

Greenhouse culture experiments on *Kuehneromyces mutabilis*

JOUNI ISSAKAINEN, KATI PIHLAJA and JENNI SMOLANDER

ISSAKAINEN, J., PIHLAJA, K. & SMOLANDER, J. 2017: Greenhouse culture experiments on *Kuehneromyces mutabilis*. – *Karstenia* 57: 17–32. HELSINKI. DOI <http://doi.org/10.29203/ka.2017.480>. ISSN 0453–3402.

Jouni Issakainen & Kati Pihlaja, Herbarium, Biodiversity Unit, University of Turku, FI-20014 Turku, Finland; e-mail: kati.pihlaja@utu.fi (corresponding author)

Jenni Smolander, Turku, Finland

Key words: *Kuehneromyces mutabilis*, Strophariaceae, mushroom cultivation, culture methods, edible, boreal, low-tech, wood decay

The edible, wood-decaying mushroom *Kuehneromyces mutabilis* was studied in 3 years' culture experiments in a climate-controlled greenhouse. Pre-incubated wood blocks and sawdust cylinders were buried in soil in test buckets. Tested variables included inter-strain variation, inoculation methods, wood disinfection, dimensions of wood blocks and mixtures of soil. *K. mutabilis* readily colonized various kinds of wood blocks and produced fruiting bodies regularly in unsterile culture conditions. It was found to be a promising species for low-tech culturing assuming further collection of efficient strains and refinement of methods. Sterilized sawdust bricks submerged in soil were most effective for strain testing. Blocks of young trees, including thin branches yielded well, suggesting that the species can be cultured on fractions of wood which have been considered less valuable in traditional forestry. Pasteurization of the wood blocks shortly in boiling water and adding the spawn as potato jelly were found to be useful, low-cost methods of inoculation. Too much acidic peat in the soil mixture was unfavorable for the production but its effects were compensated by adding CaCO₃ in the soil.

Introduction

Kuehneromyces mutabilis (Schaeff.: Fr.) Singer & A.H. Sm. is a lignicolous basidiomycete of the family Strophariaceae. It grows on dead wood of many species of deciduous trees, rarely on coniferous wood. The distribution extends from temperate to subarctic vegetation zones (Jacobsson 1990, Knudsen & Vesterholt 2012).

K. mutabilis causes white rot and tolerates added nitrogen in the substrate (Leatham & Kirk 1983). As far as known, it is apathogenic to living trees (Luthardt 1969). Fruiting bodies grow in dense clusters on stumps and logs which usu-

ally have a contact with soil. It probably complements the wood-derived nutrients with soil minerals, using mycelial connections (Luthardt 1969).

K. mutabilis has been used for food for decades. It is presented as an edible mushroom in numerous European mushroom guides (e.g. Courtecuisse & Duhem 1995, Ryman & Holmåsén 1998, Vesterholt 2004, Salo et al. 2006, Korhonen & Penkkimäki 2012) and has been subject to large scale commercial cultivation in Germany (Luthardt 1969, Gramss 1978). No cases of adverse health effects are known to us. Due to its good track record, *K. mutabilis* was

included in the officially approved list of Finnish commercial mushrooms (e.g. Korhonen 1977). However, its usage among beginning mushroom hunters is limited by the difficulty in recognizing the species in the wild. Due to the risk of confusion, especially with the poisonous *Galerina marginata* (Batsch) Kühner *sensu lato*, usage of wild *K. mutabilis* has been recommended for experienced pickers only. Therefore, it has not recently been recommended for wild mushroom trade by Finnish authorities (von Bonsdorff et al. 2013). Cultivation of *K. mutabilis* by farmers knowing the species would facilitate its safe usage.

Biology of *K. mutabilis* and inexpensive culturing methods were studied especially by Walter Luthardt in post-war Eastern Germany. In his mass production methods, thick *Fagus* blocks were usually inoculated with axenic mycelium paste, pre-incubated in piles in cellars and planted outdoors. (Luthardt 1969). Gerhard Gramss continued refining the culture methods, showing e.g. the necessity of light for sporulation (Gramss 1974, 1978). Later on, large scale culturing of *Agaricus* and other, rapidly growing taxa in controlled conditions, were developed into a more profitable business fading the commercial culturing of *K. mutabilis* in Germany (Lelley et al. 1976, Lelley 1991). In other countries, research on *K. mutabilis* culturing has been continued, showing that its cultivation in controlled conditions is possible (Ulinski & Szudyga 2005). In Finland, especially Veikko Hintikka has shown that it can be cultured outdoors on solid wood blocks using simple methods (Hintikka 2009). He has shown that fruiting of wild *K. mutabilis* on a single stump can continue for 8–13 years (Hintikka 1993).

Considering the wide distribution of *K. mutabilis* and its acceptance of many woody substrates, we wanted to study its potential for especially low-tech culturing in the boreal vegetation zone. We wanted to create a first image of several culture variables and potential sources of error, to direct future studies. This was made in a three-year study using an adjustable research greenhouse.

Material and methods

I General methods

Strain collection. Strains of *K. mutabilis* were collected from Finnish nature during 2009–2010, between latitudes 60° and 64° N (Table 1). Pieces of cap center context of young, fresh fruiting bodies were detached aseptically using a scalpel and cultured on malt agar (20 g/l malt extract; 20 g/l glucose, 1 g/l peptone) with 0.1 g/l chloramphenicol. A laminar flow cabin was used either for all stages of isolation or, at least, for subculturing, depending on facilities near the collection site. A dried voucher specimen of the fruiting bodies of each strain was deposited in Åbo Akademi Herbarium (TUR-A). The natural poorly sporulating strain 7 was obtained from E.-M. Savonen (Finnish Forest Research Institution, Parkano). Herbarium specimens of this strain were collected from the cultured fruiting bodies. During the study, subcultures of each strain were grown in room temperature on the same type of agar as when isolated. A living culture of each strain was deposited at the HAMBI/FBCC Culture Collection, University of Helsinki (<http://www.helsinki.fi/hambi/>). Collection numbers of each strain are given in Table 1 (page 25).

Spawn production. Sawdust-based inocula of each strain were produced for the study by our order in a commercial mushroom culturing company (Polar Shiitake, Rääkkylä, Finland). In short, moist sawdust of *Alnus* (grain diameter ca. 2 × 3 mm) with ca. 3 % wheat flour was steam-sterilized in ca. 2 liters (1 kg, ca. 35 × 8 cm) plastic foil tubes. Liquid inoculum of each strain was injected aseptically in the tube and the holes were covered with a porous tape. These were incubated for ca. 2–3 months until fully colonized into cylindrical “bricks” and delivered for the experiment. After reception, they were visually quality-controlled for purity and for full colonization and stored in +4 °C until used.

Acquisition of wood substrate. The solid wood blocks were obtained during thinning of a young, about 20 years old, spontaneously emerged hemiboreal *Betula* forest (90 % *B. pendula*, 10 % *B. pubescens*) on the premises of Botanical Garden, Ruissalo, Turku. The trees were harvested and cut into 1–3 m long logs in the beginning of September 2010 and sorted out in desired thickness classes. Logs which were visually suspect of decay were discarded. A dark brown central core of maximally 2.5 cm diameter was allowed in the cut surfaces of the thickest logs. They were cut into final block lengths ca. 2 weeks after harvesting and stored outdoors in a shadowy site on pallets, under a well-ventilated tarpaulin rain cover. In round block studies, any blocks which showed broken bark in any stage before final soil planting, were discarded. Due to technical obstacles, the wood blocks had to be stored 2 months before inoculation, resulting in moisture ratio of 28–34 % (g water / g oven-dried wood). During the storage period, a slight, visible mould contamination emerged on some surfaces. This was accepted as an additional challenge for the competitiveness of *K. mutabilis* in low-tech culturing. For the double-board arrangement (Fig. 1B) 5 × 5 × 1.5 cm bark-free boards of *Betula* wood were machine-sawn longitudinally from suitably sized logs. Solid wood blocks were weighed as fresh and la-

beled according to each study group. When bunches of thin twigs were tested, each twig was measured separately but after binding each bunch was labeled and handled as a single study unit, like one block.

Inoculation. The round wood blocks (fruiting position shown in Fig. 1C) were inoculated in a non-sterile laboratory by spreading a thin layer of spawn on both sawn ends of the blocks, corresponding with ca. 5 g (10 ml) spawn grains or 20 ml of spawn gel / cut end. Consistency of spawn (dry or gelatinous) and methods of surface disinfection (hot water, steam or none) varied since they were among the studied variables as described separately. Each inoculated end was placed tightly against the end of another block or against a temporary disc sawn of another log. These were bound together for the pre-incubation period with a polyethene stretch film. The film also limited evaporation of water from the spawned ends. The double-board cultures (fruiting position in Fig. 1B) were inoculated by placing a layer of spawn gel between each pair of wood boards which were then bound together with the stretch film. The ready-made spawn cylinders (fruiting position in Fig. 1A) were used without further inoculation.

Test greenhouse. Pre-incubation and the 3-year fruiting experiment were performed in a semi-controlled 6.5 × 7 m room of a test greenhouse of the Ruissalo Botanical Garden of University of Turku (Fig. 2). The room was equipped with floodable irrigation tables. Growth conditions were controlled by an automated greenhouse control system (ITU CAG, Itumic Oy, Jyväskylä, Finland). Controlled variables included the position of sun screens, position of roof ventilation lids, electrical fans, temperature (by heat-radiating pipes) and relative humidity (by misting). Glass walls and roof were chalked to diminish sunshine. The room was not fully controlled, since the ventilation was based on outdoor air and the room was at times overheated during hot summer days. A lightweight, nearly opaque tarpaulin (green with a silvery outside), supported by a plastic gage, was used on the southern and upper sides of each table as a basic sunscreen, at all times. In periods when full darkness was tested, a fully opaque plastic foil (outside white, inside black) was added.

Pre-incubation. Before planting in the final fruiting position, all inoculated solid wood substrates were pre-incubated without soil, in order to let the mycelium colonize the wood before soil contact. Round wooden blocks were incubated horizontally on the greenhouse tables under fully covering tarpaulin shadow. The air moisture of the room was kept between 75–85 % RH and temperature between + 18–20 °C. The tables were not flooded but wetted tissues were kept under the tarpaulins to secure sufficient moisture. The inoculated double boards were pre-incubated on the same tables in empty buckets with partly closed lids, resulting in moistures 90–100 % RH inside buckets. The ready-made sawdust spawn cylinders were not pre-incubated. Sufficient duration of the pre-incubation was assessed by visual inspection and by microscoping thin slices of some test blocks using Cotton Blue stain in different depths from the sawn end, at different radial distances from the bark. After 2 months' incubation, the blocks were colonized at least to the depth of 4 cm from the inoculated surfaces, corresponding with longitudinal penetration speed of ca. 2 cm/month. Based

on this, the blocks were considered ready for planting in the soil. At the end of pre-incubation, the blocks were detached from each other. At the end of the pre-incubation, each block was visually studied as to the degree of superficial colonization of both inoculated ends. Presumable *K. mutabilis* was identified based on e.g. white hyphae with brown pigmented patches. Percentage of the area correctly colonized was estimated and recorded in ten percent intervals at both ends of the block, in order to understand, whether some yield variations would originate from the early stages of colonization.

Test buckets and initial soil mixture. White, semi-transparent 5 liter HD polyethene plastic buckets (LM 470, Lanka ja Muovi Oy, Pori, Finland) were used as study containers for all the substrates planted in soil (Figs. 3, 4). The buckets were modified by drilling holes to the bottom of each bucket. A round piece of non-woven agricultural polypropylene filter cloth was placed on the bottom, to prevent leakage of soil. An initial soil mixture was specially designed for the study, by previous pilot testing with a larger number of mixtures. The aims included to provide the fungus with sufficient nutrients by adding some garden soil, to approximate its natural conditions in Finland by adding some acidic peat, and to allow sufficient draining of water by adding sand. Pilot tests showed that efficient draining called for a high proportion of sand. The selected initial soil mixture consisted of 60 volume % coarse silicate sand, 20 volume % of commercial *Sphagnum* peat (pale, slightly decayed, unfertilized, type "KTv", pH4, VAPO Oy, Jyväskylä, Finland), 20 volume % of unfertilized in-house "garden soil" and 1 volume % of CaCO₃.

The "garden soil" component was chosen to be non-defined to correspond with low-tech cultures. It was routinely prepared in large volumes at the Garden, by mixing of about 2 to 3 volume units of clay mull prevalent in the area, 1 volume unit of in-house organic plant compost, 2 volume units of peat and 1 volume unit sand. Volumes of these materials were slightly adjusted from batch to batch, based on the type of clay mull available.

Planting and maintenance. After the pre-incubation, all substrates were placed in soil as shown in Fig. 1A–C. Three main culture designs were used: 1/3 portions of the ready-made sawdust cylinders (number of buckets = 30) (Fig. 1A); boards of *Betula* wood used in a double-board ("sandwich") arrangement (modified from Gramss 1972, 1978) (n=20 buckets, Figs. 1B, 3); and various sizes of solid, round blocks of *Betula* with intact bark (n=170 buckets) (Fig. 1C).

After removal of the plastic foil tube, the ready-made sawdust spawn cylinders were cut transversely into three equally-sized (in average 0,63 liters, 350 g fresh weight, 100 g dry weight) parts which were kept otherwise intact and planted vertically, one in each soil bucket, the top of the substrate chunk on the level of soil surface (Fig. 1A). The double-boards were planted vertically, keeping each pre-incubated doublet intact, four doublets of same handling in the same bucket, about half of each "sandwich" buried and half visible (Fig. 1B, Fig. 3). The inoculated round wood blocks or twig bunches were sunk in the soil in a slightly tilted position, so that the highest edge was about 2 cm above the soil. A direct borderline between the soil and the upper, sawn block end was aimed at, fear-

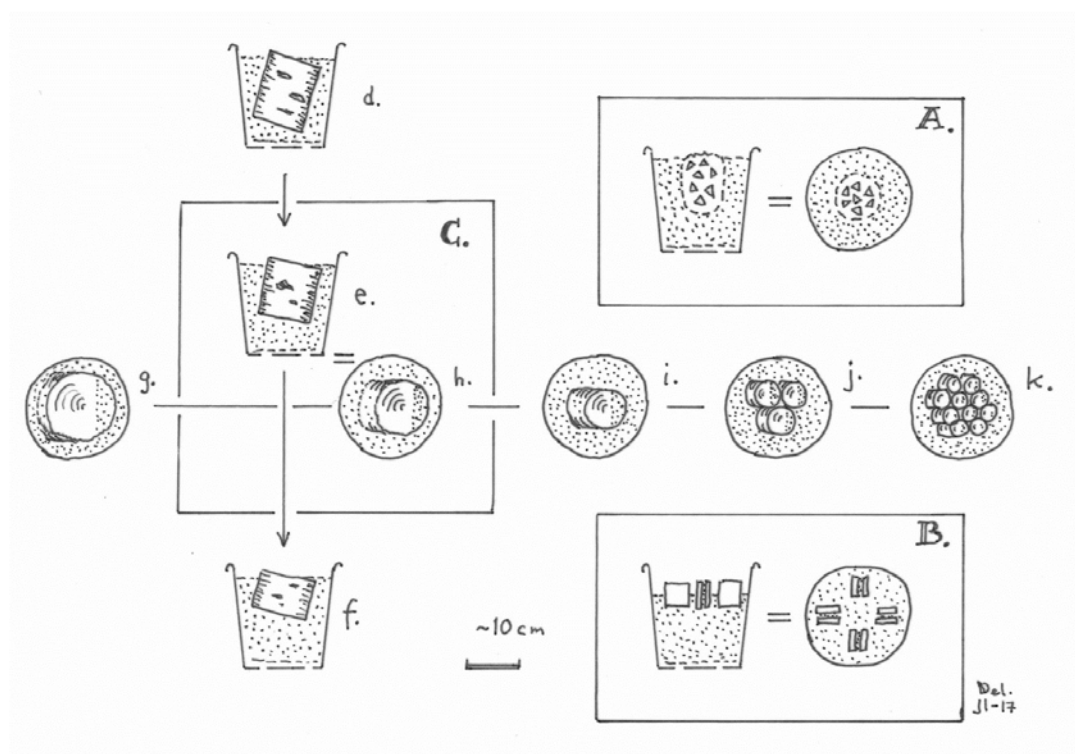


Figure 1. Spatial arrangement of 5-liter test buckets, with pre-incubated substrates planted in soil. Schematic, scale approximate. Sign “=” connects images of the same bucket as seen from above and from aside. A: Sawdust spawn cylinders. B: Double-boards. C (= e,h): Basic protocol with 10 x 10 cm solid wood blocks, used as a common reference for the two size variables (length and width) below. Vertical arrows d-e-f: Decreasing block lengths 13, 10 and 7 cm, respectively. Horizontal lines g-h-i-j-k: Decreasing block widths 13, 10, 7, 5 and 3 cm, respectively, the thinnest tied in bunches. All blocks were slightly tilted as shown.

ing that in a straight position the fresh bark might hinder the penetration of mycelia into the soil (Fig. 1C). All soil mixtures were thoroughly wetted and mixed in a cleaned construction mixer, just before planting. The original code labels were kept under the soil and duplicated as a tape label outside the bucket.

The sawdust cylinders and double-boards were used mainly for comparing the strain-specific characters of all the 5 strains. The more extensive solid wood tests were used for studying several alternative culture techniques or substrate characters. These were divided in several sub-groups according to the variables shown in Table 2. These tests relied on two strains, only (strains 4 and 7). Identical tests were performed on both these strains.

Watering and other maintenance protocols were varied during the study, since optimal conditions and growth cycles of the species in this setting were not known. The yields recorded in this paper are summed from all these stages of the study. Shortly, during the first 11 months after planting in buckets, optimal conditions were searched for, by adjusting the bucket lids, shadowing, watering and other conditions. Relative humidity of the room was var-

ied between RH 40–70 %. Air temperature was kept at ca. +20 °C. Between study months 12–20, a rapid (4 weeks) maintenance cycle was attempted during which several variations in ventilation, temperature and irrigation and lid position were tested for fruiting initiation. Natural illumination was not hindered. At the growing month 21, a slower fruiting cycle was commenced, consisting of a one-month resting period with total darkening of the cultures at +20 °C and a one-month fruiting period at +18 °C, RH 75–80 %, starting with profuse irrigation. Irrigation and air moisture were gradually lowered towards the next resting period. This cycle was continued during the remaining 15 months of the study. Lids of the buckets were used in different positions for bucket-specific moisture adjustment if needed, e.g. for preventing drying of primordia.

Protocol for observations and harvesting. All test buckets were studied at one week intervals during the whole study except the periods of full darkening. Approximate number of primordia and the number of morphologically mature fruiting bodies (defined as cap diameter > 1 cm) were counted. Diameter of the largest cap in each



Figure 2. The adjustable greenhouse room and general setting of the trial. Floodable irrigation tables and shadowing tarpaulins are shown with test buckets. South is in the direction of the next room seen behind the wall on the left. – Photo: T. Willstedt.

bucket was measured to follow the growth process. Additional observations, such as the condition of sporocarps, occurrence of any other fungi or potentially harmful other organisms were recorded. Overall presence of flying insects was monitored during the entire study by having a glue paper trap hanging in the room.

When each fruiting flush in a given bucket was coming to an end, the fruit bodies were harvested in the nearest weekly inspection day, by detaching entire sporocarps from soil or wood. After careful removal of any visible soil from the stipe base, fruit bodies of each bucket were air-dried. Total dry weight of the yield of each bucket, including stipes and eventual primordia within the bunches, was measured using 0.1 g accuracy. Conditions of the growing room such as daily minimum, maximum and mean values of air temperature and humidity were recorded by using the automated monitoring system of the greenhouse.

“Succeeded” buckets and “Success rate”. In the end of the study, test buckets which had not produced any fruiting bodies of *K. mutabilis* during the whole 3 years’ time were defined as “unsucceeded”. Correspondingly, buckets which produced any number of *K. mutabilis* fruiting bodies were defined as “succeeded”. This techni-

cal classification does not take any position on economical profitability of a given method. It is made only for discussing different mechanisms which may lead to poor yield. Yield comparisons were made between the “succeeded” buckets of a given group, since the total loss of fruiting bodies might also be caused by other factors than the studied variable. The term “success rate” is used for the percent of succeeded buckets / all buckets, within a given study group.

Degrees of purity. The isolated strains and ready-made inocula were axenic. The described disinfection methods, in the study groups where this was carried out, temporarily diminished the growth of surface microbes. All remaining stages of the study were intentionally unsterile to test the viability of the methods for low-tech culturing. E.g. earthworms and other invertebrates of the in-house soils were not controlled in any way. There was no prevention of flying insects or contaminating spores. Ventilation of the greenhouse relied on automated lids without filters.

Ending the study. At the end of the entire study, the remaining contents of each bucket was disassembled and studied. The decayed wood material was cleaned of soil and split when necessary, in order to understand the decay structure. Types of decay were estimated visually. Pres-

ence of any visible organisms such as worms inside the soil or wood was recorded.

II Variables studied with solid wood blocks

Principle of wood block comparisons. Due to strict constraints of e.g. room space, each subgroup with wood blocks was cross-compared with a single, common reference method. The arbitrary term “basic protocol” (BP) was coined for this set of material and methods. BP was applied to 20 buckets (10 of both strains 4 and 7) and consisted of a 10×10 cm block, one per bucket, block disinfection by boiling, gelatinous inoculum with *Alnus* grains, initial soil mixture as such, and capillary bottom irrigation by flooding the table. Each of the other subgroups (10 buckets each, 5 of both strains 4 and 7) differed from BP by one factor only. These were species of inoculum wood, dry or gelatinous inoculum, the method of wood disinfection, soil composition, block width, block length and methods of irrigation (Table 2). Details of each subgroup are given below.

Inocula. In BP the spawn was made of *Alnus* grains. In the only compared alternative, *Alnus* grains were replaced with sawdust of *Betula*. The *Betula* sawdust obtained by the manufacturer was fibrous and of finer sort (grains ca. $1 \times 1 \times 4$ mm) and not as fresh as the regular *Alnus* grains.

In BP the sawdust spawn was mixed with previously boiled and cooled potato flour gel (25 g sawdust spawn and 4 g potato flour / 100 ml water) to achieve good fastening of the spawn and to ensure adequate moisture. About 20 ml of gelatinous spawn, containing ca. 5 g of dry spawn, was applied on each block end. In the only compared alternative, the dry sawdust spawn was crumbled into a ca. 0.5–1 cm thick layer on the sawn wood surfaces as such, without any gelatinizing agent. During the horizontal pre-incubation, both types of spawn were kept against opposing wood surfaces using the mentioned stretch foil.

Block disinfection and soil composition. The BP was to submerge each piece of solid wood (mainly blocks but also the subgroup of double-boards for which disinfection was scheduled) in a pot of boiling water, then waiting until the water started to boil again, then removed for a short cooling, immediately before inoculation. In one compared alternative, only the sawn ends of each block were surface-disinfected by applying hot steam (30 sec / cut surface, proceeding with systematic zig-zag pattern) on the surfaces to be inoculated, using a steam cleaner. Another compared alternative was not to disinfect the blocks at all.

In BP the initial soil mixture was used as such. In one compared alternative, the CaCO_3 enrichment of the initial mixture was omitted, to test if garden soil, as such, makes the mixture sufficiently nutritious. In another alternative, the garden soil of the initial mixture was replaced with peat, resulting in a poorer and more acidic soil with 40% peat and 60 % sand. The CaCO_3 enrichment was kept in the mixture, to test if CaCO_3 , alone, would compensate for the peat's acidity and for the missing garden soil nutrients. As a third alternative, the garden soil of the initial mixture was replaced with peat and CaCO_3 was omitted. With this mixture, poor and acidic soil types of some

Finnish forest types and peatlands were mimicked.

Wood block width and length. All studied dimensions of wood were designed to fit in the same type of bucket, leaving space for the soil to surround the wood from all directions. The purpose of the width test (Fig. 1, horizontal line from g to k) was to test if less valuable, thinner wood fractions might be used for culturing. In this subgroup, all blocks or pieces of twigs were 10 cm long (all measures given with tolerance of 1 cm).

In BP the single solid wood block of each bucket had a diameter of 10 cm (Fig. 1C). In one compared alternative, the single block was 13 cm thick (Fig. 1g). In another alternative, the single block was 7 cm thick (Fig. 1i). In another alternative, each block was 5 cm thick. They were bound in bunches of 3 blocks per bucket to keep the soil/wood ratio reasonably constant between groups (Fig. 1j). In the last alternative, each block, or rather a stick, was only 3 cm thick, representing the thinnest fractions of wood harvesting. They were bound in bunches of 10 sticks per bucket, for the reason given above (Fig. 1k).

The purpose of the length test (Fig. 1, vertical line from d to f) was to test how long blocks would be optimal for culturing, thinking of, e.g., different speeds of mycelium growth into different directions of the wood, or potentially hampered transport of air or nutrients in a long block. In this subgroup, all blocks were 10 cm thick. A single block was placed in each bucket. In BP the wood block was 10 cm long (Fig. 1C). In one compared alternative, the block was 13 cm long (Fig. 1d). In another alternative, the block was 7 cm long (Fig. 1f).

Irrigation. In BP the test buckets were watered by flooding every irrigation table once a week with a few cm deep layer of water for 1–2 hours, thus letting the soil to draw in capillary water. In one compared alternative, the buckets were irrigated by flooding but also moistened weekly from above, by applying fine water mist, using a misting bottle. In case primordia were present at the time, these were not directly misted. In another alternative, the test buckets were permanently raised above the flooding level. Water mist was applied weekly as the only irrigation.

RESULTS

I General observations

Isolation and pure culturing. Radial growth rate on agar was tested precisely with three strains, the others being visually in the same magnitude. In primary isolation, a slow, one-week adaptation phase first occurred, during which the radial speed in $+20^\circ\text{C}$ averaged about 1 mm/day. After adaptation, the radial speed in $+20^\circ\text{C}$ varied between 16–22 mm/10 days, corresponding with roughly 2 mm/day. Radial speed of ca. 1 mm/day was observed permanently if an adapted colony was kept in $+4^\circ\text{C}$. In sum, about 5 cm of radial growth occurred in $+20^\circ\text{C}$ during the



Figure 3. A test bucket with the double-board setting. The illustrated flush was exceptionally good for the double-board tests. *K. mutabilis* strain no. 7 with naturally deficient geotropism and pale gills due to sparse spore formation. – Photo: T. Willstedt.

first month, the adaptation phase included. Contaminants were sparse and easily controlled by a second transfer. Colonies of *K. mutabilis* were generally white, with moderate aerial hyphae and variable yellowish pigments on underside. In some older colonies, small cylindrical conidia consistent with those observed by Jacobsson (1989) were formed.

Features of spawn. The sawdust spawn cylinders had white mycelium with cinnamon-brown

surface patches of velvety texture, typical of the species. The cylinders were easy to crumble for spawning and had pleasant, fresh, sweetish smell typical of the species.

Penetration of mycelium during pre-incubation. Microscopic examination of some reserved control blocks (round wood with intact bark) after 2 months pre-incubation showed that *K. mutabilis* spreads most rapidly under the bark, most profusely in cambium, secondarily in inner



Figure 4. A typical test bucket with a solid wood block (poorly visible) immersed in soil. A fruiting flush of *K. mutabilis*, strain no. 4, emerging along the round contact border between the block and the soil. Plastic sticks are temporary reminders of e.g. previous flushes or for follow-up of observed primordia. – Photo: T. Willstedt.

phloem layers. When penetrating into the xylem, it preferred ray cells for radial penetration and vessels for longitudinal penetration. Wood fiber cells were colonized most slowly. Also the white, laminar outer bark typical for *Betula* was penetrated, but slowly.

Degrees of superficial colonization. After the 2 months' pre-incubation, the sawn end of round blocks showed consistent colonization of visually identified *K. mutabilis* mycelium. The overall

coverage of correct mycelium in all blocks regardless of study subgroup ($n=170$) was 35–100 % of the sawn surfaces (average 84%). The areas without correct colonization were typically covered with green-spored *Trichoderma* sp. In this stage, a few deformed *K. mutabilis* fruiting bodies already grew on some blocks.

Characteristics of fungal strains. Our bucket-based modification of the double-board method was not satisfactory for testing of strains, regard-

Table 1. Strains of *K. mutabilis* used in the study. For abbreviations of the culture collection and of the Herbarium, see text.

Strain numbers in this study	Origin	Living strains HAMBI/ FBCC no.	Dried reference specimens Herbarium no.
1	Stump of <i>Populus</i> , Turku	1426	TUR-A 183778
2	Stump of a deciduous tree, Turku	1427	TUR-A 190697
4	Stump of <i>Betula</i> , Sysmä	1429	TUR-A 203730
5	Stump of <i>Betula</i> , Pyhäsalmi	1430	TUR-A 203729
7	Stump of <i>Betula</i> , Parkano	2158	TUR-A 198114, TUR-A 198115 TUR-A 198117, TUR-A 198120

less of surface disinfection of boards (data not shown). The double-board buckets generally suffered from excess moisture. Except for some better initial flushes (Fig. 3), the sporocarps were sparse, small and occurred irregularly. Strain-specific features were compared using the sawdust cylinders, since these produced substantial amounts of well-developed fruiting bodies in a regular pace. Yields of each strain are shown in Table 3. Main observations follow:

Strain 7 (Fig. 3) produced the best yield (43 g dry mushroom/kg fresh substrate) and largest sporocarps (dry weight in average 0.17 g per fruiting body). Strain 4 was a steady performer with total yield 34 g/kg, yielding moderate-sized fruiting bodies with the highest number (7) of flushes during the study. The morphology of this strain was typical for the species in the nature. Strains 2 and 5 both yielded 31 g/kg due to a combination of more delicate fruiting bodies and fewer flushes. Strain 1 formed moderate-sized fruiting bodies but the smallest number (4) of flushes, resulting in the smallest total yield (25 g/kg). Strain 7 (Fig. 3) had a characteristic morphology. It had pale gills due to deficient sporulation and showed defective geotropism, i.e. the caps grew in a tilted position. It showed these characteristics already in the nature. While sporeless strains are in general interesting for allergy prevention, we do not recommend this strain for commercial cultivation before further testing, since the tilted position seemed to make the sporocarps more sensitive to e.g. excess moisture.

Further comparison between the strains 4 and 7 when cultured on solid wood blocks, are given in Table 2 and Figures 5–6.

Influence of culture design and substrate form. The sawdust cultures had a very good success rate. One totally non-fruiting strain was omitted from data. Out of the remaining 30 buckets only one remained without any fruiting. The first proper flush on sawdust cylinders started at 3 weeks after planting in the soil buckets. Each flush contained in average 24 fruiting bodies, with an average dry weight 0.11 g / fruiting body. Mushroom production of the sawdust cylinders faded during the second year after planting and ceased totally at growing month 20. Mushroom production on the double-boards started at 19 weeks from planting. The success rate was lower (75 % of double boards showed any mushrooms). For reasons stated previously, the growth on double-boards was not consistent enough for sensible comparisons between strains or between disinfection methods. Production practically ceased within 2 years. Yields on round wood blocks accumulated slower than on sawdust but lasted longer than on other substrates. On the round wood, first flushes started at 10 weeks of planting. Production diminished gradually towards end of the study but scattered fruiting still occurred at 2 years 11 months, when the study was ended. Yields and success rates on round wood varied according to several variables tested, as shown in Table 2.

Table 2. Effects of different culturing methods and materials on the success and yield of *K. mutabilis*. Strains 4 and 7, solid wood blocks immersed in soil. Basic protocol (boiling water disinfection, block length 10 cm, block width 10 cm, gelatinous alder spawn, bottom watering, initial soil mixture) except for one given variable studied at each group. Success rate = percentage of fruiting buckets / all buckets of the given group. Yield given for succeeded buckets, as g dry mushroom / kg fresh wood.

Tested variables	Strain 4					Strain 7					Mean yield (both strains)
	Yield dry weight (g) / wood fresh weight (kg)					Yield dry weight (g) / wood fresh weight (kg)					
	n	Success rate (%)	min	max	mean	n	Success rate (%)	min	max	mean	
Basic protocol	10	60	18,5	32,4	25,1	10	70	1,9	34,1	18,8	21,7
No disinfection	5	60	3,2	20,6	14,5	5	20	3,0	3,0	3,0	11,6
Steam disinfection	5	60	20,1	31,4	26,1	5	80	4,3	25,5	17,1	20,9
Dry spawn	5	80	19,5	37,3	26,6	5	80	4,1	21,8	13,2	19,9
Birch spawn	5	60	0,3	22,1	8,6	5	40	2,7	24,3	13,5	10,6
Block length 7 cm	5	80	15,2	33,0	23,5	5	40	26,3	26,7	26,5	24,5
Block length 13 cm	5	20	25,6	25,6	25,6	5	80	0,7	12,2	3,7	8,1
Block width 3 cm	5	100	10,7	43,7	25,8	5	80	1,4	23,0	17,0	21,9
Block width 5 cm	5	100	11,6	32,8	21,4	5	100	1,6	14,2	7,8	14,6
Block width 7 cm	5	100	23,2	43,1	33,1	5	80	0,3	48,8	25,5	29,7
Block width 13 cm	5	60	1,2	20,6	8,2	5	100	3,4	19,4	11,2	10,1
Surface watering	5	60	12,4	12,8	12,7	5	80	5,2	26,2	17,5	15,4
Bottom + surface watering	5	40	11,9	14,3	13,1	5	40	2,0	15,7	8,8	10,9
Sand+peat without CaCO ₃	5	100	4,5	21,6	14,0	5	60	1,9	5,8	3,6	10,1
Sand+peat with CaCO ₃	5	60	21,9	40,4	30,7	5	100	17,4	28,0	21,3	24,8
Initial soil mixture without CaCO ₃	5	100	9,0	26,2	17,3	5	80	8,8	15,4	11,6	14,8

Decay analysis. In the dissection of wood blocks at the end of the study, the decay types were classifiable in two alternative processes, with a gradient of intermediate forms between. In the optimal type of decay, the block had lost majority of its weight, in the end. *K. mutabilis* had formed soil-mixed mycelium crusts on the sawn ends and patchily on the bark, so to protect the integrity of the block. Some white mycelium could also be seen in the surrounding soil. The wood was aerobic, whitish and fresh-smelling inside. The decay seemed to have started with a concentric decay (characterized by detachable, cylindrical slices due to degradation between each annual growth ring) and had then softened the entire volume equally. A somewhat gelatinous intermediate stage with grain-like cavi-

ties occurred in a few blocks. In the end, only a sparse whitish fiber network remained of the wood.

In practice, the traffic of worms and other soil animals would break the integrity of the colonized spaces during late stages of the softening. This resulted in secondary darkening of the already fluffy decay, due to its mixing with soil. Eventually, all wood had disappeared and soil gradually crumbled down to fill the empty space.

The undesired type of decay was anaerobic. These wood blocks had not lost significant volume or weight during the 3 years. Such wood was hard, darkish and water-logged and had a foul smell when cut. Another version of the anaerobic decay was an equally foul-smelling, relatively hard but concentric decay. This may have

Table 3. Strain-specific characters of *K. mutabilis*, tested with axenic sawdust spawn cylinders immersed in soil. Success rate = percentage of fruiting buckets / all buckets, using this method. Yield given as g dry mushroom / kg fresh substrate. Weight of a fruiting body (fb) given as g of dry weight. Number of flushes (bursts of fruiting bodies) during the entire study. (sd = standard deviation)

Strain no.	n	Success rate (%)	Yield dry weight (g) / wood fresh weight (kg)				Mean weight of a fb (g)	Mean number of flushes
			min	max	mean	sd.		
1	6	83	14,6	41,7	30,5	10,0	0,12	4,0
2	6	100	24,0	36,0	30,9	4,0	0,07	6,2
4	6	100	22,0	42,0	34,3	7,8	0,11	7,0
5	6	100	26,0	41,1	30,8	8,5	0,08	5,0
7	6	100	32,0	61,1	42,8	10,8	0,17	5,0

started as aerobic as above, but became anaerobic later. Both types of anaerobic decay resulted in very low mushroom yields. In many moderately yielding blocks, the two decay processes occupied separate 3-dimensional compartments of the block. The parts with optimal decay had been softened or nearly disappeared, leaving varying forms of fluffy cavities or tunnels “drilled” in the otherwise hard, anaerobic wood. Obviously, the volume of the soft decay in relation to the volume of the whole block was related to the yield, but this was not objectively measured.

Adjoining organisms. Due to the intentionally non-sterile conditions, a wide variety of other organisms was observed in the buckets or in the surrounding air. None of these showed any significant effect on the cultivation. Commensal or competing fungi produced fruiting bodies in a few, scattered buckets and included *Pluteus* sp., *Parasola* sp., *Pseudomerulius* sp. and *Bjerkandera adusta*. Myxomycetes (mostly *Stemonitis* sp.) and unidentified mycelial fungi occurred especially during periods of excess moisture. Concerning animal pests, the glue-trapped insects were few and corresponded with normal background. Neither phorid flies nor sciarid flies were detected in the traps. Small amounts of *Cecidomyiidae* sp. were detected but showed no relationship with the cultured mushrooms. Snails were observed rarely, occurred singly and were removed by picking. No visual signs of snail or insect damages were seen in the mushrooms. Small-sized species of earthworms (*Lumbricidae*) were common in the bucket soil. Enchytraeid worms and white-coloured springtails (*Collembola*) were seen on some water-logged

wood surfaces. Ferns and seedlings of flowering plants occurred randomly on bucket soil. These were cut in case of excessive growth but not otherwise eradicated. In most buckets, the soil began slowly be covered by mosses (e.g. *Marchantia polymorpha*). Mosses were not removed. No correlations between plant coverage and the mushroom yields were observed.

II Variables studied with solid wood blocks

Influence of species of inoculum wood. Cultures spawned with alder sawdust performed better in all respects than birch. They were in average better colonized (82 vs. 58 %), had slightly better success rate (65 vs. 50 %), gave more fruiting bodies per wood kg (148 vs. 39) and yielded more dry weight per wood weight (22 vs 11 g/kg), respectively (Table 2). The tree species was not the only factor involved, however, since the *Betula* spawn obtained by us did also differ in grain structure and freshness, and had not been optimized by the spawn producer.

Influence of gelatinized vs. dry inoculum. Cultures spawned with dry inoculum showed a better success rate than the gelatinized one (90 % vs. 65 %, respectively) but mushroom yields of the succeeded blocks were better using the gelatinized spawn (21.7 g/wood kg) than dry spawn (16.7 g/wood kg). This suggests that gelatinized spawn is a promising alternative for dry spawn, but block disinfection should perhaps be more rigorous and other variables be more optimized, if gelatinous spawn is used.

Influence of block disinfection. The method of disinfection had an effect on the superficial colo-

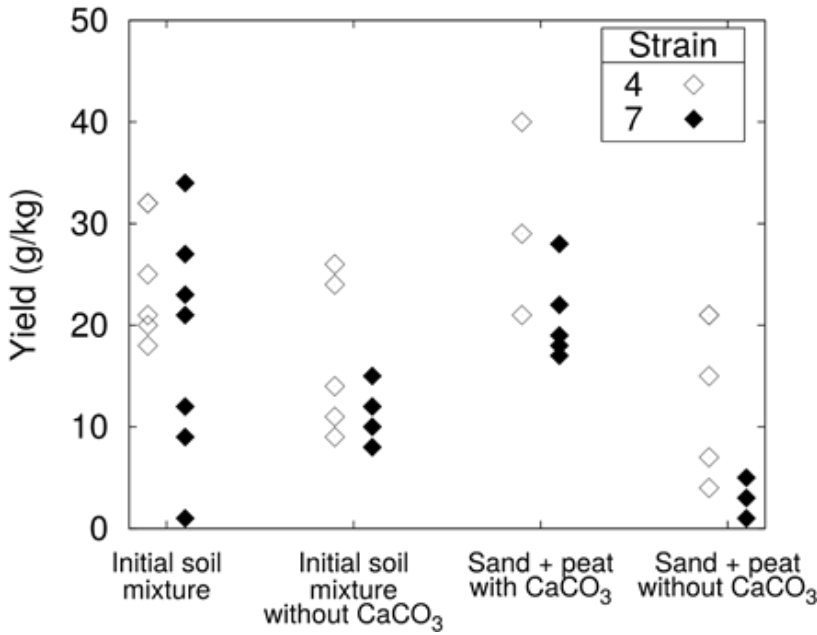


Figure 5. Effect of soil composition on mushroom yield. 10 cm wide solid wood blocks immersed in the soil types shown. Scatter diagram of strains 4 and 7. Data of fruiting, i.e. “succeeded” buckets shown, only. Yield given as g dry mushroom / kg fresh wood. For numerical data, see Table 2.

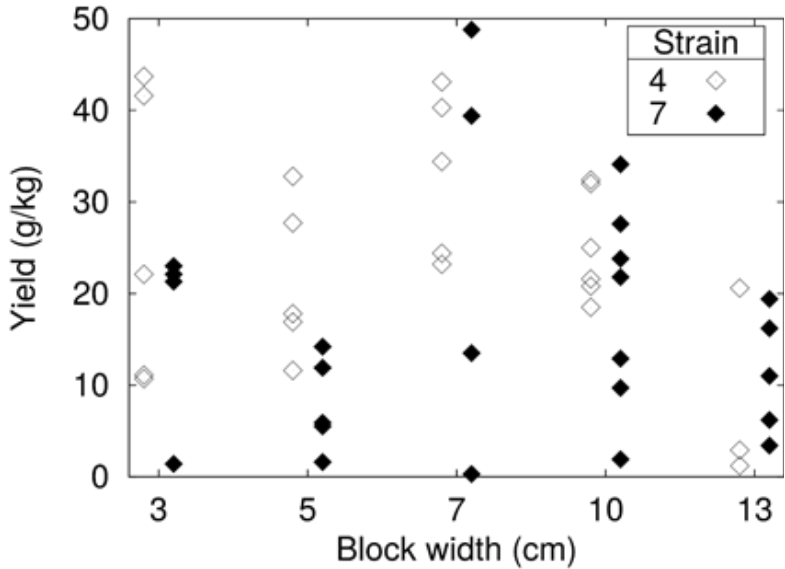


Figure 6. Effect of the block width on mushroom yield. Solid wood blocks immersed in initial soil mixture. Blocks of 3 and 5 cm width were bound in bunches of 10 and 3, respectively (see Fig. 1 g-k). Scatter diagram of strains 4 and 7. Data of fruiting (“succeeded”) buckets shown, only. Yield given as g dry mushroom / kg fresh wood. For numerical data, see Table 2.

nization seen after pre-incubation. Boiled blocks showed more extensive colonization (average 82 %) than the steamed (55 %) and the undisinfected (59 %) ones. This was not reflected in the later success rate of each group, however: The boiled group (success rate 65 %) was in the same magnitude as the steamed group (70 %). The undisinfected group showed only 40 % success rate. The total number of flushes was highest in the steamed group (average, 8.1) and lowest in the undisinfected group (5), but the differences were not significant. The number of fruiting bodies per substrate weight was highest in the boiled group (average 148 fruiting bodies per kg of fresh wood). In the steamed group the number was slightly lower, 117 fb's / kg, while the undisinfected group had the lowest number, 63 fb's / kg. Mushroom yield per fresh substrate weight is shown in Table 2. In the succeeded buckets, the boiled and the steamed group performed similarly (21.7 and 20.9 g/kg, respectively). The undisinfected group yielded only half of that amount (11.6 g/kg). Inclusion of the unsucceeded buckets in the calculation did not essentially change the difference between any kind of disinfection and the omission of disinfection (about 14 vs. 5 g/kg, respectively).

Influence of soil composition. Omission of CaCO_3 , and simultaneous replacement of garden soil with peat clearly worsened the yields (mean 10.1 g/kg dry weight of succeeded pots vs. 21.7 g/kg in the initial soil mixture (Fig. 5). The negative effects of garden soil omission and peat addition were compensated by the presence of chalk, even so that the chalk + peat recipe performed better than the initial soil mixture (24.8 g/kg). Use of garden soil without CaCO_3 enrichment was not optimal. It yielded 14.8 g/kg. Similar differences between the soil mixtures were also observed in other signs of performance such as colonization, success rates and fruiting body counts.

Influence of wood block width. There was no significant trend as to superficial colonization, corresponding with the wood thickness. All subgroups were well colonized (82–99 %). As to the success rates, the width used in BP had poorest success (65 %). All other thicknesses had equal and good success rates (80–100 %). As to dry weight yield / wood kg, the best yielding thickness was 7 cm (only succeeded ones counted, mean 29.7 g/kg). Second best widths were 3 and

10 cm, without clear difference (21.9 and 21.7 g/kg, respectively). 5 cm thickness was still poorer and the 13 cm poorest of all (Fig. 6). When also the unsucceeded buckets were counted, relations of each group were otherwise similar but yields from the thinnest 3 cm sticks were better than those from 10 cm blocks (19.7 vs. 14.1 g/kg, respectively), due to the better fruiting success on thin wood. As to numbers of flushes, the thickness groups behaved in other respects as above, but the group of thinnest 3 cm sticks gave a larger number of flushes (mean of succeeded buckets, 8 flushes) than the others. Maximum flush numbers of the whole study (19 flushes / 3 years) were obtained using these 3 cm thin sticks.

Influence of wood block length. Colonization and success rates were not affected by wood block length. As to average yields (dry weight per substrate fresh weight, only succeeded ones counted), the shorter the block was, the more it yielded. The shortest ones gave 25 g/kg, the BP (10 cm) length 22 g/kg and the longest blocks only 8.1 g/kg. When also the unsucceeded blocks were included in the results, yields of the two shortest groups were equal (14.1–14.7 g/kg) and those of the longest group only 4 g/kg. The number of fruiting bodies per wood weight was also equal among the two shortest groups (137.5/kg for the length of 7 cm and 147.8/kg for the length of 10 cm). The longest blocks yielded only 40.8 fruiting bodies per kg of wood. Concerning the number of flushes, the two shorter block types (7 and 10 cm) both had more flushes (6.2 flushes each) than the longest 13 cm blocks (3.4 flushes). The flushes of longest blocks started about six months later than those of both shorter blocks.

Influence of irrigation. The BP group showed highest dry yields per wood fresh weight (average 21.7 g/kg in the succeeded buckets). The second best was mere surface watering (15.4 g/kg). The group which was watered from both directions showed lowest yields 10.9 g/kg. Similar differences between irrigation groups were observed also in other variables such as success rates, flush numbers and fruiting body counts. In final analysis of the decayed wood blocks, the cultures watered from both directions suffered more often of excess moisture and of anaerobic conditions, and the blocks remained hard.

Discussion

In the present study, *K. mutabilis* proved to be a feasible mushroom species for various low-tech culturing methods. It has the potential for creating subsidiary income for e.g. suitably trained farmers. Useful features of *K. mutabilis* included its capability to colonize solid wood in spite of several disturbing factors such as unoptimal substrate quality, deficient pasteurization and the presence of competing microorganisms.

Characteristic limitations of *K. mutabilis* in commercial cultivation include its slow growth (Lelley 1991). The cost of fully controlled growing facilities calls for more rapid production cycles. Therefore, focus of future research should be in semi-controlled culture arrangements which may include capillary irrigation and shading. If flushes are meant to be adjusted, periods of total darkening of the fruiting beds seem to be the most feasible way to do it. This relationship with light is consistent with observations of Gramss (1974, 1978).

We identified several useful low-tech culturing tricks. The paste-like mycelial suspension, developed by Luthardt (1969) for block inoculation can, to some extent, be replaced with spawn suspended in easy-to-make potato flour jelly. Apparently, however, our block colonizations and success rates would have been higher with an actively growing mycelium paste. Dipping solid wood blocks in boiling water was shown to be sufficient for surface pasteurization. In subsequent low-tech trials with *K. mutabilis* on Finnish farms (Issakainen 2015) we have used boiling water (staying to cool ca. 10 min) successfully also for wood chips.

In Finnish nature, wild *K. mutabilis* seems to avoid the most acidic or peaty soils (Issakainen & Pihlaja, unpublished data). Many such sites would, however, be interesting for low-tech culturing due to their high prevalence and availability of water and thin birch. Therefore, it was a promising finding that a peat-rich soil could be turned suitable for low-tech fungal cultivation by adding CaCO_3 . This requires further testing in nature.

Optimal size limits of the wood block were of interest, since branches and wood removed in thinning would be readily available as low-value side products of forestry. Luthardt (1969) had cultured the species mainly on thick *Fagus* blocks.

We wondered if the profuse bark on small twigs would be poorly penetrated by mycelium, or if it would contain too much contaminants. However, the smallest 3 cm diameter sticks proved to be among the best performers. To achieve this, surface pasteurization and careful tightening of the pre-incubated stick bunches in the soil are probably obligatory, since in natural conditions thin sticks easily get too dry. Reasons for the poorer performance of exactly 5 cm thick blocks remain unsolved in the present study. As to the largest dimensions of the block, our results were probably influenced by the limited size of our test bucket and by our tendency to over-irrigate the buckets in the learning stage. Final dissection of the wood blocks showed that many of the wider or longer blocks suffered from anaerobic conditions. The mycelium was only capable of forming cavities in the block. We learned that solid wood releases moisture very slowly, and next time we would let the cultures dry better between flushes. Most likely, *K. mutabilis* can be grown on almost any size of wood block, assuming local optimization.

Any watering from above caused problems. In addition to possibly disturbing the primordia, it increased the risk of over-irrigation. In any soil-containing, closed, irrigated systems, the soil should be very sand-rich and of rapidly draining type, to keep the block aerated. The usage of mineral soil can most likely be circumvented by developing mineral-rich substrates for bag cultures. In low-tech outdoor cultures, the surrounding soil is often a natural part of the system and has also a moisture-balancing and capillary function around the substrate. The use of an automated greenhouse in the present study is not a recommendation for using such facilities in commercial cultivation. Glass walls allow unnecessary solar energy in and simple ventilation lids are not cost-efficient for all-year climate control. Hence, the facility costs are high for a slow-growing species. Also, the present bucket-based study design was tailored for research, not production. As promising culture sites for *K. mutabilis* we would see areas with access to fresh thinned wood and low-cost land. It can be cultured under the cover of forest trees. The thin sporocarps have a rather short shelf life. Therefore, they are best suited for immediate drying or other processing after harvest (Issakainen 2015).

While the traditional pre-incubation of the

block (Luthardt 1969) is useful, we have shown elsewhere that *K. mutabilis* can be inoculated on e.g. non-pasteurized blocks immediately after falling and planted directly on soil surface in suitable types of forest (Issakainen 2015). A 10-cm layer of *Phragmites* reeds or similar material on the outdoor bed is in some bulk substrate settings useful for making the microclimate change more gradual near the soil surface (Issakainen 2015). Observations thus far suggest that *K. mutabilis* could be grown in forest nearly unattended. Such outdoor arrangements could be improved by submerging solid wood blocks in purposefully designed soil beds which on acidic sites can be chalk-enriched.

Various low-tech methods for watering and shadowing can be considered, especially in garden-like cultures which are more often attended. Profuse surface irrigation with long intervals can be tested for inducing flushes during dry weather. However, if the flushes of *K. mutabilis* are aimed to be regulated, the beds should be totally darkened during resting weeks. For this purpose, closable culturing sheds are worth studying.

The genetic variation between strains naturally plays a significant role in optimization of culturing methods (e.g. Stamets 2000, Chang & Miles 2004). Luthardt (1969) already noticed that some strains of *K. mutabilis* were able to sporulate only once, others up to 3 times per season. We recommend soil-immersed sterile sawdust spawn cylinders for rapid testing of strains, instead of the double-board setting of Gramss (1972, 1978). Dr. Päivi Lehtonen (personal communication) has for several years observed up to 5 flushes of wild *K. mutabilis* per season in a single stump in SW Finland. Wild strains should be actively searched for and isolated and culturing methods should be tested in the timescale of decades. This would be wise, considering the global ecological and social potentials of mushroom culturing (Stamets 2000, Oei 2003, Chang & Miles 2004).

Acknowledgements: Pekka Niemelä, and Simo Laine kindly provided facilities at the Ruissalo Botanical Garden. Marjo Anttila and Ismo Sainio at the Garden assisted the study with long-term technical maintenance. Sirpa Nissi and Tuomas Venäläinen gave important help by volunteering in critical stages of the study. The Aerobiology Unit of the University of Turku, as well as Seppo Huhtinen and Jukka Vauras at the Herbarium have supported us in several ways. Kaj Winqvist identified Diptera. Many

other colleagues, friends and trainees of the Botanical Garden have contributed to the study for shorter periods of time. A group of Finnish small-scale entrepreneurs have kindly supported the study with a grant. We are very grateful for all the support.

References

- von Bonsdorff, T., Hopsu-Neuvonen, A., Huhtinen, S., Korhonen, J., Kosonen, L., Moisio, S. & Palmén, J. 2013: Sienimetsästä markkinoille. Opetushallitus. Helsinki. 174 pp.
- Chang, S.-T. & Miles, P.G. 2004: Mushrooms. Cultivation, nutritional value, medicinal effect, and environmental impact. 2nd ed. CRC Press. Boca Raton. 451 pp.
- Courtecuisse, R. & Duhem, B. 1995: Mushrooms & toadstools of Britain and Europe. HarperCollins. London. 480 pp.
- Gramss, G. 1972: Methode zur Feststellung der Virulenz bei den Zuchtstämmen holzbewohnender Speisepilze. – Zeitschrift für Pilzkunde 38: 89–97.
- Gramss, G. 1974: Die Abhängigkeit einiger holzbewohnender Speisepilze von Umweltfaktoren in der generativen Phase. – Zeitschrift für Pilzkunde 40: 127–136.
- Gramss, G. 1978: *Kuehneromyces mutabilis*. – In: Chang, S.T. & Hayes, W.A. (eds.). The Biology and cultivation of edible mushrooms, 424–443. Academic Press. New York.
- Hintikka, V. 1993: Occurrence of edible fungi and other macromycetes on tree stumps over a sixteen-year period. – Acta Botanica Fennica 149: 11–17.
- Hintikka, V. 2009: Small-scale cultivation of wood-rotting fungi (in Finnish, Engl. Abstract). – Sienilehti 61: 42–51.
- Issakainen, J. 2015. Herkkuruokaa puusta. Syötävien lahohtajasienten viljely maatalouden sivuelinkeinona. Turun Ammattikorkeakoulun Raportteja 216: 1–228.
- Jacobsson, S. 1989: Studies on *Pholiota* in culture. – Mycotaxon 36: 95–145.
- Jacobsson, S. 1990: *Pholiota* in Northern Europe. – Windahlia 19: 1–86.
- Knudsen, H. & Vesterholt, J. (eds.). 2012: Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp. Copenhagen. 1083 pp.
- Korhonen, M. 1977: Ruokasieniopus. Sienilehti 29 (2): 3–66.
- Korhonen, J. & Penkkimäki, P. 2012: Suomalainen sienikirja. Tunnista sienet kasvuympäristön mukaan. Readme.fi, Helsinki. 275 pp.
- Leatham, G.F. & Kirk, T.K. 1983: Regulation of ligninolytic activity by nutrient nitrogen in white-rot basidiomycetes. – FEMS Microbiology Letters 16: 65–67.
- Lelley, J. 1991: Pilzanbau. Biotechnologie der Kulturspeisepilze. 2nd ed. Eugen Ulmer. Stuttgart. 404 pp.
- Lelley, J., Schmaus, F. & Musil, V. 1976: Pilzanbau. Eugen Ulmer. Stuttgart. 318 pp.
- Luthardt, W. 1969: Holzbewohnende Pilze. Anzucht und Holzmykologie. A. Ziemsen Verlag, Wittenberg. 122 pp.
- Oei, P. 2003: Mushroom cultivation. Backhuys, Leiden. 429 pp.

- Ryman, S. & Holmåsén, I. 1998: Svampar. En fälthandbok. Interpublishing. 3rd ed. Stockholm. 718 pp.
- Salo, P., Niemelä, T. & Salo, U. 2006: Suomen sieniopas. WSOY, Helsinki. 512 pp.
- Stamets, P. 2000: Growing gourmet and medicinal mushrooms, 3rd ed. Ten Speed Press, Berkeley. 574 pp.
- Ulinski, Z. & Szudyga, K. 2005: Air temperature and substrate moisture content influence growth and yielding of sheathed woodtuft (*Kuehneromyces mutabilis*). – Vegetable Crops Research Bulletin 63: 161–168.
- Vesterholt, J. 2004: Danmarks svampe. Gyldendal Fakta. Copenhagen. 473 pp.