Fructification of *Langermannia gigantea* in artificially inoculated field soil

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Gryndler M., Borovička J., Gryndlerová H. and Gryndler E. (2008): Fructification of *Langermannia gigantea* in artificially inoculated field soil. – Czech Mycol. 60(2): 231–242.

Cultures of *Langermannia gigantea*, a fungus potentially important for human alimentation and biotechnology purposes, were isolated from wild-growing specimens and a spawn was produced using autoclaved soil as a cultivation substrate. The spawn of two isolates was placed into the soil in two field experimental plots. Fructification was first observed after 4 years in only one plot, where 3 normal fruitbodies were produced. Analysis of the rDNA cassette of the 3 fruitbodies revealed that they were identical and bore the attributes of both introduced isolates. The fungus is able to persist in the inoculated soil for years and is dispersed over the soil volume. This is the first report of successful fructification of *L. gigantea* introduced at a locality where this species had never been observed before.

Key words: *Lycoperdaceae*, *Langermannia gigantea*, *Calvatia gigantea*, spawn, cultivation, rDNA.

Gryndler M., Borovička J., Gryndlerová H. a Gryndler E. (2008): Fruktifikace houby *Langermannia gigantea* v uměle inokulované půdě. – Czech Mycol. 60(2): 231–242.

Z plodnic sebraných v přírodě byly získány izoláty vatovce obrovského (*Langermannia gigantea*). Jako substrát pro přípravu sadby izolátů této houby byla použita autoklávovaná půda a sadbou byly inokulovány dvě pokusné plochy. Pouze na jedné z těchto ploch byla po 4 letech poprvé pozorována fruktifikace a celkem se vytvořily 3 normální plodnice. Analýza kazety rDNA v DNA extrahované z těchto 3 plodnic ukázala, že jsou geneticky identické a nesou znaky obou použitých izolátů. Houba byla schopna přetrvat v inokulované půdě po několik let a v půdním objemu se šířila. Toto je první zpráva o úspěšné fruktifikaci *L. gigantea* vnesené na lokalitu, kde tento druh nebyl nikdy předtím pozorován.

INTRODUCTION

Among basidiomycetes, several species are significant on the global market scale, since they produce edible fruitbodies which are either collected in natural habitats or cultivated. Many other species are picked in the wild and consumed lo-

cally. They represent not only a valuable supplement of human nutrition but are also used in conventional and traditional medicine. One of the fungal species which is potentially interesting from this point of view is the giant puffball – *Langermannia gigantea*.

Two names of this species are used in modern literature: *Calvatia gigantea* (Batsch: Pers.) Lloyd and *Langermannia gigantea* (Batsch: Pers.) Rostk. The opinions concerning the generic position of the species are controversial (e. g. Kreisel 1992, Gube 2007). For the purpose of this study, the name *Langermannia gigantea* is used (Gube 2007) and *Calvatia gigantea* is considered a synonym.

Langermannia gigantea is common in the temperate zone (Kreisel 1994). Habitats and ecological characteristics of this species were extensively studied by Rimóczi (1987a) on the territory of Hungary. It is evident from his study that the fungus is indifferent to soil pH as well as to temperature (it is thus not thermophilous), prefers soils with moderate humidity and is strongly nitrophilous.

Formation of fruitbodies is probably induced by a rather high level of soil humidity. At least 40-50 mm of precipitation during 2 months (Rimóczi 1987b) or 150-200 mm during the period from April to August (Sørensen and Thorbek 1980) is necessary. Other factors affecting fructification remain unknown. Production of fruitbodies in large fairy rings whose diameter increases with time is occasionally observed (Sørensen and Thorbek 1980). This suggests the perennial character of the vegetative mycelium in the soil. Growth and development of the fruitbodies continue at a mean air temperature of 17.5 °C for 5 to 11 days (Juhász 2003, Rimóczi 1987b).

Langermannia gigantea grows in localities rich in mineral nutrients, such as abandoned pastures (Rimóczi 1985, Nehéz 1985, Breitenbach and Kränzlin 1986), but natural sources of organic carbon utilised for its nutrition are unknown. It is likely that the fungus is able to utilise complex forms of soil organic matter, including polysaccharides and phenolic compounds (humic substances). This is suggested by the fact that *L. gigantea* possesses various enzymes such as amylase (Kekos et al. 1987, Komnikos et al. 1988), phenoloxidases (Galiotou-Panayotou and Macris 1986, Galiotou-Panayotou et al. 1988) and lipase (Christakopoulos et al. 1992).

The production of enzymes mentioned above suggests that *Langermannia gigantea* has a potential in biotechnology. However, industrial use of an organism demands, in most cases, large scale cultivation either under axenic conditions or, as in the case of many edible cultivated fungi, on special substrates.

Several attempts to cultivate this fungus were made. On a larger scale, good substrate utilisation and growth were observed on some brewery wastes under sterile conditions (Shannon and Stevenson 1975). However, cultivation under non-sterile conditions on composts or wastes failed because the fungus is not able

to compete with other saprotrophic micro-organisms present in these substrates (Sous-Dorn 1979). This may indicate that the fungus naturally inhabits soil micro-environments where its competitors are suppressed by an unknown factor.

The conditions for non-sterile cultivation of *Langermannia gigantea* are unknown and should be elucidated. We therefore isolated a mycelial culture of this fungal species, inoculated it into non-sterile soil, and checked fructification under natural conditions. The main goal of our work was to verify the possible introduction of this fungus to a locality where it had never been observed before. This might represent the first step towards a semi-culture enabling the production of fruitbodies at a larger scale.

MATERIALS AND METHODS

Isolation of *Langermannia gigantea* strains. Eleven immature fruitbodies were collected during July and August 2001 in the České Středohoří hills (Northern Bohemia, Czech Republic), locality Hořidla, Litoměřice district, from 2 different sites where the fungus is naturally present. This locality is situated 4.5 km from the locality of the field experiment described below.

The first site (50°32'13"N, 14°16'29"E, W exposure, inclination 15°, elevation 320 m, annual cumulative precipitation approx. 440 mm), is an abandoned pasture characterised by a plant community dominated by *Fraxinus excelsior* and *Robinia pseudacacia* (80 % canopy closure) with understorey species like *Urtica dioica*, *Sambucus nigra*, *Sambucus racemosa*, *Geum urbanum*, *Arctium lappa*, *Galium aparine* and *Viola reichenbachiana*. The second site (50°32'16"N, 14°15'53"E, S exposure, inclination 5°, elevation 295 m) is colonised by *Rosa canina* (compact shrub, 100 % canopy closure) without any other accompanying plant species. The soil type at both sites is eutric cambisol on arenaceous marl. Isolates CG2 and CG11 (see below) were obtained from fruitbodies collected at the first and second sites, respectively. These two isolates were used as inoculants in the field experiment described below.

Besides this, isolate CG13 was obtained using the same method from a fruitbody of *Langermannia gigantea* collected at the locality Horní Nezly (50°34'27"N, 14°13'30"E). The locality (W exposure, inclination 30°, elevation 291 m, annual cumulative precipitation 440 mm) is dominated by *Quercus robur* and *Carpinus betulus* (90 % canopy closure), accompanied by *Sambucus nigra*. The poorly developed soil (on quartz sand) was covered by a 5-cm litter layer. This is the site of the closest (1.8 km) known record of *L. gigantea* to the locality of the field experiment described below.

Isolation was carried out by using small pieces of gleba aseptically explanted from fruitbodies 30-250 mm in diameter, which were transferred to Petri dishes with solid cultivation medium C. The cultivation medium C contained 704 mg Na_2SO_4, 384 mg K_2SO_4, 1 g $CaCl_2 \cdot 2H_2O$, 193 mg KNO_3, 1 g KH_2PO_4, 3 g $Ca(NO_3)_2 \cdot 4H_2O$, 2 g $MgSO_4 \cdot 7H_2O$, 25 mg NH_4NO_3, 8 mg FeNaEDTA, 6 mg $MnCl_2 \cdot 6H_2O$, 2.5 mg $ZnSO_4 \cdot 7H_2O$, 1 mg $CuSO_4 \cdot 5H_2O$, 2 g bacteriological peptone, 1 g yeast extract, 2 g malt extract, 0.5 mg thiamine HCl and 8 g glucose per l of deionised water. Agar was added at a concentration of 1.0–1.5 %.

Mycelial cultures were used for further multiplication and for molecular characterisation by sequencing the ITS region of the rDNA cassette.

Exsiccates of fruitbodies from which the isolates CG11 and CG13 were obtained have been deposited in the herbarium of the Mycological Department of the National Museum, Prague (PRM 909837 and 909838, respectively).

Molecular characterisation of *Langermannia gigantea*. Approximately 20 mg of aseptically explanted fruitbody tissue or 10 mg of fresh mycelium were roughly disintegrated using
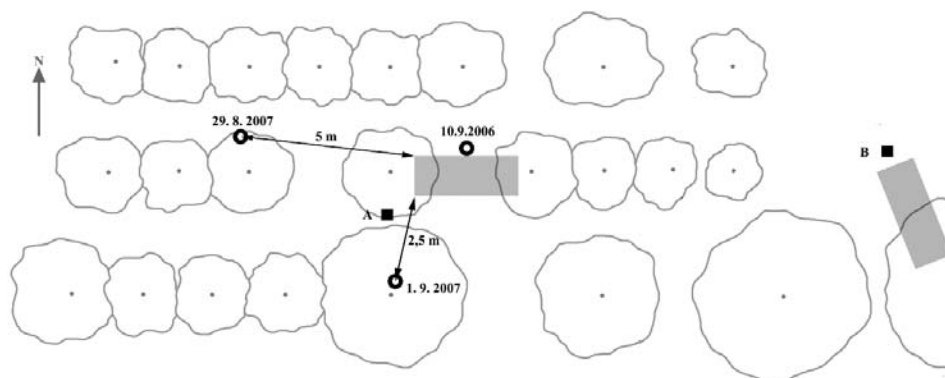
Fig. 1. Field experiment location map. Grey rectangles indicate inoculated areas in the apple orchard. A: soil sampling spot close to the development of fruitbodies. B: soil sampling spot close to non-fruiting experimental plot. Small black circles denote the spots where the fruitbodies were collected.

autoclaved mortar and pestle in a 550 µl bead solution supplied as a part of the Fecal DNA Extraction Kit (MoBio), transferred to a provided bead tube and further extracted as recommended by the supplier. Extracted DNA was further used as a template for PCR.

A fragment of rDNA (ITS region) was amplified from the extracted DNA using a pair of the primers ITS1F (5´-CTTGGTCATTTAGAGGAAGTAA-3´; Gardes and Bruns 1993) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´; White et al. 1990). The PCR mixture consisted of 25 µl REDTaq™ ReadyMix™ PCR Reaction Mix with $MgCl_2$ (Sigma R2523), 1 µl primer ITS1F (10 µM), 1 µl primer ITS4 (10 µM), 1 µl DNA extract and 22 µl water.

Thermal cycling started by initial incubation for 5 minutes at 94 °C and the mixture was further subjected to 34 PCR cycles lasting 60 seconds at 94 °C, 60 seconds at 52 °C and 120 seconds at 72 °C. Finally, the sample was incubated for 10 min at 72 °C. Amplified fragments were purified using the UltraClean™ PCR Clean-up DNA Purification Kit (MoBio).

Purified PCR products were detected using electrophoresis in TBE/1% agarose containing 1 µg/ml ethidium bromide, in which the approximate concentration and the length of the PCR product were estimated by comparison with standards. The amplified fragments were then bidirectionally sequenced on an ABI Prism 3130XL sequence analyser using the primers ITS1F and ITS4.

Production of soil inoculum – spawn. Spawn was produced in 0.75 l wide-necked glass vessels containing 0.6 l non-sieved soil collected at the locality from where the isolate CG2 was obtained. The soil contained 30 % (w/w) moisture. Filled vessels were closed with a brass lid with one hole of 5 mm diameter covered with cotton wool, autoclaved for 25 minutes at 121 °C and inoculated with a 10 × 10 × 3 mm block of *Langermannia gigantea* culture. Inoculated vessels were incubated for 6 weeks at 25 °C and then used as follows.

Establishment of the field cultivation experiment. Two experimental plots were established at the Soběnice locality (Litoměřice district, Czech Republic) on March 30, 2002, to test possible fructification in the field. The locality (50°34'07"N, 14°14'39"E, S exposure, inclination 5°, elevation 293 m, annual cumulative precipitation approx. 440 mm) was an apple orchard (50 % canopy closure) covered with mown grass composed mainly of *Poa annua*, *Taraxacum* sp., *Poa pratensis*, *Lolium perenne*, *Trifolium pratense*, *Urtica dioica* and *Dactylis glomerata*. The soil was clay-loam, recently non-tilled eutric cambisol (with the upper 30 cm modified by tillage in the past). The experimental plots were located at a distance of 12 m from each other (Fig. 1).

This locality was considered suitable for establishment of the field experiment with *Langermannia gigantea* because it was protected by a fence, was shaded by fruit trees and could be potentially used for a semi-culture of the fungus since the soil was not tilled, which eliminated possible disturbance of the soil mycelium. The only management applied was mowing performed approximately twice per year. Each plot consisted of 10 parallel rows, 1 m long, separated by 33 cm space so that the whole plot was 1 m wide and 297 cm long. Each row was inoculated with 0.6 l mixed spawn of both isolates. The spawn was deposited at a depth of approx. 7 cm and covered with soil.

C h e m i c a l   a n a l y s e s . Soil samples for chemical analyses were taken between 3 points where the fruitbodies were collected (Fig 1, point A) as well as from the proximity of the infertile plot (point B). Six subsamples of 200 g were taken from a depth of 5-10 cm, sieved through a 2– mm sieve, mixed and oven-dried for 12 h at 90 °C.

The content of macro– and trace elements in dried fungal tissue and soil samples (both 0.5 g; soil samples were ball-milled in an agate mill) was determined by long-term and short-term instrumental neutron activation analysis (INAA) as described in the literature (Řanda and Kučera 2004, Řanda et al. 2005). Where data on some elements could not be provided by INAA or the detection limit was too high, inductively coupled plasma-optical emission spectrometry (ICP-OES), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) were used. ICP-OES analyses were performed in the Laboratory of Environmental Geology and Geochemistry at the Institute of Geology of the Academy of Sciences of the Czech Republic. ICP-AES and ICP-MS analyses were performed at Acme Analytical Laboratories Ltd., Canada. Total N, C and S contents in the soil samples were analysed with a Vario-MAX Elemental Analyser (dry combustion, Elementar Analysensysteme, Hanau, Germany), oxidisable C was measured using wet bichromate combustion (Hršelová et al. 1999).

RESULTS AND DISCUSSION

Culturability of the fungus in vitro and spawn production

A total of 9 isolates were obtained from the tissue of fruitbodies collected at the Hořidla locality. From these isolates one isolate per site was chosen for further experimentation with spawn production and field culture. These isolates were marked CG2 and CG11. All the isolates, including the two used for spawn production, grew 4 mm per week at 25 °C), possessed the characteristic smell of fresh giant puffball fruitbodies and formed pure white colonies with a light brown reverse, becoming creamy to light brownish during ageing. Cultures sometimes produced clearly distinct hyphal cords (false rhizomorphs) making the shape of the colony irregular. The mycelia are insensitive to mechanical damage and are thus easily subculturable. Besides the above-mentioned isolates, an other isolate, CG13, was obtained from the fruitbody collected at Horní Nezly. The isolates CG2, CG11 and CG13 are deposited in the Collection of Basidiomycetes (Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague) under the codes CCBAS867, CCBAS866 and CCBAS868, respectively.

When blocks of agar culture were transferred to autoclaved soil, the fungus rapidly penetrated the soil by mycelial cords and a 6-week period was sufficient to colonise the whole volume of the 0.75 l vessel at 25 °C. Spawn particles (colonised

Fig. 2. Ash-grey, dense but very fine mycelium of *Langermannia gigantea* growing throughout the soil volume. This type of mycelium is associated with fruitbodies and contains false rhizomorphs.

pieces of soil) were light grey, closely resembling the soil colonised by *Langermannia gigantea* in nature and are easily separable and dispersable.

Production of fruitbodies of *Langermannia gigantea* in the field experiment

On May 26, 2002 (8 weeks after field inoculation), several compact underground mycelial tufts resembling fruitbody primordia or small fruitbodies were observed during inspection of the buried spawn after removal of the covering soil layer at several locations in one of the experimental plots – the one which produced fruitbodies several years later. These structures were pure white and had a diameter of approx. 8-12 mm. However, during the first vegetation period (2002), the mycelium visually disappeared from the spawn particles. When inspections were performed (once a week during the first two months of cultivation and further approximately once every two months, except in winter), mycelium could not be observed for the next 3 years.

The first aboveground fruitbody was observed at the end of the 5th vegetation period (September 9, 2006), was 13 cm in diameter and was located precisely at the margin of the inoculated area which had produced primordium-resembling structures 4 years ago.

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Two other fruitbodies with diameters of 14 and 16 cm appeared on August 29 and September 1, 2007 (Fig. 1). They were located 2.5 and 5 m from the margin of the inoculated area of the same experimental plot. The positions of the fruitbodies related to the position of the inoculated area indicate that the mycelium spread at a minimum speed of approx. 1 m per year, which is about the same speed as reported for a natural colony of the same species in Denmark (0.95 m per year; Sørensen and Thorbek 1980).

Dense, ash-grey, probably nourishing mycelium was always observed under developed fruitbodies and completely filled the soil layer within depths of approx. (2-)5-12 cm (Fig. 2). This mycelium contained hyphal cords and was not preferentially accumulated around plant roots or other distinguishable organic particles.

No marks of root colonisation were observed when grass (*Poa annua*) or tree (*Malus domestica*) roots growing inside the mycelial colony were analysed microscopically. Apparently, the fungus does not possess the capacity to associate with plant roots. The grass cover of the fruiting plot is of normal height and colour, showing no symptoms of intoxication, disease or malnutrition. Our inoculants are thus probably no plant pathogens and the hypothesis supposing this species to be a root disease agent (Pennycook 1989) was not confirmed.

The second experimental plot remained infertile with no marks of soil mycelium development or primordium formation. To explain why only one of the two experimental plots produced fruitbodies, we took a mixed soil sample from the close proximity of each of them and subjected it to chemical analyses for trace elements and macro-element content. The results are presented in Tab. 1. The contents of only two mineral elements, iron and cobalt, differed by at least 100 %. The iron content was higher in the non-fruiting soil sample and the cobalt content was higher in the fruiting soil sample.

Great differences were observed between the contents of some elements in soil and fruitbody dry biomass. In accordance with earlier observations (Cocchi el al. 2006, Borovička and Řanda 2007, Stijve 2007), the fungus accumulates some heavy metals (Ag, Cu, Hg, Zn) and selenium. Mainly the content of accumulated Hg may be of great importance and should be monitored particularly if the fruitbodies are used for human alimentation purposes. On the other hand, the contents of many elements in fruitbody biomass is lower than that in soil, probably because of the low bio-availability of the element in soil or selectivity of ion absorption and transport in the mycelium.

The growth of the organisms at the experimental locality is not limited by nitrogen, because in both experimental plots *Urtica dioica*, a nitrophilous plant species, occurs. This supports the character of *Langermannia gigantea* as a nitrophilous fungal species (Rimóczi 1987a). Nevertheless, the higher total nitrogen content found in the plot not supporting fructification (Tab. 1) indicates that insufficient nitrogen content is not a reason why the fungus failed to grow and form fruitbodies.

Tab. 1. Results of chemical analyses of *Langermannia gigantea* fruitbody collected on September 1, 2007, and two soil samples – sample A taken near the spots where all the fruitbodies were collected and sample B of the soil not supporting fructification.

Element	Fruitbody	Soil sample A	Soil sample B	Concentration unit
Total C	–	2.57	3.36	%
Oxidisable C	–	2.21	2.76	%
N	–	0.226	0.309	%
K	2.05	1.9	1.95	%
P	1.063	0.1312	0.1255	%
Cl	114	62.1	77.5	mg / kg
Na	54.7	4649	5211	mg / kg
Mg	0.1109	0.5264	0.6145	%
S	400	357	440	mg / kg
Ca	0.02	1.0	1.2	%
Al	0.005	4.3	4.7	%
Sc	0.007	7.5	8.82	mg / kg
Ti	< 18	5148	6194	mg / kg
V	0.14	79.4	99.6	mg / kg
Cr	0.44	56.8	56.6	mg / kg
Mn	8.6	715	746	mg / kg
Fe	0.0034	1.3	3.1	%
Co	0.06	26.5	12.3	mg / kg
Ni	0.6	42.2	49.8	mg / kg
Cu	24.9	18. 8	17.3	mg / kg
Zn	157	103	108	mg / kg
As	3.15	10.9	11.6	mg / kg
Se	8.6	0.4	0.4	mg / kg
Br	0.95	4.89	5.62	mg / kg
Rb	1.83	89.9	97.2	mg / kg
Mo	0.36	0.75	0.51	mg / kg
Ag	4.92	0.08	0.06	mg / kg
Cd	0.28	0.34	0.30	mg / kg
Sb	< 0.01	0.95	1.17	mg / kg
Cs	0.04	4.58	4.88	mg / kg
Ba	< 5.7	532	587	mg / kg
Au	8	5	8	µg / kg
Hg	6.36	0.34	0.52	mg / kg
Pb	0.16	32.3	38.2	mg / kg
pH (H$_2$O)	–	5.8	5.8	–
pH (CaCl$_2$)	–	5.3	5.2	–

Based on the available data it does not seem likely that mineral element content is the primary cause of the soil's ability to support the growth of *Langermannia gigantea* mycelium and fructification. The only element with a concentration dra-

matically lower in non-fruiting soil (compared to the fruiting one) is cobalt. However, the concentration of this particular element is relatively high even in non-fruiting soil and its concentration in fungal biomass is extremely low so that the need of the fungus for cobalt would be easily met even in the non-fruiting plot.

The production of fruitbodies does not clearly coincide with climatic events such as increased precipitation, measured at the closest meteorological station of Doksany. The vegetation seasons 2003 and 2004 were quite dry with a cumulative precipitation of 191 and 196 mm over the period of April – August, respectively. In 2005, the cumulative precipitation calculated over the same period was 275 mm and this did not result in fructification. One year later the fungus produced its first fruitbody even though the cumulative precipitation over the months April – August was by 11% lower than in 2005.

Molecular characterisation of isolates and fruitbodies of *Langermannia gigantea*

DNA extraction and purification as well as PCR produced a high quality product suitable for sequencing. The obtained sequences (at least 633 bp in length) showed high similarity (99 %) with the sequence AJ617492 belonging to *Langermannia gigantea* published in GenBank. This confirmed the identity of the isolates. The sequences of isolates CG2, CG11 and CG13 were published in GenBank under accession numbers EF190314, EF190317 and EF190318, respectively.

Isolates CG2 and CG11 were distinguishable by two polymorphic loci (positions 233 and 307 according to reference GenBank sequence AJ617492). At position 233, the sequence of the CG2 isolate contains guanine and the sequence of CG11 contains cytosine. At position 307, the sequence of the CG2 isolate contains cytosine, whereas the sequence of CG11 contains guanine.

Interestingly, the corresponding sequence found in all three artificially cultivated fruitbodies showed the presence of both mentioned bases at both polymorphic loci (positions 233 and 307). This probably indicates that hyphal fusion of the isolates CG2 and CG11 occurred in the inoculated soil, possibly resulting in recombination of the genetic material. This process may be important for the formation of primordia and development of fruitbodies due to the higher genetic complexity of the recombinant organism and corresponding better equipment for living in hitherto "unknown" soil.

At the same time, the sequence of isolate CG13 contained cytosine at both polymorphic loci. This isolate represents the geographically closest *Langermannia gigantea* mycelial colony which could, hypothetically, spontaneously colonise ("contaminate") the fruiting experimental plot instead of developing introduced inoculants. This spontaneous colonisation might occur as a result of basidiospore dispersion by wind. However, the absence of guanine in the above-mentioned poly-

morphic loci in the sequence of the CG13 isolate suggests that this is unlikely (the guanine at both loci should have been introduced into the ITS sequence of *L. gigantea* in the experimental plot in the DNA of the inoculants). Moreover, no fruiting of *L. gigantea* was previously recorded in the experimental locality and in its proximity, so that spontaneous colonisation of soil and fructification was unlikely to have taken place.

No other sequence differences were observed between the inoculants and the fruitbodies (positions 73-684 of the reference AJ617492 sequence).

Tab. 2. Nucleotide sequences of a part of ITS region of *Langermannia gigantea* isolates under study and of fruitbodies produced in the field experiment. Fruitbodies are identified by the date of collection. Nucleotides are numbered corresponding to the base numbering of the reference sequence found in GenBank under accession number AJ617492. Polymorphic loci are highlighted by shading. Symbol "S" indicates that both G and C bases are identified at the locus at the same time.

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            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
              185        195        205        215        225        235
CG2         ATGTGGCCTT TCCGGATGTG AGGATTGCTG AGTGCGAAAG CATACAGCTC TTGTCAAAGC
CG11        ATGTGGCCTT TCCGGATGTG AGGATTGCTG AGTGCGAAAG CATACAGCTC TTCTCAAAGC
CG13        ATGTGGCCTT TCCGGATGTG AGGATTGCTG AGTGCGAAAG CATACAGCTC TTCTCAAAGC
10.9.2006   ATGTGGCCTT TCCGGATGTG AGGATTGCTG AGTGCGAAAG CATACAGCTC TTSTCAAAGC
29.8.2007   ATGTGGCCTT TCCGGATGTG AGGATTGCTG AGTGCGAAAG CATACAGCTC TTSTCAAAGC
1.9.2007    ATGTGGCCTT TCCGGATGTG AGGATTGCTG AGTGCGAAAG CATACAGCTC TTSTCAAAGC


            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
              245        255        265        275        285        295
CG2         GACTTGTAAA CCTCTCCTTC GAGCACTATG TTTTCATATA CCACATAGTA TGTTGTAGAA
CG11        GACTTGTAAA CCTCTCCTTC GAGCACTATG TTTTCATATA CCACATAGTA TGTTGTAGAA
CG13        GACTTGTAAA CCTCTCCTTC GAGCACTATG TTTTCATATA CCACATAGTA TGTTGTAGAA
10.9.2006   GACTTGTAAA CCTCTCCTTC GAGCACTATG TTTTCATATA CCACATAGTA TGTTGTAGAA
29.8.2007   GACTTGTAAA CCTCTCCTTC GAGCACTATG TTTTCATATA CCACATAGTA TGTTGTAGAA
1.9.2007    GACTTGTAAA CCTCTCCTTC GAGCACTATG TTTTCATATA CCACATAGTA TGTTGTAGAA


            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
              305        315        325        335        345        355
CG2         TGTGATCAAT GGGCCTATGT GCCTATAATA ATCTTATACA ACTTTCAGCA ACGGATCTCT
CG11        TGTGATGAAT GGGCCTATGT GCCTATAATA ATCTTATACA ACTTTCAGCA ACGGATCTCT
CG13        TGTGATCAAT GGGCCTATGT GCCTATAATA ATCTTATACA ACTTTCAGCA ACGGATCTCT
10.9.2006   TGTGATSAAT GGGCCTATGT GCCTATAATA ATCTTATACA ACTTTCAGCA ACGGATCTCT
29.8.2007   TGTGATSAAT GGGCCTATGT GCCTATAATA ATCTTATACA ACTTTCAGCA ACGGATCTCT
1.9.2007    TGTGATSAAT GGGCCTATGT GCCTATAATA ATCTTATACA ACTTTCAGCA ACGGATCTCT
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CONCLUSIONS

Our results indicate that *Langermannia gigantea* can be introduced at new localities and this is the first report of a successful fructification of this species in semi-culture. The fungus is able to persist in the inoculated soil for years and may be dispersed over the soil volume in the form of visually undetectable tenuous hyphae. Under favourable conditions, the fungus produces dense mycelium and fruitbodies.

The conditions necessary for massive development of the dense mycelium in non-sterile soil remain unknown and need further investigation. It is particularly necessary to determine the organic substrates which are exploited by the mycelium as a natural source of energy and organic nutrition. The results of our experiment might represent the first step towards a large-scale cultivation of this fungal species in the future.

ACKNOWLEDGEMENTS

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