PhD THESIS

Sándor Kecskeméti

Budapest

2022

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

THE EFFECT OF DIFFERENT OLFACTORY AND VISUAL STIMULI ON THE FUNGUS GNAT *LYCORIELLA INGENUA*

Sándor Kecskeméti
Budapest
2022

THE DOCTORAL SCHOOL:

Name:	Doctoral School of Horticultural Sciences
Scientific Branch:	Agriculture and Horticulture
Head of School:	Dr. Éva Németh Zámboriné (PhD) Professor, DSc MATE, Institute of Horticulture, Department of Medicinal and Aromatic Plants
Supervisors:	Dr. András Geösel Associate Professor, PhD MATE, Institute of Horticulture, Department of Vegetable and Mushroom Growing
	Dr. József Fail Associate Professor, PhD MATE, Institute of Plant Protection, Department of Zoology

…… ……..

Head of School ……..

Supervisors

I. BACKGROUND AND OBJECTIVES

Mushroom cultivation is an important sector in agriculture worldwide. It re-utilises agricultural wastes and produces nutritious food. Because of several positive content qualities, mushrooms can be easily inserted into our modern, healthy dietary practice.

As with other cultivation, mushroom production is also hindered by pests, on which the "back-bone" of crop protection is based. Fungus gnats can cause devastating losses in mushroom farms worldwide, and to this point, no effective solution has been given against them. Larvae consume the mushroom compost and adults vector several fungal diseases inside cultivation facilities.

The chance of a new pesticide's approval for mushroom growers in Hungary is very slim, furthermore the EU regulations will demand the reduction of pesticide output as well. With this in mind, it will be extremely important to develop alternative plant protection methods that ensure chemical-free / reduced-pesticide cultivation. Alternative methods include traps, which operate via different mechanisms. Foreign countries have already placed great emphasis on researching pest specific chemical lures. Kairomone based traps lure insects around them, so only these "hot-spots" require chemical protection. Or, if the traps are put on "border-plants", than it is sufficient to only treat these plants with pesticides. Another alternative is when the lure is laced with pesticide, so that only the pests that feed on the bait are killed. In addition to chemical processes, traps that emit visual stimuli should not be forgotten. In addition to the most well-known light traps, there are a number of other control methods that take advantage of the pest's vision.

Selectivity is a serious problem in trap development. Non-target species are likely to fall victim to chemical/visual traps on field. Mushroom cultivation provides ideal conditions in this respect, as both button- and oyster mushrooms are grown in completely enclosed facilities.

Nevertheless, the origin of trap development lies on basic research, focusing on the pest's biology, life cycle, physiology etc. It is imperative to know which volatile or visual cues are the most attractive for the targeted pest. These questions still remain unknown for fungus gnats, we know very little about the chemical and visual ecology of these insects. In my dissertation I aim to answer these questions by conducting several electrophysiological and behavioural experiments with the most common fungus gnat *Lycoriella ingenua*. I hope to provide basic data to establish the development of a fungus gnat mass trapping device.

In my thesis it was my goal to:

- analyse the headspace volatile profile of the materials used in mushroom cultivation.
- use the volatile collections in electrophysiological experiments and to detect the antennally active components of *Lycoriella ingenua*.
- identify and validate the antennally active components.
- use the active components in behavioural experiments.
- determine the spectral sensitivity of *Lycoriella ingenua* with electrophysiology.
- determine the fototaxis of *Lycoriella ingenua* with multiple behavioural experiments.

II. MATERIAL AND METHODS

2.1. Olfactory experiments

2.1.1. Insect material

I have used laboratory colonies of *L. ingenua* for the electrophysiological and behavioural experiments. The colonies were maintained in the Department of Vegetable and Mushroom Growing of MATE since 2016.

2.1.2. Mushroom cultivation materials.

I have used the following mushroom cultivation materials in the olfactory experiments:

- phase two button mushroom compost
- phase three button mushroom compost
- casing material
- button mushroom colonised casing material

2.1.3. Volatile collection

Open-strip (dynamic) volatile collection method was used to capture components from the headspace of cultivational materials. The volatile components were captured on active charcoal filters. Captured volatiles were eluated from the filters with 100 µl dichloromethane, and the extracts were stored on – 40 Celsius.

To determine the ratio and volatile profile of the active components in the phase III button mushroom compost, I used solid micro extraction method. SPME fibers had DVB/PDMS/CAR coating.

2.1.4. Electrophysiology (GC-FID/EAD)

I used gas chromatography coupled (GC-FID) electroantennograpy (EAD) to determine the antennally active compounds.

In the experiments, I have removed the head of 1-3 days old female *L. ingenua* specimens. The head was mounted on the electrolyte filled capillary tube (reference electrode), while the antennae were inserted into the recording capillary tube, with the aid of micro manipulators. The electric discharge expressed inside the sensilla and the signal of the gas chromatograph was recorded simultaneously with the GC-EAD software.

2.1.5. Mass spectrometry

The collected volatiles were identified with gas chromatography coupled mass spectrometry.

Volatiles were identified according to the mass spectrum consistency inside NIST 11 MS library, with the aid of ChemStation program. Later, the antennally active components were validated with synthetic compounds as well.

2.1.6. *Behavioural experiments.*

In order to compare the behavioral effect of cultivation materials and antennally active compounds two-choice bioassays were conducted in modified, custom-made static-air olfactometers made from two glass vials connected to a Petri-dish. The vials served as pitfall

traps containing the test materials to compare, while the Petri-dish served as the main compartment chamber where the insects were placed. A total of ten experimental arenas were used, and in each experimental arena, 10 two days old females were released per experimental trial. Each trial was replicated five times, in total 500 female specimens of *L. ingenua* were tested per trial. Each assay lasted for 45 min. The list of experiments and further parameters are detailed in Table 1.

Table 1: The parameters of the two-choice bio-assays

Vial 1 (Treatment 1)	quantity (g)	Vial 2 (Treatment 2)	quantity (g)	dosage/dispenser (µg)
phase II (ph II)	4	phase III (ph III)	4	-
phase II (ph II)	4	phase II + 1-octen-3-ol (ph II + 1octol)	4	100
phase II (ph II)	4	phase II + 3-octanone (ph II + 3octone)	4	100
phase II (ph II)	4	phase II + 1-hepten-3-ol (ph II + 1heptol)	4	100
phase II (ph II)	4	phase II + 1-hepten-3-ol + 1-octen-3-ol + 3-octanone (ph II + syntmix)	4	3+1+96
phase II (ph II)	4	empty vial (blank)	0	-
phase III (ph III)	4	empty vial (blank)	0	-
phase III (ph III)	4	sterile distillate water (dw)	4	-
empty vial (blank)	0	epty vial (blank)	0	-
casing material (cas)	4	empty vial (blank)	0	-
casing material (cas)	4	hyphae colonized casing (casmyc)	4	-

2.2. Visual experiments

Electroretinography

For the experiments I have used custom made electroretinography device.

For measurements, I have inserted the insects with protruding heads into a pipette tip, and immobilized the head movement with a droplet of melted wax. I have inserted two tungsten electrodes into the compound eyes of the fungus gnat. The recording electrode was inserted into the eye, which was closer to the light source, while the reference electrode was pierced into the other compound eye.

Light stimuli were created by a custom-made light source containing 14 monochromatic LEDs. LEDs could produce light stimuli between 346 and 744 nm spectral threshold with adjustable intensity.

The electrical signals of the photoreceptors were recorded by a modified USB soundcard, with the aid of Audacity 2.2.1 software in lossless format.

In every measurement I have used dark adapted specimens. During the measurements, the compound eyes were stimulated with distinct wavelengths and increasing light intensities. Each stimulus lasted for 500 ms with 3 s long inter-stimulus intervals. I have started from the shortest wavelength (with increasing intensity) and moved to longer and longer wavelengths. Photon flux of the applied light stimuli varied between $2.4 \cdot 10^{11}$ and $2.4 \cdot 10^{15}$ photons/cm^2/s.

Response amplitudes were considered as the magnitude of negative jumps in the potential in the first 100 ms of the 500-ms-long light stimulus. For a given stimulus sequence, sigmoid exposure-response curves were fitted to the measured response amplitudes as a function of log photon flux for all available wavelengths. Spectral sensitivity was calculated by obtaining the reciprocals of photon flux required for inducing a standard response criterion for all wavelengths. Finally, all spectral sensitivity curves corresponding to each repetition of the stimulus sequence were averaged and normalized with the maximal value.

Behavioural assays

The wavelength dependence of phototaxis was tested in two separate experiments. The first **(a)** experiment was focusing on the determination of the lowest intensity (to a given wavelength) which induces a phototactic response. While in behavioural experiment **(b)**, I have evaluated the attraction effect of several wavelengths with the same intensity at once, in a six-choice arena.

a) Action spectrum of phototaxis

I have used a custom-made light source, which could produce 11 monochromatic light stimuli in the range between 368 and 637 nm with adjustable intensity.

Insects were put inside a 3 mm depression in a fiberboard piece lined with black cardboard (arena). The illuminating surface of the light source faced downward to the arena centre from a distance of 19 cm. The movement of insects were recorded with an infrared sensitive web camera. An infrared light source (940 nm) was also fixed above the arena.

30-44 dark adapted insects were put inside the arena. A series of 30-s-long light stimuli separated by 180-s-long dark periods were presented to the insects. Stimuli of 4–5 different light intensities were applied at all 11 wavelengths. First, at the lowest light intensity, stimuli of different wavelengths were randomly applied. Then, all available wavelengths were tested again with 1 log unit higher light intensity, and so forth.

According to the camera recordings, attraction of *L. ingenua* to a given stimulus was quantified by calculating the centroid of the insects relative to the arena centre (centroid bias Δx) at the beginning and end of the stimulus. In total I have conducted 50 experiments, using 1834 *L. ingenua* specimens. The photon flux of light stimuli ranged from $7{,}51\times10^{2}$ and $1{,}53\times10^{13}$ photon/cm^2/s

b) Attraction of simultaneous wavelengths at the same intensity

A six choice experimental arena was used to determine the most attractive wavelength for fungus gnats. The experimental arena had a centre and peripheral unit:

The centre unit was a hexagon shaped hollow box made from foam core. One glass vial was connected with a silicone tube to each side of the hexagon. The inner surface of the hexagon box was lined with matte black cardboard. A hexagon lid covered the centre unit. Insects were placed inside this centre unit during an experiment. There was no lightsource inside the centre unit.

The peripheral unit was a much larger hexagon shaped box, which was divided into six identical chambers with side panels. The inner surface was lined with matte aluminium foil. The centre unit could be fitted into the peripheral unit, so that each individual vial could sit inside its own visually isolated chamber. The peripheral unit was also covered with a hexagon shaped lid, but the lid had six rectangle holes near its edges. Inside these holes, the light sources could be placed in a way that the six identical chambers could be lit from above. The LED light sources could be re arranged randomly at any given time. The used light sources were: UV, blue, green, red wavelengths and a 3000 K white LED was also used.

During the experiments, insects located in the central unit could choose between the light stimuli flowing through the glass vial and the silicone tubes connecting it. The photon flux of the light filtered through the tubes was the same for all light stimuli ((2.7×10^{13} foton/cm^2/s ($\pm$ 6.9%)).

After adjusting the LED strips, I placed 50 dark-adapted fungus gnats in the central unit, and after 45 minutes, the number of gnats trapped in the glass vials was recorded.

I divided the experiments into several experiment types. In **Type 1 experiments**, 5 out of the 6 chambers of the station were illuminated, while one chamber had no light, which served as a control. **In type 2 experiments**, UV light was removed from the options, and two control chambers were used in this series of experiments. **In Type 3 experiments**, fungus gnats could choose from only two light stimuli (the remaining four chambers served as control chambers). Of the two stimuli, green was always apparent, while the second option was one of the remaining stimuli. In Type 1 and Type 2 experiments, the arrangement of light stimuli was always randomized for each repetition, while in Type 3 experiments, the two stimuli were located opposite each other.

III. RESULTS

3.1. Olfactory experiments

3.1.1. Electrophysiology and identification (GC-FID/EAD and GC-MS)

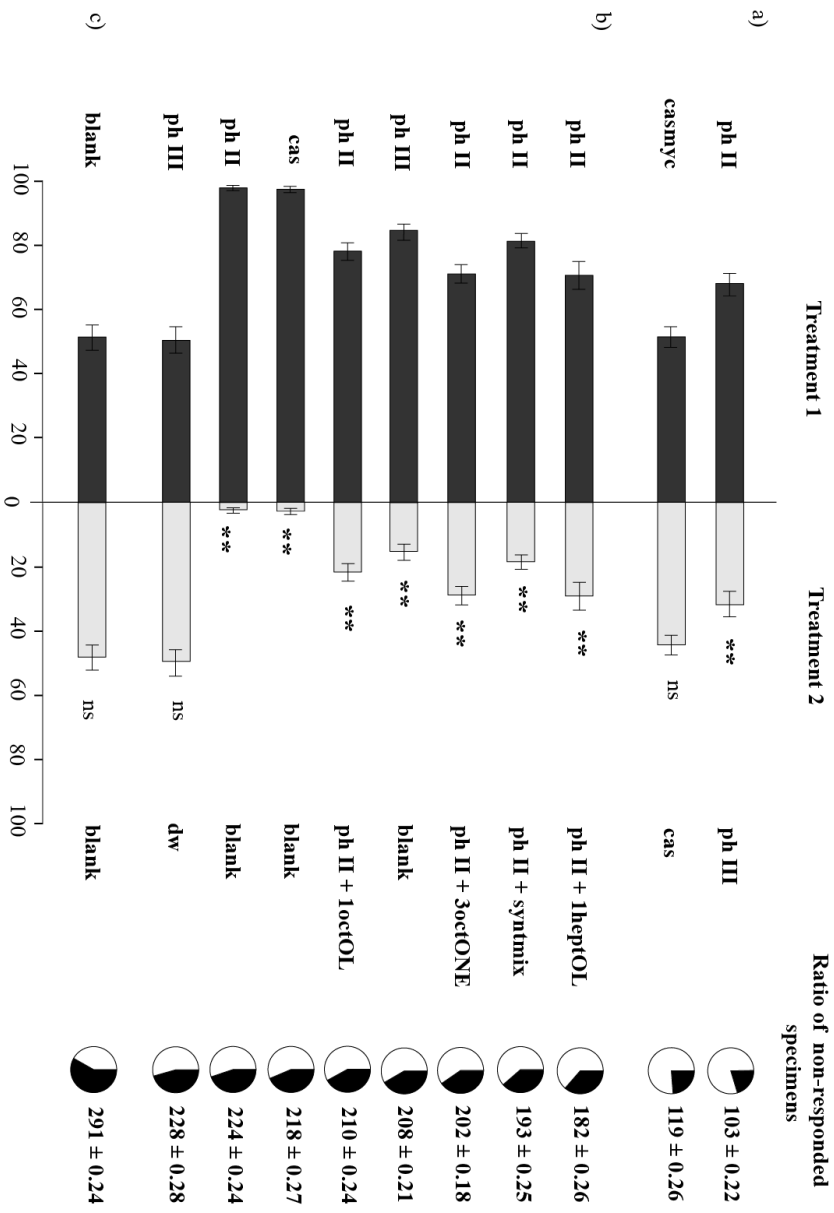
From the headspace of phase III button mushroom compost, 3 compound triggered consistent responses from female *L. ingenua* antenna. According to GC-MS the componds

were: 1-hepten-3-ol (CAS 4938-52-7), 1-octen-3-ol (CAS 3391-86-4) and 3-octanone (CAS 106-68-3). Later, these were verified by synthetic compounds. The volatiles identified in phase II compost and casing materials is presented in Table 2.

Table 2.: Volatile compounds identified in phase III (Ph III) phase II (Ph II) compost, mycelia colonised casing (Casmyc) and casing (Cas) material.

#	Retention Time Nist	Compounds	CAS	Ph III	Ph II	Casmyc	Cas
				Area %	Area %	Area %	Area %
1	875	m-xylene	108-38-3	0.38	17.06	0.20	0.00
2	890	2,6-dimethylpyridine	108-48-5	0.38	0.00	0.72	0.00
3	892	1-hepten-3-ol	106-35-4	0.65	0.00	0.00	0.00
4	987	1-octen-3-ol	3391-86-4	18.94	8.49	20.93	0.00
5	993	3-octanone	106-68-3	66.84	0.00	64.40	0.00
6	1000	3-octanol	589-98-0	3.25	0.00	2.34	0.00
7	1034	2-ethylhexanol	104-76-7	0.63	20.80	4.72	100.00
8	1037	limonene	138-86-3	0.44	7.92	0.13	0.00
9	1082	(Z)-linalool oxide	5989-33-3	1.57	1.68	0.00	0.00
10	1092	3-nonanone	925-78-0	0.52	0.00	0.06	0.00
11	1097	(E)-linalool oxide	34995-77-2	0.48	0.00	0.00	0.00
12	1106	linalool	78-70-6	1.22	4.65	5.80	0.00
13	1127	unknown 1	-	0.27	0.00	0.00	0.00
14	1286	unknown 2	-	0.23	7.27	0.00	0.00
15	1332	unknown 3	-	0.22	7.96	0.00	0.00
16	1469	β-barbatene	53060-59-6	1.99	5.50	0.71	0.00
17	1482	2,6-di-tert-butylquinone	719-22-2	1.46	10.79	0.00	0.00
18	1487	α-cedrene	469-61-4	0.35	1.47	0.00	0.00
19	1579	unknown 4	-	0.00	6.42	0.00	0.00
20	1745	unknown 5	-	0.18	0.00	0.00	0.00
	Sum			100.00	100.00	100.00	100.00

3.1.2. Behavioural experiment

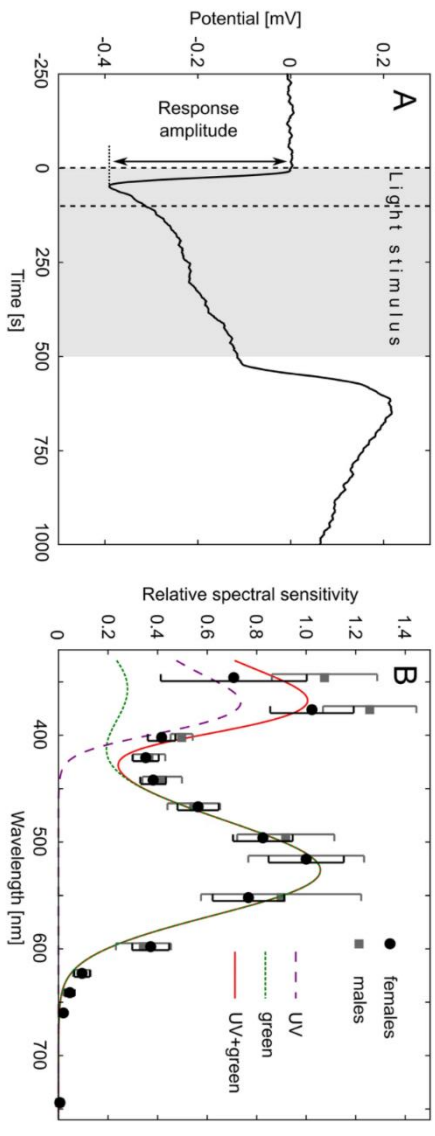


Percentage (±SEM) of female *Lycoriella ingenua* flies attracted to differently treated mushroom cultivation materials in two-choice, static-flow olfactometer bioassays. Each horizontal bar is representing the ratio of responded insects while pie charts show the percentage (as well as the number) of non-responded specimens (black segment) to flies responded (white segment) for each corresponding treatment. In total, 500 females' (50 replicates 10 females/ treatment/replicates) choice was observed per treatment. Stars indicate significant behavioral response towards test material (Games-Howell, $p < 0{,}05$) and

lowercase letters show the responsiveness groups based on non-responding specimens (a: high, b: medium, c: low; Tukey, $p < 0,05$)

3.2. Visual experiments

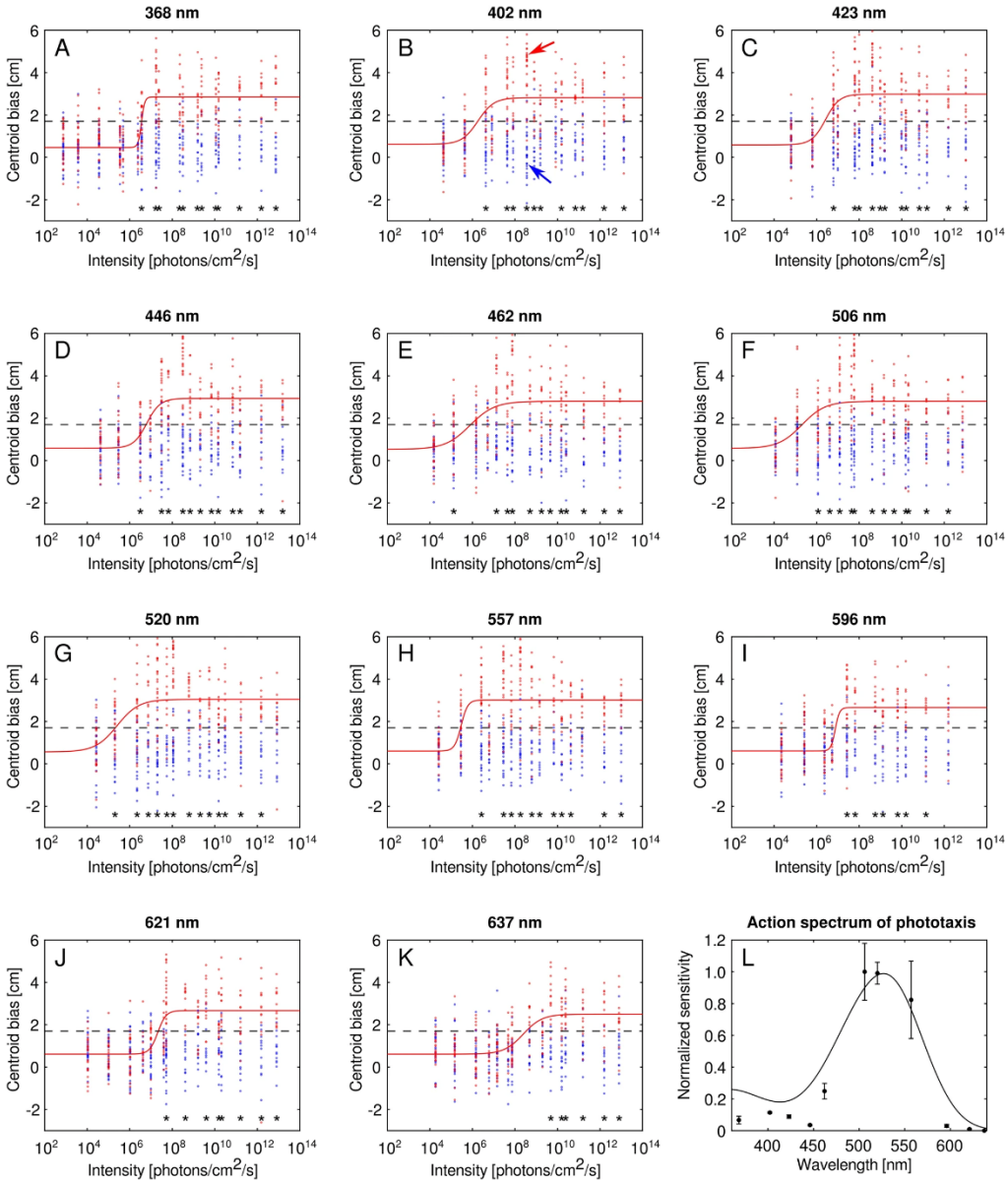
3.2.1. Electroretinography



Electroretinogram of L. ingenua. (**A**) Time course of a typical receptor potential measured from a female L. ingenua eye preparation. Grey region represents the 500-ms-long light stimulus, and the pair of vertical dashed lines show the 100-ms-long period where the response amplitude was determined. (**B**) Mean relative spectral sensitivity of 12 female and 4 male L. ingenua eye preparations with vertical bars denoting SD. The red continuous curve shows the fitted sum of two A1-based pigment templates[41] with peak wavelengths of $\lambda_{max,UV} = 370.1$ nm (dashed purple curve) and $\lambda_{max,G} = 526.3$ nm (dotted green curve), respectively. Curve fitting was performed on the pooled data of females and males.

4.2.2. Phototactic response of fungus gnats to differing wavelengths

Behavioural experiment (a)

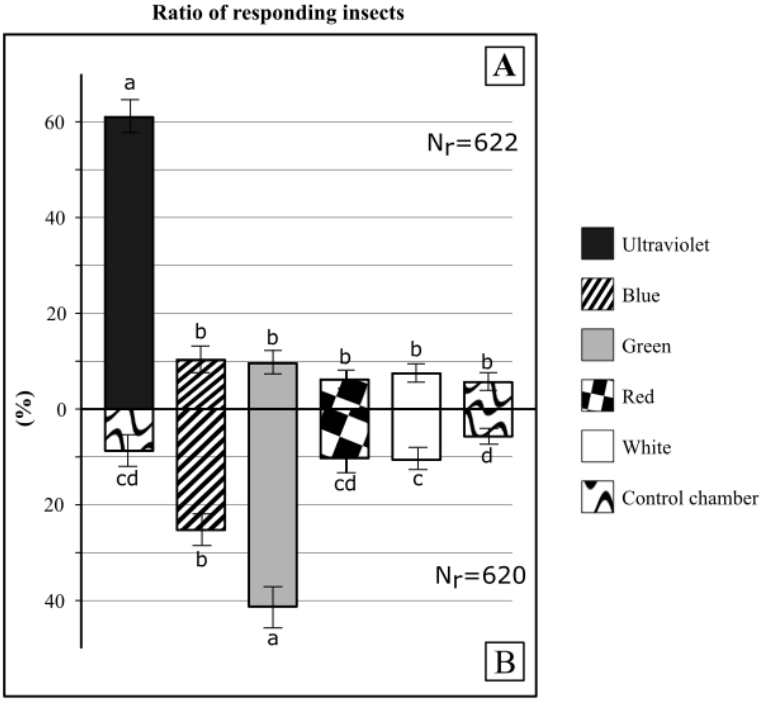


Phototactic responses of *L. ingenua* in behavioural experiment 1. (**A**–**K**) Responses with fitted sigmoid exposure-response curves at 11 wavelengths. Blue and red dots represent the centroid bias values (Δx) measured at the beginning and end of the applied light stimuli. Sigmoid exposure-response curves were fitted to the Δx values obtained for the end of the stimuli (red dots). Dashed lines show the critical response criterion ($\Delta x_c = 17.0$ mm), and asterisks indicate whether distribution of the Δx values at the end of a light stimulus (red dots for a given light intensity) significantly differ from the corresponding distribution of Δx values measured at the beginning of the same stimulus (blue dots for the same light

intensity) at $\alpha = 0.005$ significance level. (**L**) Calculated action spectrum of phototaxis with error bars denoting 95% confidence intervals. Curve shows a fitted A1-based pigment template with a λ_{max} of 526.6 nm.

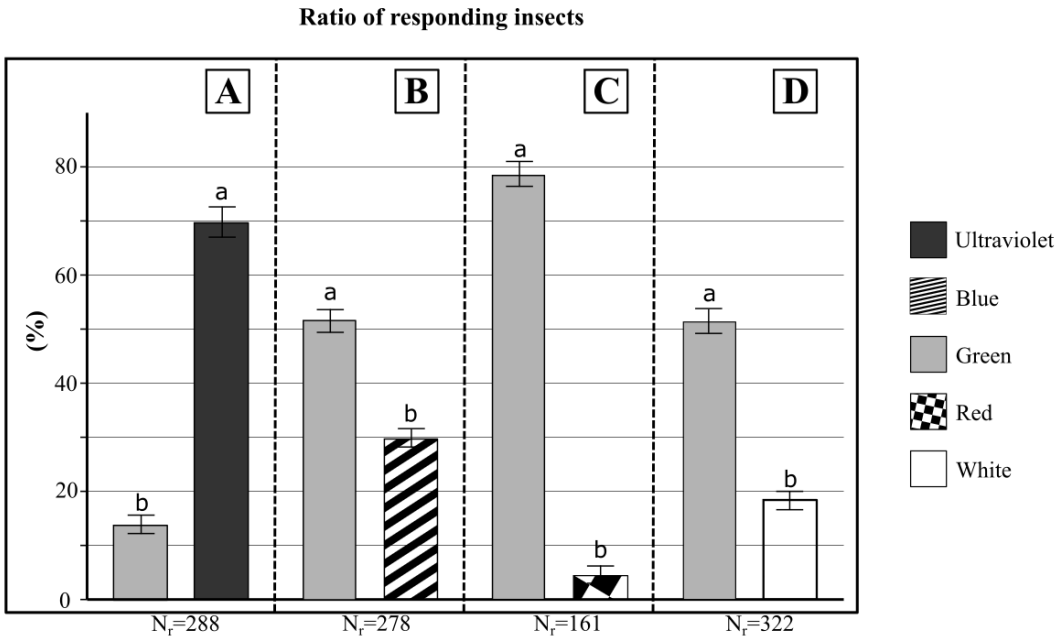
Behavioural experiments (b)

Type 1 and 2 experiments:



Choice percentages for the different chambers of the choice-box in trial types 1-2 in behavioural experiment 2. (**A**) Results for type 1 trials with vertical bars denoting SD. Lowercase letters indicate statistically homogeneous groups at $\alpha = 0.05$ significance level revealed by Tukey's post-hoc test. N_r is the number of respondent fungus gnat individuals out of the total number of 1000 tested specimens. (**B**) Same as A, for type 2 trials. Total number of trials was 20 in case of both type 1 and type 2 trials.

Type 3 experiments:



Percentage of choices of fungus gnats for the four different stimulus pairs in type 3 trials in behavioural experiment 2. (**A**) Results for the green-UV stimulus pair (Fig. 3C) with vertical bars denoting SD. Lowercase letters indicate statistically homogeneous groups at $\alpha = 0.05$ significance level revealed by Games-Howell post-hoc test. Choice percentages for the four unexposed chambers are not displayed. N_r is the number of respondent fungus gnat individuals out of the total number of 500 tested specimens.

IV. DISCUSSION

4.1. Olfactory experiments

Fungus gnats are considered to be one of the most important pests of mushroom cultivation. As generally with insects, volatiles are pivotal cues in finding the most favourable habitat for the next generation. To identify a sufficient oviposition medium a vast array of environmental factors should be considered. Fungal and bacterial volatile compounds were suggested to mediate the oviposition behavior of *Bradysia impatiens*. The fungi *Scytalidium thermophilum* and *Chaetomium* spp. found in mushroom compost was favorable for oviposition and larval development of *L. ingenua*. Even though various fungi were shown to increase the attractiveness for oviposition and enhance larval development, the high mycelial density of white button mushroom (*Agaricus bisporus*) decreases the preference. In contrast with *Bradysia impatiens*, *Lycoriella castanescens* has shown no preference for colonized or uncolonized compost in olfactometer bioassays. In the case of *Lycoriella ingenua* mycelial colonisation of compost was also observed to be indifferent.

We observed that colonized compost was not suitable for the oviposition or development of *L. ingenua*, as imagoes did not emerge from compost when only colonized compost was offered for females. From the previous findings, we may suspect that phase III compost is

not suitable for *L. ingenua* larval development. Moreover, we might assume, that females would avoid phase III, if the possibility of choice is given.

This hypothesis was supported by the results of our behavioral bioassays because females significantly avoided colonized compost when uncolonized compost was also available. The olfactory cues behind this phenomenon were screened with GC-EAD on female imagoes; 1-hepten-3-ol, 3-octanone and 1-octen-3-ol were identified as antennally active compounds in the colonized compost volatilome. 3-octanone and 1-octen-3-ol are derivatives of fungal oxylipin-synthesis, and the former compound was reported to be present in the headspace of A. bisporus colonized compost and fruiting bodies. Interestingly 1-hepten-3-ol was not identified earlier in *A. bisporus* related studies, but it was present in the headspace of fruiting bodies of *Lactarius camphoratus* and *Boletus edulis*. The behavioral activity of these antennal active volatiles was further supported in behavioral bioassays with *L. ingenua* adults.

The preference was clear towards phase II compost in all tested pairwise comparisons: adding physiological active volatiles to phase II both separately and in combination, in order to mimic phase III volatile profile, resulted in clear avoidance. Mushroom alcohol (1-octen-3-ol) is counterintuitively repellent for most of the studied fungivorous insects, but it is suggested, that these observations were biased by the applied unnaturally high concentrations. Furthermore, phorid females of the fungivore species *Megaselia halterata* were either attracted or repelled by 1-octen-3-ol and 3-octanone in a concentration-dependent manner. We can deduct that low abundance of these compounds may indicate actively growing mycelia, but the high abundance shows excessive mycelial damage, caused by an overpopulation of fungivorous larvae in the compost hindering sciarid development.

When we compared the attractiveness of uncolonized and *A. bisporus* colonized casing material for *L. ingenua*, contrary to phase III, colonized casing was not avoided significantly. This difference might be explained by the lower abundance of the behaviorally active volatiles in colonized casing. This could also explain that *Agaricus* colonisation of solid synthetic growing medium was indifferent for *L. ingenua* in respect of oviposition choice. Furthermore, Cantelo found that the number of *Lycoriella auripila* larvae was higher in the casing material than in the compost over the post-casing phase. Our findings show that the high abundance of these fungal volatiles is a reliable indicator of *A. bisporus* colonized compost, thus an unsuitable habitat for larval development.

We may further suspect that the negative correlation between the amount of *A. bisporus* mycelia in the compost, and the low survival rates of fungus gnat larvae is caused by the calcium oxalate content of mycelium. In the work of Whitney and Arnott, they state that acicular calcium oxalate crystals appear on the surface of the mycelium, originating within the cell wall. Both White and Binns concluded that the addition of calcium oxalate to mushroom compost delayed and reduced the emergence of fungus gnat adults. The high amount of active olfactory cues may indicate the high amount of mycelial growth (subsequently the high amount of calcium oxalate) in a substrate for the female, that avoids oviposition as a result.

Colonized compost, and casing material have relatively high-water content, 45–65% for fresh compost and 75–86% for casing, and larvae of sciarid species tend to thrive when the humidity is high. This might explain the significantly avoided blank treatment in favour of anything else. Additionally, colonized compost was always avoided, except when no other

medium was offered. This effect was diminished when colonized compost was paired against sterile distilled water. As a conclusion, humidity for *L. ingenua* could be even more important than the presence of mycelia in a substrate. It is worth mentioning that more number of insects chose distilled water, than colonized compost (152 vs 120 specimens) however the difference was not significant.

The analysis of non-responding specimens may serve as an indication of luring efficiency. Paring casmyc against cas and ph II against ph III resulted in the lowest non-responders' rate, hence we may conclude that the most effective lures were natural materials without synthetics. The highest rate of non-respondents occurred when no test materials were offered. We suggest that excluding non-responding specimens when analyzing the results of a choice bioassay may lead to losing vital information.

We suggest that female *L. ingenua* is not primarily attracted to volatiles emitted by mycelia of *A. bisporus*, in fact, the high concentration of certain volatiles elicit avoidance. In the future, we wish to determine the dosage dependency of *Lycoriella ingenua* avoidance to 1-hepten-3-ol, 1-octen-3-ol and 3-octanone, to quantify the limit at which this evasion occurs. Furthermore, we wish to study if there are other attractive microbial volatiles in uncolonized compost of *A. bisporus* that result in positive choice.

4.2. Visual experiments

Until now, the only fungus gnat examined with ERG was the bioluminescent New Zealand glowworm *Arachnocampa luminosa* (Skuse, 1891) with eye spectral sensitivity adapted to the emission spectrum of the bioluminescence of the individuals. The subject of our present work, *L. ingenua* is not that special as *A. luminosa*, but its significance as a crop pest is infinitely superior.

According to our ERG measurements, *L. ingenua* has two sensitivity maxima, one in the UV and another in the green spectral range. Only from dark-adapted ERG recordings one cannot unambiguously identify different photoreceptor types, although distinct sensitivity peaks may indicate that more than one photoreceptors are present. The fact that phototaxis was far the strongest for green and UV quasi monochromatic light in behavioural experiments 1 and 2, respectively, may also indicate that the presence of the peaks in the spectral sensitivity of the compound eye are caused by distinct photoreceptor types. The reason for the different attraction to light as a function of wavelength lies in the differences between the two setups.

There were three essential differences between behavioural experiments **(a)** and **(b)**. Firstly, in behavioural experiment **(a)**, only one stimulus was present at a time, while in behavioural experiment **(b)**, stimuli were simultaneously present. Secondly, the results of behavioural experiment **(a)** and **(b)** are primarily valid for different light intensity ranges. In behavioural experiment **(a)**, typical minimal photon flux values being able to elicit significant attraction depended on wavelength, but were in the 10^5–10^9 photons/cm^2/s range even in the case of the least attractive red wavelengths. These photon flux values are excessively lower than that of the stimuli used in behavioural experiment **(b)** (~10^{13} photons/cm^2/s). This is not especially surprising, because methodology applied in behavioural experiment **(a)** is based on the determination of the minimal light intensity needed for a given phototactic reaction (critical response criterion Δx_c), and photon flux of the stimuli in behavioural experiment 2 was arbitrarily chosen. The third important difference between the behavioural experiments was related to the test insects. Behavioural experiment **(a)** was made exclusively with *L.*

ingenua, while in behavioural experiment **(b)**, the proportion of this species was 76%, however, the rest 24% could not account for the high UV preference alone.

Thus, it seems that spectral sensitivity of phototaxis in *L. ingenua* depends on light intensity.

Explanation for our results may lie in the ocelli, which are simple eyes usually present in winged adult insects. Ocelli are extremely underfocused visual organs and mostly used to stabilize flight by blurry but rapidly perceiving the intensity pattern formed by the dark ground and the brighter sky. Although ocelli thought to be unable to mediate phototactic reactions alone, light reaching exclusively the ocelli can elicit phototaxis from insects after all. Because primarily green-sensitive ocelli are not uncommon among insects, and the light sensitivity of insect ocelli can be several orders of magnitude higher than that of the compound eyes, light perception through ocelli could have been dominated in behavioural experiment **(a)** resulting in the action spectrum of phototaxis with $\lambda_{max} = 526.6$ nm. Green-sensitive ocelli is a feature of mainly nocturnal insects, because night-light has very little UV content, especially under forest canopies. Among fungus gnats, both diurnality and nocturnality has already been demonstrated, but as far as we know, circadian activity of *L. ingenua* has not been studied yet. In behavioural experiment **(b)**, the higher stimulus intensities could account for the mainly UV-dominated attraction which result is in accordance with the finding of Stukenberg et al. who used practically the same light intensities for quasi monochromatic stimuli in choice experiments with the black fungus gnat *B. difformis*, being another notorious pest in mushroom cultivation. When UV stimulus was not present in behavioural experiment **(b)**, the green stimulus was the most attractive which is also in accordance with previous studies.

On the other hand, the peak of the action spectrum of phototaxis overlaps extremely well with the green sensitivity peak in the spectral sensitivity of the compound eye ($\lambda_{max,G} = 526.3$ nm), which may suggest that the compound eye could still play significant role in behavioural experiment **(a)**. It is also important to mention that in behavioural experiment **(b)**, only four quasi-monochromatic stimuli were applied and the shortest-wavelength stimulus was the UV (398 nm) one. Consequently, the wavelength of maximal attraction cannot be estimated as precisely as could be done for behavioural experiment **(a)**, but based on the ERG results it is expected around 370 nm. However, independent of the underlying mechanisms causing the light intensity dependent reactions, our experiments and ERG recordings highlight the importance of both UV (~ 370–398 nm) and green (~ 526 nm) light, or even their combination for creating visually attractive monitoring or trapping tools for *L. ingenua* at mushroom growing facilities.

In general, the spectral sensitivity obtained from ERG recordings is very informative, but the action spectrum of phototaxis is not necessarily similar in shape. Thus performing behavioural experiments are essential in the process of light trap development. The most important message of our paper is that UV and green spectral ranges attract *L. ingenua* individuals with the highest efficiency and future research should investigate the effectiveness of trap prototypes in combining these spectral regions. Based on our results, we would also like to emphasize that besides the wavelength composition of a light stimulus, light intensity is also a very important parameter, which should not be disregarded when light trapping of insects is the aim.

V. NEW SCIENTIFIC RESULTS

In my thesis I have determined that:

1. The female specimens avoid phase III button mushroom compost (produced in Hungary) and prefer phase II instead. This phenomenon was not published, or falsely, that there is no preference between the two compost phases.
2. Based on electrophysiology screening I have defined the compounds 1-hepten-3-ol, 1-octen-3-ol and 3-octanone as antennally active components.
3. I have proven in behavioural experiments that the aforementioned components (at the published dosage) induce an avoiding behaviour in the female *L. ingenua* specimens. To my knowledge, this was never published before.
4. To my knowledge, I have revealed the spectral sensitivity of the compound eye of *L. ingenua*. I have determined that the sensitivity is bimodal, the two maxima are in the UV ($\lambda_{max,UV}$ = 370.1 nm) and in green ($\lambda_{max,G}$ = 526.3 nm) spectrum.
5. Based on several bio-assays I have concluded that UV (~370 nm) and green (~526 nm) wavelength radiation attracts *Lycoriella ingenua* specimens the most.

VI. PUBLICATIONS RELATED TO THE THESIS

1. Peer reviewed scientific papers:

Kecskeméti, Sándor ; Geösel, András ; Fail, József ; Egri, Ádám
In search of the spectral composition of an effective light trap for the mushroom pest Lycoriella ingenua (Diptera: Sciaridae)
SCIENTIFIC REPORTS 11 : 1 Paper: 12770 , 12 p. (2021)
((D1), IF: 4,379)

Kecskeméti, Sándor ; Szelényi, Magdolna Olívia ; Erdei, Anna Laura ; Geösel, András ; Fail, József ; Molnár, Béla Péter
Fungal Volatiles as Olfactory Cues for Female Fungus Gnat, Lycoriella ingenua in the Avoidance of Mycelia Colonized Compost
JOURNAL OF CHEMICAL ECOLOGY 46 : 10 pp. 917-926. , 10 p. (2020)
((Q1), IF: 2,626)

2. Proofread papers (listed in MTA):

Kecskeméti, Sándor ; Szelényi, Magdolna Olívia ; Erdei, Anna Laura ; Molnár, Béla Péter
Miért nem kedvelik a gombaszúnyogok a gombakomposztot – az átszövetett csiperkegomba komposzt illatanyagok repulzív hatása a gombaszúnyog nőstényekre
NÖVÉNYVÉDELEM 57 : 4 pp. 145-155. , 11 p. (2021)

Kecskeméti, S ; Fail, J ; Geösel, A
A gombatermesztésben előforduló Sciaridok és az ellenük való védekezési lehetőségek
NÖVÉNYVÉDELEM 53 : 7 pp. 307-320. , 14 p. (2017)

Jenei, Lajos ; Fail, József ; Szukács, Gergely ; **Kecskeméti, Sándor** ; Egri, Ádám ; Geösel, András
Gombaszúnyogok válaszreakciója különböző intenzitású kék fényingerekre
KERTGAZDASÁG (1998) 51 : 4 pp. 74-85. , 12 p. (2019)

Kecskeméti, Sándor ; Fail, József ; Geösel, András
*A kétspórás csiperkegomba (*Agaricus bisporus*) termesztésben alkalmazott több dúsítóanyag hatása a* Lycoriella ingenua *fejlődésére*
KERTGAZDASÁG (1998) 50 : 4 pp. 10-19. , 10 p. (2018)

3. Full paper conferences:

Kecskeméti, Sándor ; Fail, József ; Geösel, András
Development of Lycoriella ingenua and Bradysia impatiens on different phases of Agaricus composts

REVIEW ON AGRICULTURE AND RURAL DEVELOPMENT 7 : 1-2 pp. 55-60. , 6 p. (2018). 16th Wellmann International Scientific Conference, Hódmezővásárhely, Magyarország : Szegedi Tudományegyetem Mezőgazdasági Kar.

Kecskeméti, Sándor

Gombatermesztésben károsító sciaridok preferenciája

In: Fodor, Marietta; Bodor, Péter (szerk.)
ITT - Ifjú Tehetségek Találkozója
Budapest, Magyarország : Szent István Egyetem, Budai Karok (volt KÉE) (2017) 162 p. pp. 17-30. , 13 p.

4. *Conference abstracts*:

Kecskeméti, Sándor ; Fail, József ; Geösel, András

Gombaszúnyogok (Sciaridae) felszaporítása különböző szerves közegeken

In: Dima, B; Lökös, L; Papp, V (szerk.)
Mikológiai Közlemények Clusiana: VI. Magyar Mikológiai Konferencia
Budapest, Magyarország : Magyar Mikológiai Társaság (2017) pp. 105-107. , 3 p.

Sándor, Kecskeméti ; József, Fail ; András, Geösel

Development of Lycoriella ingenua *and* Bradysia impatiens *on different phases of* Agaricus *composts*

In: Monostori, Tamás (szerk.)
16th Wellmann International Scientific Conference : Book of Abstracts
Hódmezővásárhely, Magyarország : Szegedi Tudományegyetem Mezőgazdasági Kar (2018) pp. 55-56. , 2 p.

Kecskeméti, Sándor ; Geösel, András ; Molnár, Béla Péter

Lycoriella ingenua *perifériális érzékelése egyes gombafajok illatanyaga esetében*

In: Áldorfai, György; Tóth, Tamás (szerk.)
"SZIE kiváló tehetségei" konferencia előadásainak összefoglaló kiadványa
Gödöllő, Magyarország : Szent István Egyetemi Kiadó (2018) 157 p. pp. 53-54. , 2 p.

5. Other educational writings and lectures:

Egri, Á. ; **Kecskeméti, S**. ; Geösel, A. ; Fail, J.

Fénnyel a gombaszúnyogok ellen: a Lycoriella ingenua *spektrális érzékenységének kísérleti vizsgálata*

In: Haltrich, Attila; Varga, Ákos (szerk.)
67. Növényvédelmi Tudományos Napok konferenciakiadványa: Növényvédelmi Tudományos Napok 2021
(2021) 76 p. p. 14 , 1 p.

Kecskeméti, Sándor ; Geösel, András

Gombaszúnyogok elleni védekezés a csiperketermesztésben

ZÖLDSÉG-GYÜMÖLCS PIAC ÉS TECHNOLÓGIA 20 : 2 pp. 13-14. , 2 p. (2016)

Kecskeméti, Sándor ; Geösel, András

Szúnyogok a gombatermesztésben

AGROFÓRUM - A NÖVÉNYTERMESZTŐK ÉS NÖVÉNYVÉDŐK HAVILAPJA 28 : 10 pp. 134-137. , 4 p. (2017)

Kecskeméti, S. ; Szelényi, M. O. ; Erdei, A. L. ; Geösel, A. ; Fail, J. ; Molnár, B. P.

Gombakomposzt illatanyagok szerepe a Lycoriella ingenua *nőstények viselkedési vizsgálataiban*

In: Haltrich, Attila; Varga, Ákos (szerk.)
66. Növényvédelmi Tudományos Napok
Budapest, Magyarország : Magyar Növényvédelmi Társaság (2020) 103 p. p. 24 , 1 p.