 h) in these species. Consequently, out of 30 specimens of each species collected in the field, only 12 of *O. camponoti-saundersi* and 15 of *O. polyrhachis-furcata* could be used in this experiment.

To test for the effect of extracts on spore germination, Petri dishes were prepared by dropping 50 μ l of extract in pentane + 50 μ l of extract in dichloromethane-methanol on the solid culture medium (PDA), i.e. two glands equivalent, and letting the solvent evaporate for at least 5 min. Extracts naturally spread over the surface of the PDA, forming a disc of c. 2 cm in diameter on which 10 single spores from a single fungal specimen were transferred manually under a stereomicroscope. The number of germinated spores was recorded every day for 5 d. Spore germination was tested for each fungus specimen, for each of the following conditions: untreated PDA (negative control), 50 μ l of pentane + 50 μ l of dichloromethane-methanol without gland extract (negative control), 100 μ l of a commercial fungicide (Fungizone) at the concentration of 0.1 per volume (positive control), extract of the mandibular gland from *P. furcata*, extract of the post-pharyngeal gland from *P. furcata*, cuticular

extract of *P. furcata*, extract of the mandibular gland from *C. saundersi*, extract of the post-pharyngeal gland from *C. saundersi*, cuticular extract of *C. saundersi*. The extracts of the metapleural gland of *Dolichoderus* sp. and *Pheidole* sp. were only tested against the spores of *O. polyrhachis-furcata*.

2.3. Germination tests with spores of *Beauveria bassiana*

We used the 10 following strains of *B. bassiana* from the BIOTEC Culture Collection: BCC 1666, BCC 2660, BCC 8606, BCC 13922, BCC 18191, BCC 30545, BCC 31604, BCC 31670, BCC 37938 and BCC 39761. One week before testing, strains were transferred onto new PDA using a cork borer no.3. Single spores were isolated using the streak plate method. For each of the 10 strains, 10 spores were transferred onto water agar plates that were prepared under the same conditions described above (except for the extracts of metapleural gland that were not tested). As *B. bassiana* is a very fast growing species, the number of germinated spores was recorded every six hours for 24 h.

To test for the effect of treatment and time (i.e. day or hour of observation) and accounting for the variation of performance between strains, we performed analysis of variance on linear models with repeated measurements, using generalized least-squares fitted on the data (one model for each fungal species). Pairwise comparisons were tested using least-squares means. The Bonferroni correction for multiple testing was applied. Statistical analyses were performed using RStudio vision 1.0.136 (R Core Team, 2016).

2.4. Identification of compounds from ant extracts

Gas chromatography-mass spectrometry analysis was performed on the pentane and dichloromethane-methanol extracts of mandibular and postpharyngeal glands and cuticular extracts of three colonies for each of the two focal ant species (10 glands of each type per colony per vial, see 2.1 for the extraction). Before analysis, 30 µg of biphenyl were added as an internal standard to each vial. For samples with a very low concentration of compounds, the content of the vial was evaporated under N₂ to c. 50 µl (i.e. 10 times concentration). Extracts were analysed using a gas chromatograph-mass

spectrometer Shimadzu QP2010plus (Kyoto, Japan) with a potential of 70 eV for ionization by electron impact. The temperature of the transfer line and the ion source were programmed to 280°C and 200°C respectively. The spectrometer was used in scan mode, from 38 to 450 m/z ratio. 1 µl of the solvent extract was Auto-sampler introduced (AOC20i, Shimadzu) in a Split/splitless injector, temperature set to 250°C, with a ¼ split ratio. Gas chromatography was performed using a 30m x 0.25mm x 0.25µm Optima-5MS (Macherey-Nagel, Düren, Germany) fused silica capillary column. The column was temperature programmed as follows: 60°C (hold 2 min.), from 60°C to 220°C at 6°C/min and finally upped to 280°C at 10°C/min and hold 2 min. The carrier gas was helium with a constant linear velocity set close to 36.3 cm/s. We injected a series of pure n-Alkanes for calculating retention indices. All components were area integrated using the GC-MS solution software (Shimadzu, Kyoto, Japan) and identified by comparison with mass spectral library NIST 2005, retention indices founds in the literature (Adams 2007) and a free database (www.pherobase.com). Compound quantification was performed by computing peak area of each compound relatively to the area of the peak of Biphenyl which was attributed an arbitrary value of 1000. This allowed quantitative comparison among samples.

3. Results

3.1. Spore germination tests

Regarding germination tests, the linear models fitted on the data for *O. camponoti-saundersi*, *O. polyrhachis-furcata* and *B. bassiana* showed a significant effect of time ($F = 62, 91$ and 325 for the three species respectively, $p < 0.0001$) and treatment ($F = 11, 27$ and 26 for the three species respectively, $p < 0.0001$) (Figure 1). The effects of the two negative controls (PDA only and PDA with solvent) on spore germination did not differ significantly (Figure 2). For pairwise comparisons we compared each treatment with the negative control (PDA with solvent). All the treatments inhibited the germination of the spores of *O. polyrhachis-furcata* including the metapleural gland extracts from

Dolichoderus sp. and *Pheidole* sp. that were tested only on the spores of this species. Spore germination inhibition levels were similar for metapleural glands and extracts from the two focal ant species (Figure 1 and 2). In contrast, only Fungizone inhibited the germination of the spores of *O. camponoti-saundersi* (Figures 1 and 2). Cuticular extracts of *C. saundersi* and *P. furcata* showed a tendency to inhibit germination in *O. camponoti-saundersi*, but the effect was not significant (Figure 2). Only Fungizone and the extract of mandibular gland of *C. saundersi* inhibited the germination of spores of *B. bassiana* (Figures 1 and 2). In contrast, all the other extracts tended to stimulate germination in this fungus (Figure 1). Stimulation of germination was significant for all the extracts from *P. furcata*, but only marginally significant for the extract of the postpharyngeal gland of *C. saundersi* and non-significant for the cuticular extract of *C. saundersi* (Figure 2).

3.2. Chemical content of ant extracts

For the identification of ant compounds, we used only dichloromethane-methanol extracts as pentane extracts contained higher levels of contamination making identification less reliable.

The cuticular extract of *P. furcata* was mostly composed of a series of unpaired alkanes (from tridecane to tricosane) and traces of paired alkanes (Table 1). The postpharyngeal gland contained only traces of the compounds from the cuticle. The mandibular gland contained mainly 4-methyl-3-heptanone, and traces of a few other compounds.

In comparison, the mandibular gland of *C. saundersi* had a higher diversity and larger quantities of compounds (Table 1). The three major compounds were (in order of increasing quantity): an unknown phenolic compound (95; 41/50; 67/50; 136/40; 164/30; 182/5); 2,4,6-trihydroxyacetophenone; and 2-heptanone. The latter was also present on the cuticle. The cuticle also presented a series of unpaired alkanes (from undecane to heptadecane) and a few alcohols. The postpharyngeal gland contained traces of compounds from the cuticle, as well as pentacosane.

4. Discussion

In this study, we showed that chemical extracts of two ant species, *P. furcata* and *C. saundersi*, inhibited spore germination of *O. polyrhachis-furcata*, the fungal pathogen specific to *P. furcata*. In contrast, the ant extracts had little effect on spore germination of *O. camponoti-saundersi*, the fungal pathogen specific to *C. saundersi*. Interestingly, spore germination of *B. bassiana* was stimulated by most ant extracts.

Initially, we hypothesized that the specificity of these fungal pathogens was driven by the interaction between the chemicals produced by ants and spores at the germination stage, and thus, we expected the spore germination of a focal fungal species to be inhibited by the extracts of the non-host ant species but not by those of the host species. In contrast to our expectations, one of the fungus species, *O. polyrhachis-furcata*, appeared more sensitive to any ant extract than the other fungus species, *O. camponoti-saundersi*. As the two fungi differed in their response to ant extracts and did not support our hypothesis, the chemical interaction between spores and ants in this study must not be responsible for host-pathogen specificity. As *C. saundersi* had higher diversity and quantity of chemicals, including several noxious ones (e. g.: 2,4-dihydroxyacetophenone), than *P. furcata*, its specific fungal pathogen (*O. camponoti-saundersi*) may have been under stronger selection for coping with toxic compounds. This would explain why germination was far less inhibited by ant extracts of *O. camponoti-saundersi* than of *O. polyrhachis-furcata*.

Extracts of the metapleural glands of *Dolichoderus* sp. and *Pheidole* sp., two ant species that have never found to be infected by *O. unilateralis s.l.* in nature, inhibited the germination of *O. polyrhachis-furcata* spores and also at a similar level to Fungizone. These results are not surprising because the chemical secretion from metapleural glands of ants were largely reported to be antiseptic or antimicrobial (Attygalle et al 1989, Morgan 2008, Billen et al 2011, Yek and Mueller 2011, Vieira et al 2012).

We had unexpected results for *Beauveria bassiana* which is a generalist entomopathogenic fungus that has a large range of hosts. Generalist pathogens are expected to cope with a larger diversity of chemical compounds than specialist species. Indeed, only commercial fungicides and extracts of the mandibular gland of *C. saundersi* inhibit the spore germination of *B. bassiana*. Our data show that most ant extracts stimulated *B. bassiana* germination. In effect, *B. bassiana* can grow on a

wide range of hydrocarbons as the sole source of carbon, including some of the ones described from the ants in this study; various compounds including fatty acids found on insect cuticles can affect virulence (Pedrini et al. 2010, Pedrini et al. 2013, Ortiz-Urquiza et al. 2016). We propose two possible explanations for this surprising result: (1) *B. bassiana* uses ant chemicals as a trigger for germination because they indicate suitable conditions (the presence of a host), (2) spores of *B. bassiana* use ant surface chemicals as a source of carbon, required for germination (Smith & Grula 1981, Hunt et al. 1984).

Among all the glands studied, the mandibular gland of *C. saundersi* had the strongest inhibitory effect: it strongly inhibited germination of *O. polyrhachis-furcata* and it was the only gland that inhibited germination of *B. bassiana*. Its chemical composition is highly peculiar, and several compounds may explain its strong activity. First, 2-heptanone is one of the major compounds and is known to inhibit growth and germination of a wide range of fungi, such as species in the genera *Pythium*, *Rhizoctonia*, *Diplodia*, *Fusarium*, *Penicillium*, *Gliocladium*, *Cunninghamella* and *Paecilomyces* (Cole et al. 1975). It is a minor component of the clove buds oil that inhibits growth of the human pathogens, *Candida* spp. (Chaieb et al, 2007). It is a major component of the *Pittosporium undulatum* essential oil that inhibits growth and aflatoxin production in *Aspergillus flavus* (da Silva Medeiros et al., 2011). Second, four phenolic compounds represent, by far, the main constituents of the gland. The two major compounds are an unidentified phenolic and the phenolic ketone 2,4,6-trihydroxyacetophenone. Two other phenolic ketones are present in less quantities: 2,4-dihydroxyacetophenone and 2-methyl-5,7-dihydroxychromone. These phenolics are irritants and would likely injure the opponent when a *C. saundersi* worker burst. They are also responsible for the bright whitish colour of the content of the mandibular gland, which can be seen through the cuticle and is believed to serve as an aposematic signal (Jones et al. 2004). The three identified phenolic ketones have already been reported in other species of exploding ants (Jones et al. 2004). Although these compounds evolved most likely for territorial defence, they could also be involved in protection against pathogens (Voegtle et al. 2008, Davidson et al. 2009). The various exploding ant species studied by Jones et al. (2004) differed in their chemical composition. The differences most probably reflect species-specific differences. The taxonomy of exploding ants is in need of revision. In

particular, the taxon *C. saundersi* is ill-defined. A better knowledge of species delineation would probably lead to the discovery of many new chemical compounds. The diversity of DNA markers used in taxonomy is also higher within the species *O. camponoti-saundersi* than within *O. polyrhachis-furcata* (Kobmoo et al. 2012). This may reflect diversity arising through the coevolution with the ant *C. saundersi*. This coevolution could have led to higher diversity of compounds produced by the ant and a higher overall resistance to ant extracts as previously discussed. Furthermore, exploring the effect of such compounds on *Beauveria bassiana* could provide new avenues for understanding the mechanisms of insect resistance to this fungus used for insect biocontrol.

Extraction with dichloromethane/methanol (9:1) should have yielded fatty acid if they were present. It is surprising that we did not detect any. However, a previous study (Jones et al. 2004) showed that extractions (using methanol) from nine species of the *cylindricus* complex (to which *Colobopsis saundersi* belongs) yielded fatty acids in four species only. In the five others no fatty acid was detected. It seems that the presence of fatty acids in this species complex is variable.

The reservoir of the mandibular gland of *P. furcata*, in contrast to that of *C. saundersi*, is much smaller but contains 4-methyl-3-heptanone, a compound known to inhibit growth and germination of a wide range of fungi (Cole et al. 1975). The presence of this compound might explain the inhibitory activity of this gland on germination of spores of *O. polyrhachis-furcata*.

The cuticular extracts of the two ant species tend to inhibit germination of the two fungus species, although the effect was not statistically significant for *O. camponoti-saundersi*. Cuticular extracts of *P. furcata* and *C. saundersi* contain 4-methyl-3-heptanone and 2-heptanone respectively, two compounds that are present in the mandibular gland of the respective ant species, and that have anti-fungal properties (Cole et al. 1975). Pentadecane is found in reasonable quantities in cuticular extracts of both ants and is known to reduce mycelial growth of *Fusarium oxysporum* (Yuan et al. 2012). It is a likely candidate for the inhibitory effect of cuticular extracts because inhibition of the germination of *O. camponoti-saundersi* tended to be stronger with cuticular extract than with mandibular gland extracts. It is interesting to note that pentadecane has previously been shown to have no effect on *B. bassiana* while cuticular quinones were inhibitory (Pedrini et al. 2015).

The postpharyngeal gland of the two ant species revealed very low amounts of compounds, but still, inhibited spore germination in *O. polyrhachis-furcata*. These glands contain pentadecane, which could be responsible for the inhibitory effect. Alternatively, they may contain compounds that were not detected by Gas Chromatography-Mass Spectrometry analysis.

Although we failed to demonstrate a chemical basis for the specificity of *Ophiocordyceps* fungi to their ant host, our results revealed a pattern of germination inhibition that could be partially explained by the nature of ant-produced chemicals. Other exocrine glands than those investigated could be tested. Alternatively, the germination phase might not be the stage when species-specificity is achieved. Fungal growth within the host body and manipulation of host behaviour require fine-tuned mechanisms to be achieved appropriately. Recent studies showed species-specific deployments of metabolomes by *O. unilateralis* inside the hosts' body (De Bekker et al. 2014). The screening mechanism of the successful pathogen species should now be investigated at these stages of the parasite life-history.

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Table 1: Compounds identified by gas-chromatography mass-spectrometry in the chemical extracts of the ants *Polyrhachis furcata* and *Colobopsis saundersi*. CC: cuticular extracts, PPG: postpharyngeal gland, MG: mandibular gland. Units are relative to an internal standard composed of 30 μg of biphenyl, which was attributed an arbitrary value of 1000. Values are averages over three colonies of each ant species.

Name	Retention time (min)	Retention index	<i>Polyrhachis furcata</i>			<i>Colobopsis saundersi</i>		
			CC	PPG	MG	CC	PPG	MG
2-Heptanone	5.853	884	0	0	0	88	5	1327
2-Heptanol	6.115	897	0	0	0	0	0	83
4-Methyl-3-Heptanone	6.838	928	8	7	99	0	0	0
Undecane	11.11	1100	0	0	1	527	15	134
Tridecene	15.77	1292	1	0	0	0	0	0
Tridecane	15.935	1300	65	3	2	35	5	4
Tetradecane	18.177	1400	0	0	0	0	1	0
Pentadecene	19.957	1484	1	1	1	0	0	0
Pentadecane	20.302	1500	139	7	7	103	11	8
2,4-Dihydroxyacetophenone	21.2	1544	0	0	0	0	0	48
Hexadecane	22.32	1600	9	0	0	0	0	0
1-Heptadecene	23.817	1679	24	1	1	0	0	0
Heptadecane	24.215	1700	402	6	8	64	2	8
Unknown compound 2	25.293	1759	0	0	0	0	0	5405
Octadecane	26.028	1800	6	0	0	0	0	0
1-Hexadecanol	27.42	1881	0	0	0	70	0	9
2,4,6-Trihydroxyacetophenone	27.62	1892	0	0	0	0	0	5342
Nonadecane	27.753	1900	206	4	4	0	0	0
2-Methyl-5,7-dihydroxychromone	29.182	1988	0	0	0	0	0	260
Eicosane	29.372	2000	5	0	0	0	3	0
1-Octadecanol	30.612	2089	5	0	0	46	0	8
Heneicosane	30.773	2100	231	4	4	0	0	0
1-Eicosanol	31.988		5	0	0	0	1	0
Tricosane	33.075		299	5	4	0	0	66
Pentacosane	34.98		29	17	0	0	48	108

Figure captions

Figure 1: Evolution through time of the germination on potato dextrose agar of spores of *Ophiocordyceps polyrhachis-furcata* (A and B, separated to increase visibility), of *O. camponoti-saundersi* (C) and of *Beauveria bassiana* (D) exposed to various substances.

Figure 2: Analysis of contrast of spore germination on potato dextrose agar between exposure to various substances and exposure to a negative control (application of solvents), for *Ophiocordyceps polyrhachis-furcata* (A), *O. camponoti-saundersi* (B) and *Beauveria bassiana* (C).

Figure 1

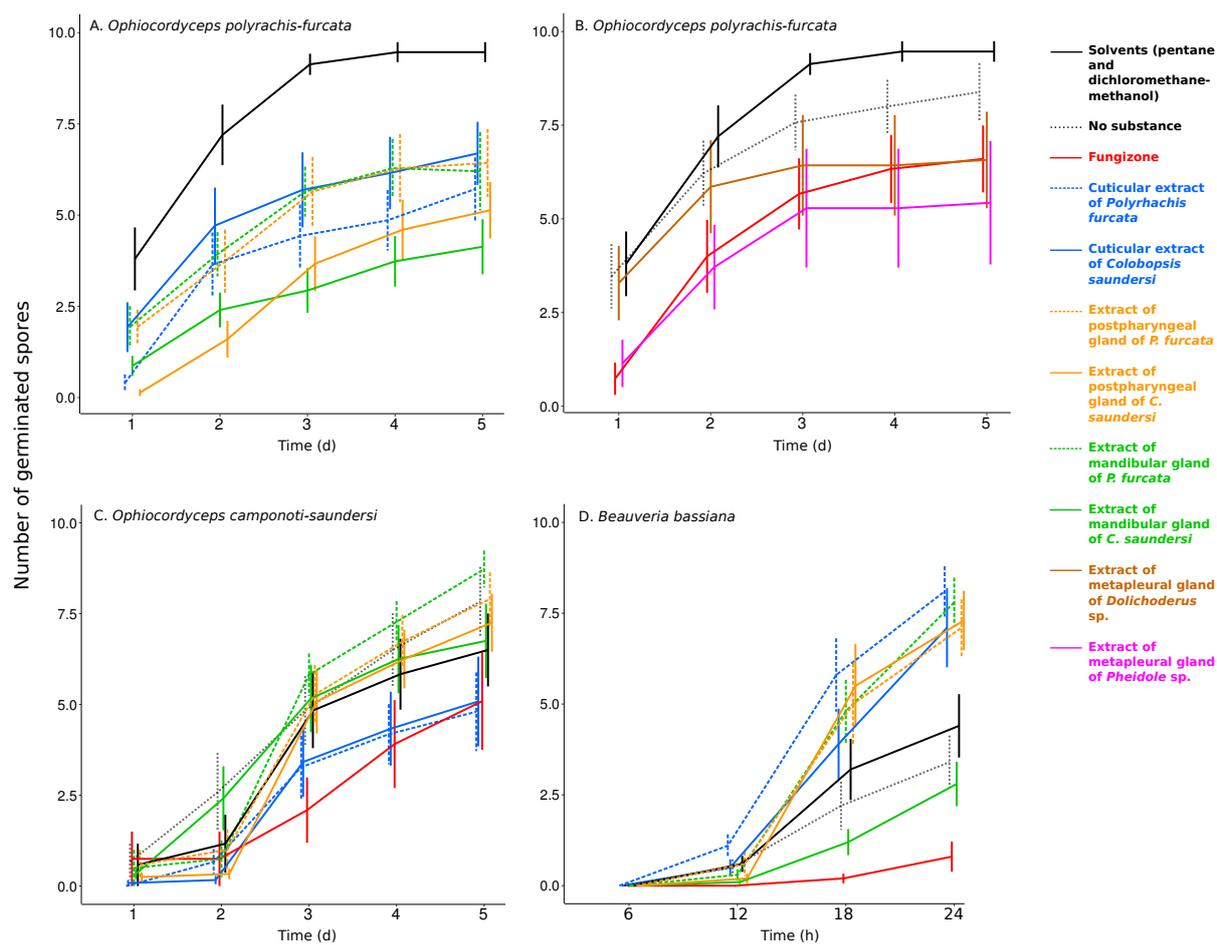


Figure 2

