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INSECTICIDAL PROPERTIES OF MUSHROOM AND TOADSTOOL CARPOPHORES

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Key Word Index—Mushroom; *Drosophila melanogaster*; *Spodoptera littoralis*; antifeedant; insecticides.

Abstract—In order to find compounds with insecticidal or antifeedant properties from mushrooms and toadstools, a wide screening was undertaken using the non-mycophagous *Drosophila melanogaster* as a model insect. Powdered fruit bodies of edible and poisonous mushrooms were incorporated with the *Drosophila*'s rearing medium, and their development was observed. Among the 175 different species of fungi tested, 79 were found to inhibit insect development, hence making the isolation of new compounds look hopeful.

INTRODUCTION

The fruit bodies of numerous species of mushrooms and toadstools are never inhabited by insects while some insects only utilize a restricted number of species of a fungal group [1]. Traditionally, some fungi have been used to control insects. For example, *Lycoperdon* spores have been used to anaesthetize bees, *Amanita muscaria* will kill houseflies when added to sugar solution, and the powder of *Trametes odorata* keeps insects away from clothing [2, 3]. These observations suggest that some fungi could contain repellent, antifeedant or even toxic compounds, possibly active against a wide range of insects.

We present here the results of a screening of 175 fungal species using two insects, *Drosophila melanogaster* and *Spodoptera littoralis*, and show that a large number of the fungi tested (nearly half) were toxic. Toxicity was variable between the different collections, suggesting that ecological conditions and/or physiological states could interfere with the presence of biologically active compounds in mushrooms.

RESULTS AND DISCUSSION

Table 1 shows the number of batches tested and the biology of all the species screened. Table 2 shows a classification of the species toxic on *Drosophila* larvae, according to the minimal lethal dose of fungi powder required to observe a 100% toxicity (LD100). Among the 175 different species tested, a total of 79 (45%) revealed themselves to be toxic for *D. melanogaster*, whereas the 96 remaining ones showed no toxicity at a concentration of 60 mg fungi powder ml^{-1} rearing medium. This concentration roughly corresponds with the freeze-dry ratio for most of the samples and, therefore, gives an immediate idea of the toxicity of the fungus.

Most of the mushrooms that were highly toxic for *D. melanogaster*, were also toxic for *S. littoralis*, but in most cases the LD100 was higher (*cf.* Table 2). The test with *S. littoralis* was less sensitive and it was not possible to add higher amounts of powder to the rearing media. Nearly all of the fungi that were non-toxic for *D. melanogaster* were also non-toxic for *S. littoralis* except for three of them, i.e. *Clitocybe odora*, *Cortinarius calochrous* and *Tricholomopsis rutilans*, which were highly toxic. The LD100 for *D. melanogaster* ranged from 0.1 to 60 mg ml^{-1}. For the more toxic species, the compounds involved could be either highly toxic, or present at high concentrations, or both at the same time. By contrast, for the others, the substances must be less active, or just as active, but only present at low concentrations.

Among the 79 toxic species, nine showed a direct toxicity on the adult flies in at least one batch: *A. phalloïdes*, *Boletus chrysenteron*, *Clitocybe nebularis*, *Hygrophorus chrysodon*, *B. badius*, *Hygrophoropsis aurantiaca*, *Cystoderma cinnabarinum*, *Pulveroboletus lignicola* and *Entoloma lividum*. Mortality was total and occurred four to seven days after the first contact of the adult flies

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Table 1. Species tested

Species	NBT	Biology
Ascomycotines		
Daldinia concentrica	1	L d
Helvella crispa	1	? d/c
Leptopodia atra	1	? d
Gasteromycetes		
Pisolithus tinctorius	1	T d/c
Vascellum pratense	1	T m
Lycoperdon perlatum	2	T d/c
Langermannia gigantea	1	T m
Astraeus hygrometricus	1	T d
Scleroderma meridionale	1	T m
Battaraea phalloïdes	1	? c
Phallus impudicus	2	T d/c
Phragmobasidiomycetes		
Pseudohydnum gelatinosum	1	L d/c
Exidia truncata	1	L d/c
Aphyllophorees		
Albatrellus cristatus	2	M c
Bjerkandera adusta	1	L d
Coltricia perennis	1	L d/c
Daedalea quercina	1	L d
Daedaleopsis confragosa	1	L d
Fistulina hepatica	4	L d
Fomitopsis pinicola	2	L d/c
Ganoderma lucidum	1	L d
*Ganoderma species**	2	L d
Phellodon niger	1	M d
Inonotus hispidus	2	L d
Ischnoderma benzoïnum	1	L d/c
Lenzites betulina	1	L d/c
Meripilus giganteus	2	L d
Merulius tremellosus	1	L d
Phellodon ferruginosum	1	M c
Piptoporus betulinus	4	L d
Polyporus brumalis	1	L d
Albatrellus pescaprae	2	M d
Polyporus squamosus	2	L d
Laetiporus sulfureus	1	L d
Postia species	1	L d
Postia caesia	2	L d
Postia styptica	1	L d/c
Pycnoporus cinnabarinus	1	L d
Sarcodon imbricatus	2	M c
Serpula lacrymans	1	L c
Trametes versicolor	1	L d/c
Cantharellus cibarius	1	M d/c
Cantharellus lutescens	1	M d/c
Cantharellus tubaeformis	1	M c
Clavariadelphus pistillaris	1	? d
Clavulina cinerea	1	? d/c
Clavulina rugosa	1	? d/c
Hydnum repandum	2	M d/c
Gomphus clavatus	1	M d/c
Sparassis laminosa	1	T d
Russulaceae		
Russula atropurpurea	1	M d
Russula chloroides	1	M d/c
Russula densifolia	1	M d
Russula mustelina	1	M c
Russula nigricans	2	M d
Russula olivacea	1	M d/c
Russula sardonia	4	M c
Lactarius blennius	1	M d
Lactarius chrysorheus	1	M d
Lactarius controversus	1	M d
Lactarius decipiens	1	M c
Lactarius deliciosus	8	M c
Lactarius mitissimus	1	M c
Lactarius pubescens	2	M d
Lactarius quietus	1	M d
Lactarius salmonicolor	1	M c
Lactarius scrobiculatus	3	M c
Lactarius torminosus	1	M d
Lactarius vellereus	2	M d
Boletaceae		
Boletus (Bol.) appendiculatus	1	M d
Boletus (Bol.) edulis	2	M d
Boletus (Bol.) erythropus	1	M d
Boletus (Bol.) luridus	2	M d
Boletus (Lec.) corsicus	1	M d
Boletus (Xer.) badius	3	M d/c
Boletus (Xer.) chrysenteron	2	M d
Boletus (Xer.) subtomentosus	1	M d
Pulveroboletus lignicola	1	L c
Boletus (Chal.) piperatus	5	M c
Boletus (Bol.) radicans	1	M d
Boletus (Suill.) granulatus	1	M c
Boletus (Suill.) grevillei	2	M c
Boletus (Suill.) luteus	2	M c
Boletus (Suill.) bellinii	2	M c
Boletus (Suill.) bovinus	1	M c
Strobilomyces floccopus	1	M d
Gomphidiaceae		
Gomphidius glutinosus	5	M c
Chroogomphus rutilus	2	M c
Paxillaceae		
Paxillus involutus	1	M d
Hygrophoropsidaceae		
Hygrophoropsis aurantiaca	2	L d/c
Tricholomaceae		
Armillaria mellea	2	L d
Armillaria ostoyae	2	L c
Clitocybe nebularis	3	T d/c
Clitocybe odora	1	T d/c
Lepista inversa	1	T c
Lepista nuda	3	T d/c
Lepista panaeolus	1	T m
Leucopaxillus giganteus	1	T m
Leucopaxillus paradoxus	1	T d
Tricholoma atrosquamosum	1	M d/c
Tricholoma aurantium	1	M c
Tricholoma bufonium	1	M d/c
Tricholoma caligatum	1	M c
Tricholoma auratum	1	M c

Table 1. (*continued*)

Species	NBT	Biology	
Tricholoma focale	1	M	c
Tricholoma fulvum	1	M	d
Tricholoma imbricatum	1	M	d/c
Tricholoma pseudonictitans	1	M	c
Tricholoma saponaceum	2	M	d/c
Tricholoma sejunctum	1	M	d/c
Tricholoma striatum	3	M	c
Tricholoma sulphureum	1	M	d/c
Tricholoma terreum	1	M	c
Tricholoma vaccinum	1	M	d/c
Tricholomopsis rutilans	3	L	c
Cystoderma amianthinum	1	T	d/c
Cystoderma carcharias	1	T	c
Cystoderma cinnabarinum	1	T	d
Phaeolepiota aurea	1	T	d
Laccaria amethystea	1	M	d/c
Laccaria laccata	1	M	d/c
*Pleurotus ostreatus**	1	L	d
Lentinus tigrinus	1	L	d
Marasmiaceae			
Oudemansiella longipes	1	T	d
Oudemansiella radicata	1	T	d
Collybia butyracea	1	T	d/c
Collybia maculata	2	T	d/c
Collybia peronata	1	T	d/c
Megacollybia platyphylla	1	L	d/c
*Flammulina velutipes**	1	L	d
Marasmius alliaceus	2	T	d
Marasmius oreades	1	T	m
Mycena pura	1	T	d/c
Hygrophoraceae			
Hygrophorus agathosmus	2	M	c
Hygrophorus chrysodon	2	M	d/c
Hygrophorus limacinus	1	M	c
Hygrophorus niveus	2	T	m
Hygrophorus pudorinus	2	M	c
Amanitaceae			
Amanita citrina	2	M	d/c
Amanita junquilla	1	M	d/c
Amanita muscaria	1	M	d/c
Amanita ovoïdea	1	M	d/c
Amanita pantherina	1	M	d/c
Amanita phalloïdes	1	M	d
Amanita rubescens	1	M	d/c
Agaricaceae			
Agaricus romagnesii	1	T	d/c
Agaricus xanthoderma	3	T	d/c
Lepiota ignivolvata	1	T	d/c
Macrolepiota rhacodes	1	T	d/c
Cortinariaceae			
Cortinarius aleuriosmus	1	M	d
Cortinarius auricilis	1	M	d
Cortinarius calochrous	1	M	d
Cortinarius elatior	1	M	d
Cortinarius evernius	1	M	c
Cortinarius ionochlorus	1	M	d
Cortinarius largus	1	M	d
Cortinarius malicorius	1	M	c
Cortinarius purpurascens	1	M	d/c
Cortinarius semisanguineus	1	M	c
Cortinarius venetus	1	M	d/c
Hebeloma crustiliniforme	1	M	d
Hebeloma sinapizans	1	M	d
Rozites caperata	2	M	d
Inocybe calamistrata	1	M	c
Strophariaceae			
Gymnopilus spectabilis	1	L	d
Pholiota destruens	1	L	d
Pholiota lenta	1	L	d
Pholiota squarrosa	1	L	d
Stropharia aeruginosa	1	T	d/c
Hypholoma fasciculare	3	L	d/c
Hypholoma sublateritium	2	L	d/c
Coprinaceae			
Coprinus comatus	1	T	m
Rhodophyllaceae			
Clitopilus prunulus	2	?	d
Entoloma lividum	1	M	d
Entoloma nidorosum	1	M	d

NBT: number of batches tested; L: lignicolous; T: terrestrial; M: mycorrhizian; d: deciduous tree; c: coniferous; m: meadow.

with larval medium. This mortality was observed for higher doses than the ones stated in Table 2, which gives only toxicity on larvae. The compounds involved here are, therefore, of an insecticidal nature.

Variability was observed between batches of the same species, but corresponding to a different harvest; 40% of the batches showed an at least three-fold varying toxicity. Likewise, of the 48 species that have also been tested in a former screening by Besl *et al.* [4], 14 species gave a different result. It can be noted here that four of our most toxic species (*A. phalloïdes*, *Xerocomus chrysenteron*, *X. badius* and *Megacollybia platyphylla*) were also found to be highly toxic by Besl *et al.* [4]. On the other hand, *A. muscaria* and *A. pantherina*, considered as toxic,

Table 2. Toxicity of fungi on insects

	Drosophila				Spod. H	
Fungus	LD100	nbt	var.	Th.	1	
Amanita phalloïdes	0.1	1	—	R	2	yes
Boletus (Xer.) *chrysenteron*	0.5	2	no	R/S	30	no
Boletus (Bol.) *radicans*	2	1	—	S	16	no
Clitocybe nebularis	3	3	yes	R	20	?
Hygrophorus chrysodon	3	2	yes	R	> 29	no
Clitopilus prunulus	4	2	no	S	23	no
Hygropphorus niveus	5	2	no	S	> 17	no
Lepista inversa	5	1	—	R	10	no
Megacollybia platyphylla	5	1	—	—	—	no
Tricholoma sulphureum	5	1	—	R	—	no
Lepista nuda	5	3	no	R	> 22	no
Hygrophoropsis aurantiaca	6	2	no	S	> 23	no
Boletus (Xer.) *badius*	8	4	no	S	36	no
Leucopaxillus paradoxus	8	1	—	S	26	no
Hebeloma sinapizans	8	1	—	R	6	?
Ganoderma species	8	2	no	—	> 27	no
Polyporus squamosus	10	2	no	S	> 33	no
Cystoderma cinnabarinum	10	2	yes	—	> 16	no
Cystoderma amianthinum	10	1	—	S	> 23	no
Boletus (Bol.) *edulis*	10	2	yes	S	31	no
Lepista panaeolus	10	1	—	R	23	no
Cystoderma carcharias	10	1	—	S	> 23	no
Lepiota ignivolvata	10	1	—	S	> 19	no
Boletus (Suil.) *bovinus*	12	1	—	S	> 24	no
Boletus (Xer.) *subtomentosus*	12	1	—	S	26	no
Serpula lacrymans	12	1	—	S	28	no
Pulveroboletus lignicola	13	1	—	R	24	?
Phaelepiota aurea	14	1	—	S	> 25	no
Tricholoma saponaceum	15	2	yes	R	> 21	?
Cortinarius semisanguineus	15	1	—	R	> 18	?
Agaricus romagnesii	17	1	—	R	27	yes
Cortinarius aleuriosmus	17	1	—	S	24	no
Piptoporus betulinus	20	4	yes	S	—	no
Cantharellus tubaeformis	20	2	no	—	> 11	no
Agaricus xanthoderma	20	3	yes	S	> 26	yes
Entoloma lividum	20	1	—	S	34	yes
Albatrellus cristatus	21	3	—	S	> 42	no
Boletus (Bol.) *luridus*	23	2	—	S	21	no
Tricholoma focale	25	2	—	S	17	no
Boletus (Bol.) *erythropus*	29	3		R	> 38	no
Cortinarius purpurascens	29	1		S	> 33	no
Hebeloma crustiliniforme	30	1		—	> 19	yes
Cortinarius malicorius	30	1		—	—	no
Amanita citrina	30	3		S	23	no
Clavulina cinerea	30	1		S	> 22	no
Tricholoma equestre	30	1		S	24	no
Macrolepiota rhacodes	30	1		R	> 22	no
Tricholoma sejunctum	35	1		—	16	no
Boletus (Lec.) corsicus	38	1		—	> 29	no
Vascellum pratense	38	1		S	> 20	no
Lenzites betulina	40	1		S	> 25	no
Russula chloroides	40	1		—	> 21	no
Polyporus pescaprae	40	2		S	26	no
Chroogomphus rutilus	40	1		S	—	no
Pholiota destruens	40	1		—	> 17	no
Gymnopilus spectabilis	40	2		S	> 12	?
Helvella crispa	40	1		S	—	yes
Pisolithus tinctorius	42	1		R	> 29	no
Pseudohydnum gelatinosum	42	1		—	—	no

Table 2. (*continued*)

	Drosophila				Spod.	H
Fungus	LD100	nbt	var.	Th.	1	
Tricholoma pseudonictitans	42	1		S	—	no
Mycena pura	42	1		S	> 23	no
Pleurotus ostreatus	45	1		S	> 17	no
Leptopodia atra	45	1		S	> 29	no
Laccaria laccata	45	1		S	> 27	no
Stropharia aeruginosa	50	1		—	> 20	?
Phellodon ferruginosum	50	2		S	> 29	no
Lentinus tigrinus	50	1		—	—	no
Hypholoma sublateritium	52	1		S	> 28	?
Amanita rubescens	53	1		S	> 29	no
Collybia butyracea	60	1		S	> 18	no
Entoloma nidorosum	60	1		—	> 8	?
Cortinarius venetus	60	2		R	25	no
Pholiota squarrosa	60	1		S	—	no
Boletus (Suil.) *grevillei*	60	2		S	> 20	no
Collybia maculata	60	2		S	28	no
Inocybe calamistrata	60	2		—	—	no
Fomitopsis pinicola	60	3		S	—	no
Russula nigricans	60	4		S	> 23	no
Tricholoma striatum	60	2		—	> 23	no

LD100: minimal lethal dose required to obtain 100% toxicity; nbt: number of batches tested; var: variability; 'yes' means that the minimal toxic dose differed at least 3-fold between two batches (therefore it could only be estimated for toxicities $< 20\ mg\,ml^{-1}$); Th: thermolability; R: resistant; S: sensitive; Spod: LD100 for *Spodoptera littoralis* indicates the highest dose tested without toxicity; H: toxicity for humans.

appear inactive in our results, whereas *H. aurantiaca*, belonging to our more active species, appeared inactive in their study [4]. Artefacts could be a factor since mushrooms and toadstools are well known to accumulate heavy metals or other toxic substances. This pollution may be responsible for some toxicities observed. The variability may also have a natural origin. Such a variability has already been observed in plants studied for their insecticidal properties [5]. Activity may depend on time or place of harvest. The physiological state of the fungus is important [6] and sometimes defences increase according to insect attacks [7]. Furthermore, several ecotypes could exist, indistinguishable by morphological characteristics, in different geographical regions.

Toxic compounds have already been isolated from higher fungi screened here. Amatoxins and phallotoxins are highly toxic cyclopeptides, which can be found essentially within the genus *Amanita* [8], and recent HPLC determination has shown that up to five amatoxins with six phallotoxins can be identified in a single *A. phalloïdes* toadstool [9]. Amatoxins are known to inhibit irreversibly RNA polymerase II [10] and are, therefore, toxic to all eukaryotes with the exception of a few mycophagous insects [11]. These compounds are, therefore, most likely responsible for the toxicity observed with *A. phalloïdes* in our screening. In the past, small amounts of amatoxins also have been reported in *A. citrina*, *A. rubescens* and *B. edulis* [12], but these data have been questioned subsequently [9]. It is therefore likely that other compounds are responsible for the toxicity observed with these three species.

Other compounds have been isolated from the same fungal group. Ibotenic acid is a fly-killing molecule encountered in *A. muscaria* and *A. pantherina* [8] and has been reported as an important glutamate agonist [13]. Another neurologically active compound is 2-amino-3-(1,2-dicarboxyethylthio) propanoic acid, which is an antagonist of NMDA-sensitive glutamate receptors, and isolated from *A. pantherina* [14]. Neither *A. muscaria* nor *A. pantherina* displayed toxicity in our screening. This suggests that the insect's NMDA receptors are insensitive to 2-amino-3-(1,2-dicarboxyethylthio) propanoic acid.

L-DOPA has already been isolated from *Strobilomyces floccopus* [15] and from *Hygrocybe conica* [16] and has shown insecticidal activity against *Prodenia eridania* [17]. However, neither *S. floccopus* nor *H. conica* showed toxicity towards *D. melanogaster* larvae. A nucleoside with strong insecticidal activity (clitocine) has been isolated from *Lepista inversa* [18] and this fungus showed severe toxicity against *D. melanogaster* and *S. littoralis* in this study.

A cytotoxic lanostane-type triterpene ester; 3-β-acetyl-2α-(3′-hydroxy-3′-methyl) glutarylcrustulinol has been isolated from *Hebeloma sinipizans* and *H. crustuliniforme* [19], and the toxic effect of this compound, found also in other *Hebeloma* species, was shown to affect not only the central nervous system, but also the autonomic nervous system in mice [20]. Both fungi displayed significant toxicity in our screening, and *H. sinapizans* was among the most toxic species for the two insects. *Tricholoma saponaceum* was one of the more toxic species encountered in the *Tricholoma* genus. Cytotoxic C_{30}-terpenoids (saponaceolides A, B, C and D) have been found in this suspect fungus [21, 22].

4-Hydroxybenzenediazonium ions with antibiotic activity have been isolated from the poisonous *Agaricus xanthoderma* [23], but although extracts of this fungus are known to be cytotoxic, it is not certain that these compounds are responsible for the activity observed against *D. melanogaster* larvae in our screening.

An impressive number of terpenoids with cytotoxic, antibiotic, antifeedant and mutagenic activities have been isolated from *Hypholoma fasciculare* and *Merulius tremellosus* as well as from a large number of species belonging to the *Lactarius* genus [24]. Whereas some end-products possess cytotoxic activity [25], others have been reported to possess antifeedant activity against storage pests [26, 27]. In our screening, no toxicity was found in the *Lactarius* genus, or in the other species. The precursor of all the sesquiterpenes isolated from fruit bodies of the pungent *Lactarius* species is velutinal, present in the intact fruit body as stearylvelutinal, a highly labile compound [28]. It has already been reported that the pungent taste of *L. vellereus* is rapidly lost when the mushroom is frozen [29]. It is, therefore, not surprising that no toxicity was observed in our screening within this genus, as all the samples were frozen and lyophilized before testing.

Interestingly there are several mushrooms that are non-toxic for humans, but that are highly toxic for insects. Fungal toxicity for humans, however, as found in the literature, concerns generally speaking the cooked carpophores, and as showed in Table 2 only 17 species remained toxic for *Drosophila* after the thermolability test. Of these, 10 are particularly interesting because they are non-toxic for humans. Therefore, if the toxicity does not only reflect the higher sensitivity of insects towards a compound that is toxic for all eukaryotes, then these fungi would be a valuable source for new candidate insecticides.

EXPERIMENTAL

Mushrooms and toadstools. Freshly gathered fungi were cleaned and frozen after identification [30]. By freeze-drying for 48 hr on a Serail-type Sublivac apparatus, dehydrated fruit bodies were obtained. All material was preserved in desiccators and thoroughly pulverized before testing. Mushrooms and toadstools were classified by species, and themselves subdivided into different batches, gathered the same day and in the same place. Table 1 shows all the species screened and the number of batches tested for each species.

Insects. Drosophila melanogaster strain Canton S has been chosen here as a model insect because it is easy to rear and it has a short generation time. The rearing medium was 2.5% agar, 10% glucose, 10% powdered barm and 0.5% *p*-methylhydroxybenzoate, dissolved in H_2O. The mixt. was previously boiled and maintained at 55° before melting in rearing tubes. The insects were added after solidification of the media at room temp. *Spodoptera littoralis* was chosen because it represents a major agricultural pest. It was reared essentially as described in ref. [31]. The rearing medium was composed of 1.6% agar, 13% polenta, 3.3% wheat germ and 3.3% yeast extract; 0.2% *p*-methylhydroxybenzoate and 0.2% benzoic acid were added as preservatives.

Toxicological tests. Fungi powder (600 mg) was added to 10 ml *D. melanogaster* rearing medium. 5 males and 5 females were enclosed, and the development of their progenies was followed at 25°. The fungus was considered as non-toxic when the larval development was completed (i.e. when pupae were observed 2 weeks after deposition of the adults). If failure in larval development was observed by the absence of pupae, then the fungus was considered as toxic. A smaller quantity of fungus was then added to a new nutritive medium and the test was repeated as previously described. Repetition of this procedure was performed in order to determine the minimal concn of fungus necessary to inhibit totally larval development (LD100). Normal development of larvae was followed with controls containing only the rearing medium. For some fungi, several batches of the same species were tested. In these cases only the most toxic batch was presented, and the variability estimated. It was considered that there was a variability when the minimal toxic dose differed at least 3-fold between 2 batches. As the maximal concn tested was 60 mg ml^{-1}, variability could only be estimated for species where the maximal toxicity was found under 20 mg ml^{-1}. Toxicity for *S. littoralis* was tested using the same protocol. Powder was added to the rearing medium before solidification at a final concn of 20 to 30 mg ml^{-1}. Between 50 and 100 hatching larvae were kept on the medium until reaching the 3rd larval stage.

Thermolability test. Insecticidal compounds present in toxic fungi were tested for their thermoresistance. The media were prepared as previously described, but the mixt. was maintained at 100° for 15 min in a heat bath before final solidification.

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