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Heat resistance, biology and prevention of Diehliomyces microsporus in crops of Agaricus species

ANNEMARIE VAN ZAAYEN[1] and BERNARDA VAN DER POL-LUITEN[2]

[1] Mushroom Experimental Station, Horst-America (L)
[2] Laboratory of Phytopathology, Agricultural University, Wageningen

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Abstract

Soon after its introduction the mushroom species *Agaricus bitorquis*, which is immune to virus disease and prefers a warm climate, was threatened by the competitor *Diehliomyces microsporus*, false truffle. This fungus also likes warmth, and used to occur in crops of *A. bisporus*.

Mycelium and ascocarps were grown on several nutrient media. Optimum temperatures for mycelial growth were 26°C and 32°C, with a slight depression at 30°C. In trials in isolated growing-rooms strain Somycel 2.017 of *A. bitorquis* was generally used since it appeared to be highly sensitive to the competition of false truffle. Inoculation with mycelium, ascocarps or ascospores of *D. microsporus* nearly always resulted in the presence of the competitor and in decreased mushroom yields. Even ten spores per m^2 caused *D. microsporus*. The time of inoculation was most important: irrespective of the kind of inoculum, inoculation only resulted in both false truffle and yield loss, if applied from spawning until a few days after casing. Inoculation at a later date could result in false truffle, but yield was not decreased.

As germination in vitro of ascospores failed, even after addition of various triggers, ascospore suspensions were treated at various temperatures for several periods. Then mushroom growing trays spawned with Somycel 2.017 were inoculated with the treated suspensions giving $7-11 \times 10^7$ spores/m^2. The ascospores could not withstand 85°C for 0.5 h, 80°C for 1 h and 70°C for 3 h. Spontaneous incidence of false truffle, however, could not always be prevented and interfered with the results of these trials. It is possible that the thermal death-point of the ascospores is below 85°C. Fruiting bodies and ascospores did not survive 'peak-heating' at the beginning and cooking out (compost temperature 12 h at 70°C) at the end of a crop. After cooking out, however, *D. microsporus* could still be present in the wood of trays and contaminate a following crop if no wood preservative was applied.

Yield of Somycel 2.017 was reduced by the competition of *D. microsporus* much more than yields of other strains of *A. bitorquis*. The least sensitive were the highly productive strains Horst K26 and Horst K32.

The effects of fungicides on *D. microsporus* in vitro and in growing trials did not correspond. The fungicides tested so far could not prevent or control *D. microsporus*. Growing of less sensitive strains of *A. bitorquis* together with sanitary measures early in the crop and at the end of the crop, however, can prevent the competitor.

Introduction

Soon after the first crop, in 1974, of a commercial strain of *Agaricus bitorquis* (Quél.) Sacc. that is immune to mushroom virus disease (Van Zaayen, 1976), a competitor even more dreaded than this disease re-appeared. *Diehliomyces microsporus* (Diehl & Lambert) Gilkey, 'false truffle', was first observed in crops of the cultivated mushroom *Agaricus bisporus* (Lange) Imbach in Ohio in 1929 (Lambert, 1930) and

described by Diehl and Lambert (1930) as *Pseudobalsamia microspora*. Gilkey (1954) reclassified the fungus from the Tuberales into the Eurotiales and named the genus *Diehliomyces*. Spores of *D. microsporus* were reported to withstand at least 82°C for 5 h (Lambert, 1932). Beach (1937) mentioned an optimum temperature for mycelial growth of 28°C, which covered the frequent association of false truffle with warm weather.

Over the years, the fungus caused a substantial annual loss in crops of *A. bisporus* in the United States and Great Britain (Kligman, 1944). *D. microsporus* was not reported in the Netherlands until 1956 (Bels-Koning and Bels, 1958).

In recent years false truffle was hardly noticed in crops of *A. bisporus*, most probably because environmental conditions were better controlled, e.g. temperature in the growing-rooms. During the hot summers of 1974 and 1975, the fungus was observed in crops of *A. bisporus*, but yield was only slightly reduced (Olivier and Guillaumes, 1975).

However, *A. bitorquis* requires higher growing temperatures than *A. bisporus:* 30°C during mycelial growth in the compost and 25°C during cropping (Hasselbach and Mutsers, 1971; Pompen, 1975). These conditions appeared to be most favourable to false truffle. From 1974, crop yield of *A. bitorquis*, commercial strain 'Somycel 2.017', was considerably reduced. The present study deals with properties of *D. microsporus* in vitro and in vivo and trials to control or prevent this fungus.

Materials and methods

Experiments in vitro

Mycelial cultures were obtained from fruiting bodies of *D. microsporus* occurring on commercial mushroom farms. Ascocarps were disinfected in 2% bleaching liquor for about 1 min and plated on malt agar at 26°C. Mycelial growth and formation of fruiting bodies at 26°C was compared on various media in Petri dishes (Table 1). To suppress bacterial growth, all agar media were supplied with 50 µg/ml of oxytetracyclin (commercially available as Vendarcin).

The influence of temperature on mycelial growth was determined on malt agar (Oxoid CM 59, pH 5.4) in a serial thermostat. The plates were inoculated with an inverted disc (diam. 5 mm) of agar with mycelium. Discs were cut from the periphery of 5 day-old cultures. Temperatures in the agar plates were verified with thermocouples. Radial growth increase was measured in each of three to eight replicates. The experiment was repeated three times. The most representative data of one experiment in Petri dishes 150 mm in diameter are given in Fig. 2.

Formation of fruiting bodies with ascospores was compared in 1 l bottles or flasks with autoclaved grain or compost media:

- sorghum grains: to 1 kg sorghum grains, boiled in 1 l water for 5–10 min, 5.3 g chalk ($CaCO_3$) and 21.2 g gypsum ($CaSO_4$) were added (Stoller, 1962);
- rye grains: to 1 kg rye, boiled in 2.5 l water, 5 g chalk and 20 g gypsum were added;
- peak-heated (i.e. pasteurized and conditioned), ground and wetted compost;
- wetted, spent compost.

Flasks or bottles containing such media were autoclaved, inoculated with pieces of agar with mycelium and incubated at ca. 28°C for 3 to 8 weeks depending on the

formation of fruiting bodies. Some bottles were incubated in a mushroom growing-room during mycelial growth of *A. bitorquis* at 27–28°C (air temperature). Sterile ascospore suspensions with 10^4 to 2×10^5 spores per ml were prepared from fruiting bodies in bottles or flasks containing grain or compost media. Since the ascospores failed to germinate properly on sterilized agar media, the following triggers were added: furfural in various amounts (Emerson, 1948); acetic acid, sodium hydroxide and furfuryl alcohol (Sussman and Halvorson, 1966, p. 168–169) and orange juice. In some of the trials basidiospores or discs of agar with mycelium of *A. bisporus* or *A. bitorquis* were added to the plates to test for any stimulative effect of *Agaricus* sp. on spore germination (Ferguson, 1902). Rate of germination, however, was not improved by any of these means. Consequently trials to determine thermal death-points of the ascospores in vitro failed because of the low percentage of germination.

Therefore, unsterile ascospore suspensions from fruiting bodies occurring in mushroom crops (usually *A. bitorquis*) were treated in vitro at various temperatures and times and then used as inocula for trays, spawned with *A. bitorquis*. Ascocarps of *D. microsporus* were disintegrated in distilled water in test tubes with a whirl mixer. The suspension was filtered through cotton wool to remove mycelial fragments. Ascospore suspensions with 9.7×10^5 to 1.5×10^6 spores per ml were treated in 20 ml aliquots in test tubes in a Grant SB15 thermostatic water bath. Temperatures in the water bath were checked with a stamped and verified thermometer and with thermocouples. Tests were in 9 to 10 replicates per temperature treatment. Temperatures in the test tubes were checked with thermocouples set in veterinary injection needles (prepared by the Technical and Physical Engineering Research Service, Wageningen), inserted in the spore suspensions at various depths. All 12 thermocouples were connected with a Honeywell Electronic 16 or a Speedomax recorder (sensitivity $\pm$ 0.15°C). The temperatures measured in water bath and test tubes were recorded. The test tubes with ascospore suspensions were placed in water of the desired temperature. The suspensions reached the desired temperature in 3–5 min and then the stopwatch was started for a certain period. Cooling down was at room temperature. The spore suspensions were then stored overnight at ca. 10°C until inoculation. Each tray of 0.27 m^2 was inoculated with 20 ml of spore suspension, yielding 7 to 11×10^7 spores per m^2.

Trials in mushroom growing-rooms

Mushroom growing. Mushrooms were grown by the single zone system. Standard mushroom trays (0.27 m^2) were filled with 21.6 kg (ca. 80 kg/m^2) compost. Preparation and composition of the compost has been described by Gerrits (1977). For some trials, the moisture content of the compost was varied during composting. After filling, the compost was peak-heated i.e. pasteurized and conditioned for 9 to 10 days. A maximum air temperature of 57°C was recorded for 10–12 h on the first day. After cooling down to 30°C, the compost was spawned with laboratory-prepared or commercial spawn of *A. bitorquis*. The spawning rate was 8 litre spawn/1000 kg compost. In most trials the commercial strain Somycel 2.017 (Poppe, 1972) was used. Occasionally other strains of *A. bitorquis*, mainly originating from breeding work (Fritsche, 1976), were tested. Preparation of spawn was described by Van Zaayen (1976). During mycelial growth, the trays were covered with thin plastic sheets. The

compost temperature was kept at 30°C. Twelve days after spawning the compost was fully colonized by mushroom mycelium. It was then cased with a 4 cm layer of a mixture of black peat (ca. 60% v/v), sphagnum peat (ca. 34%), sand (ca. 3%) and marl (ca. 3%). From 1976 the mixture consisted of black peat and sphagnum peat (together 75%) and lime cakes (25%), a waste product of sugar-beet factories.

Mushrooms appeared 3 to 4 weeks after casing, depending on the strain. The compost temperature during cropping was 25°C. For the next 4 to 5 weeks mushrooms were harvested as cut mushrooms every 2 to 3 days and weighed for each of usually 8 to 10 replicates in randomized blocks. The yields were then adjusted for 100 kg compost/m^2 and processed by analysis of variance.

In trials to determine thermal death-points and in trials on the infectivity of ascospores another layout was used to prevent mutual contamination of trays, for instance during watering. A growing-room contained 10 rows of 9 trays each. Nine different treatments were applied to the trays of each row, beginning with no treatment (control) at one end of the rows, via weakly inoculated to severely inoculated at the other end. Consequently such trials usually were in 10 replicates.

At the end of each trial all trays were carefully checked for the presence of false truffle and then the growing-rooms were cooked out (compost temperature 12 h at 70°C). After being emptied the trays were dipped in 4% sodium pentachlorophenate (SPCP), unless otherwise stated. All growing experiments with false truffle were in isolated growing-rooms. To prevent other growing-rooms from becoming infected, precautions were taken according to Dieleman-van Zaayen (1972).

Inoculum of D. microsporus:

a. Compost and casing soil from invaded mushroom crops, with mycelium and fruiting bodies of false truffle. Per tray 50 g and later 2 g was used mainly in preliminary trials.
b. Mycelium. Inoculum was prepared on autoclaved sorghum grains as described above for the formation of fruiting bodies with ascospores in 1 l bottles or flasks. After 10 to 14 days at ca. 28°C, grains were covered with mycelium of *D. microsporus;* 2 g per tray was used.
c. Ascospores. Ascospore suspensions were prepared as described above for determination of thermal sensitivity. In most trials, about 10^6 spores per m^2 were inoculated, unless otherwise stated.
d. Infected trays. In trials to determine whether the usual disinfection procedures in growing-rooms killed the infectious material, trays that had contained *D. microsporus* were emptied before or after cooking out, wrapped in plastic bags after possible treatment with SPCP and stored at room temperature. In the next trial the trays were filled with peak-heated and spawned compost.

Inoculation. Compost and casing soil with mycelium and ascocarps, or mycelium of *D. microsporus* on autoclaved sorghum grains, was put in the centre of a tray in an isolated growing-room, at a depth of 5 cm in the compost. Ascospore suspensions were sprinkled over the surface of the compost. Inoculation was one day after spawning unless otherwise stated. Uninoculated trays spawned with *A. bitorquis* acted as control. To prevent trays not to be inoculated from becoming infected during or after inoculation, all trays were covered with plastic sheets immediately after

spawning. During inoculation the sheets were only lifted in some places and not removed before casing.

Fungicides. The effect of benomyl, captafol, chlorothalonil and vinclozolin on mycelial growth of *D. microsporus* was tested on malt agar. Determination of in vitro sensitivity of mycelium to fungicides has been described by Bollen and Van Zaayen (1975).

In mushroom growing trials benomyl (0.75 g a.i./m^2) and captafol (2.4 g a.i./m^2) were applied at spawning or at spawning and at casing. Mancozeb (0.3 g a.i./m^2) was tried at spawning and at casing. Copper sulphate (Gandy et al., 1953) was mixed with the compost just before filling, at the rate of 200 g and 400 g per 1000 kg compost (= 20 and 40 g a.i./m^2).

Experiments and results

Experiments in vitro

The morphological characteristics of mycelium, isolated from ascocarps of *D. microsporus* from commercial mushroom farms, and of ascocarps conformed to the description of *Diehliomyces microsporus* (Gilkey, 1954), which was compiled largely from the original description by Diehl and Lambert (1930). All authors, however, mentioned the presence of 'chlamydospores occasionally within hyphae of the ascocarp, spherical, 13 μ in diameter, content golden-brown with epispore 2 μ thick, smooth to fine granular'. Comparisons were made with type specimens of *Pseudobalsamia microspora*, deposited in the National Fungus Collections, Beltsville, USA (BPI) in 1929. Mycelium and ascospore dimensions of *D. microsporus* used in this study and those of the type specimens were similar. In the type material, chlamydospores were not observed within hyphae of the ascocarp. Our isolates of *D. microsporus* did not form chlamydospores on sterile media. The description of chlamydospores probably fits aleuriospores of *Torula thermophila* Cooney & Emerson frequently present in mushroom substrate with or without *D. microsporus* and also present in the type material from 1929 we studied.

Mycelial growth and formation of ascocarps. Table 1 presents details on the mycelial growth of *D. microsporus* and the formation of small ascocarps (Fig. 1A) on various culture media in Petri dishes. Growth rate on the media was highly variable, with protruding sectors and wefts of aerial mycelium, and often difficult to measure. Formation of ascocarps as well as the development of ascospores some weeks later, were highly variable too. Best growth was obtained on oatmeal agar and MTA (mushroom tissue agar, Schisler et al., 1968), whereas the most regular growth occurred on malt agar and PDA. Water agar, CMA and wheat extract agar (Fritsche and Von Sengbusch, 1962) resulted in a rather tenuous mycelial growth.

Small fruiting bodies with ascospores were formed after 3 to 8 weeks in bottles or flasks with autoclaved sorghum grains and with peak-heated, ground and autoclaved compost. Incubation in growing-rooms with *A. bitorquis* yielded fruiting bodies with ascospores after a much shorter time, i.e. several weeks, probably because of a more suitable climate. Larger ascocarps were occasionally formed in flasks with autoclaved,

Table 1. Mycelial growth in 3 days and formation of fruiting bodies of *D. microsporus* on various culture media in Petri dishes at 26°C.

Culture medium	Composition	pH[1]	Average[2] diameter of colony in mm	Subsequent formation of ascocarps
Biomals agar, a malt product	1/50, v/v	6.3	31.1 ± 14.84	+
Cellulose	Whatman powder CF_{11} (20 g/l, w/v)	7.0	58.8 ± 6.12	−
Cherry decoction agar	cherry juice ($\frac{1}{4}$, v/v)	4.0	30.2 ± 14.19	−
CMA (=cornmeal agar)	Difco B386	6.0	61.7 ± 6.02	+
Czapek-Dox agar	Oxoid CM95	6.8	59.7 ± 12.83	−
Malt agar	Oxoid CM59	5.4	19.1 ± 1.44	+
MTA (= mushroom tissue agar)	Schisler et al., 1968	6.4	50.6 ± 9.91	+
Oatmeal agar	Oatmeal (60 g/l, w/v)	6.3	> 80	+++
PDA (= potato-dextrose agar)	Oxoid CM139	5.6	18.0 ± 1.58	−
Water agar		6.5	45.5 ± 25.47	−
Wheat extract agar	Fritsche and Von Sengbusch, 1962.	7.0	54.0 ± 10.92	++

[1] pH was determined as indicated after autoclaving.
[2] Averages of 10 replicates with standard deviations.

Tabel 1. Myceliumgroei na 3 dagen en vorming van vruchtlichamen van D. microsporus op verschillende voedingsbodems in petrischalen bij 26°C.

spent compost (Fig. 1B). Contrary to the statement of Kligman (1944) that false truffle did not grow in a rye spawn medium, mycelial growth was abundant on autoclaved rye grains (moisture content: over 50% of the total weight) but ascocarps were not produced.

Effects of temperature on mycelial growth. The optimum temperature for mycelial growth was 26°C. A second, slightly lower optimum occurred at 32°C, preceded by a depression at 30°C (Fig. 2). At 10°C and at 35°C the mycelium did not grow. All three experiments gave similar results. To verify whether this pattern might be caused by a mixture of different strains of the fungus, 50 hyphal tips consisting of one single cell were transferred to malt agar plates but failed to grow.

From the mycelial cultures treated at 26°C and at 32°C transfers were made and incubated at 25°C, 26°C, 30°C and 32°C for 6 days. The new isolates showed the same pattern, as did a probable monosporous isolate, viz. optimum at 26°C, depression at 30°C and second but lower optimum at 32°C. The results with our isolates

Fig. 1A. Mycelium en vruchtlichamen van D. microsporus op moutagar.
Fig. 1B. Grote vruchtlichamen in gesteriliseerde, afgeoogste compost.
Fig. 1C. Mycelium van D. microsporus op de dekaarde.
Fig. 1D. Vruchtlichamen in compost en dekaarde. Een zijplank is verwijderd.
Fig. 1E. Asci met ascosporen. De maatstreep geeft 10 µm aan.

Fig. 1A. Mycelium and ascocarps of *D. microsporus* on malt agar.
Fig. 1B. Large ascocarps in sterilized, spent compost. Photograph: IPO, Wageningen.
Fig. 1C. Mycelium of *D. microsporus* on the casing soil. Photograph: Th. G. M. Pompen, Horst.
Fig. 1D. Ascocarps in compost and casing soil. A sideboard was removed. Photograph: M. A. Ruissen, Wageningen.
Fig. 1E. Asci with ascospores. The bar represents 10 μm.

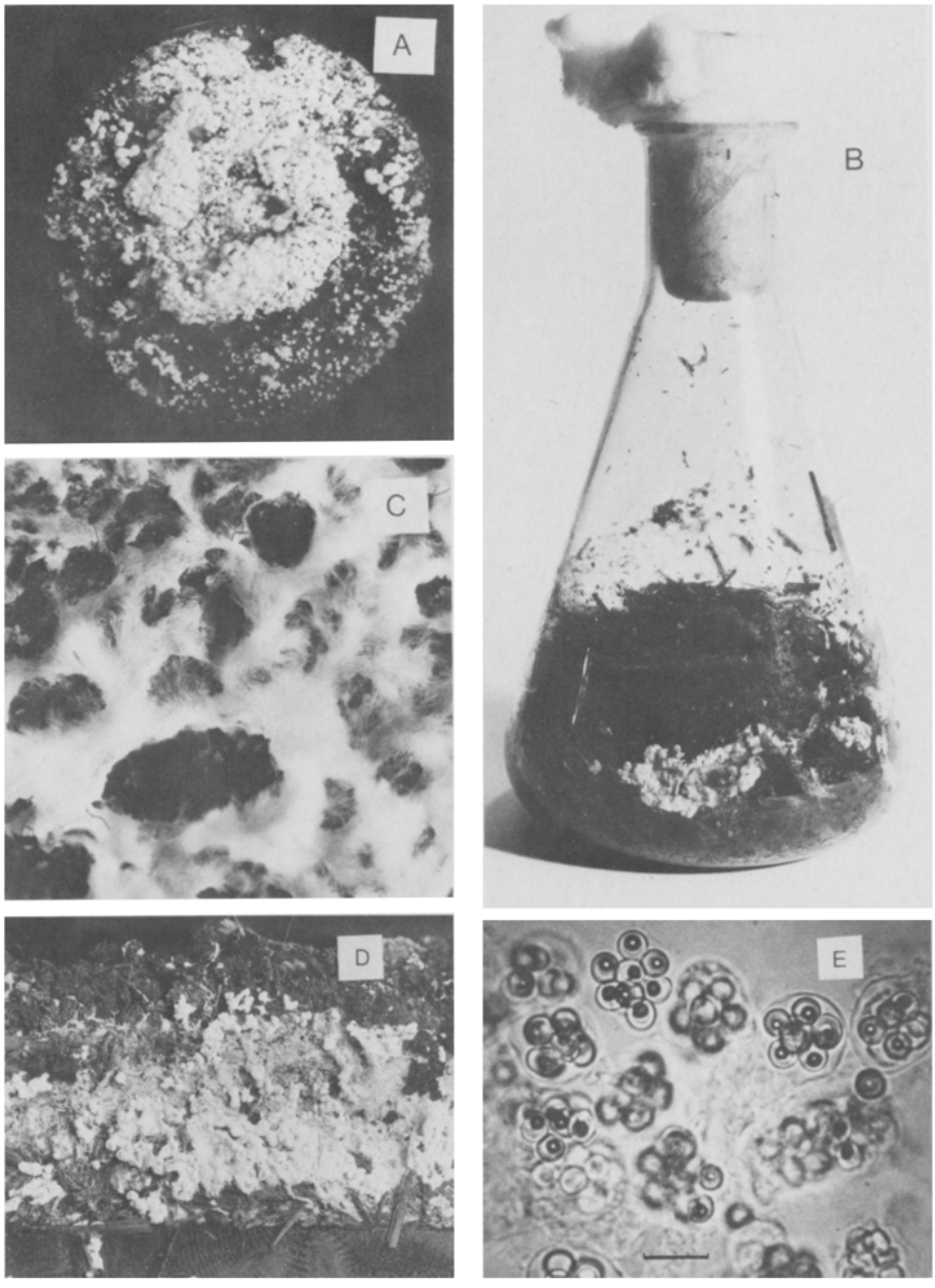

Fig. 2. Effect of temperature on mycelial growth of *D. microsporus*. Radial growth on malt agar was measured after 3 days (●– – –●) and 7 days (▲——▲). Standard deviations are indicated.

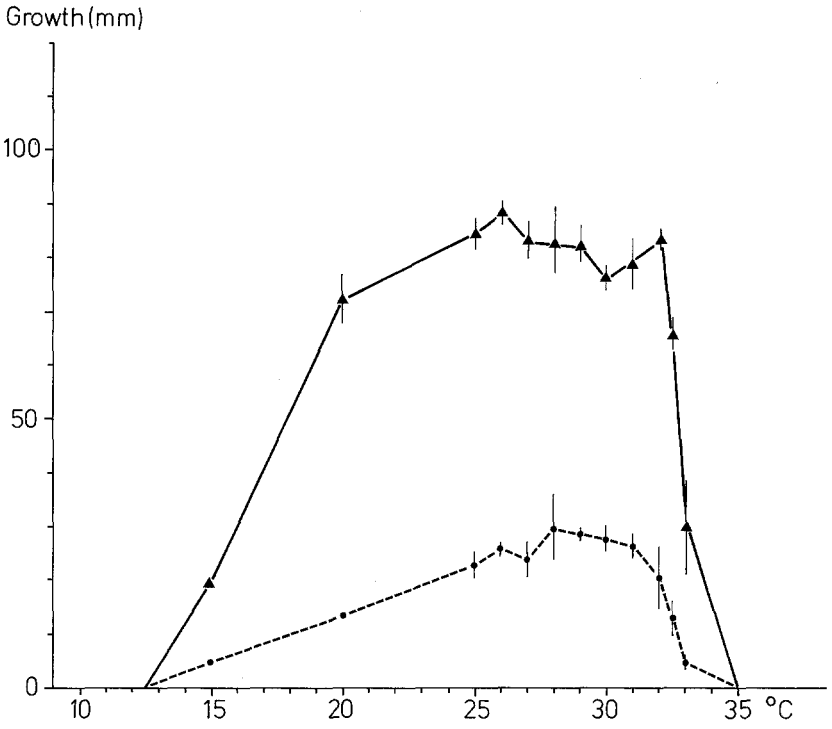

Fig. 2. Invloed van de temperatuur op de myceliumgroei van D. microsporus. De radiale groei op moutagar werd gemeten na 3 dagen (●– – –●) en na 7 dagen (▲——▲). De standaardafwijkingen zijn aangegeven.

differ from the observations of Beach (1937): he reported an optimum temperature for mycelial growth of 28.3°C. After 3 days of growth on malt agar, however, the optimum temperature was 28°C (Fig. 2), so he may have judged too early.

Trials in mushroom growing-rooms

Symptoms after successful inoculation. Symptoms and morphologic features of mycelium and ascocarps of *D. microsporus* in crops of *A. bisporus* have been detailed by Lambert (1930, 1932), Diehl and Lambert (1930) and Kligman (1944). In trays spawned with strain Somycel 2.017 of *A. bitorquis* and inoculated with *D. microsporus* at spawning, ascocarps generally appeared during the cropping period. Mushroom yield was sometimes delayed and usually significantly reduced. Prior to the appearance of false truffle, young mushrooms turned yellow and died. Then cottony wefts of mycelium of *D. microsporus* grew in the small space between substrate and sideboards of the trays. Occasionally such mycelium grew on top of the casing soil (Fig. 1C). About 4 to 5 days later ascocarps (Fig. 1D) with ascospores

(Fig. 1E) were formed along the sideboards. Fruiting bodies were first found on the border between compost and casing soil, and later on top of the casing.

Decreased yield compared with that of the uninoculated control and/or presence of ascocarps indicated a successful inoculation in these trials. Yields in the isolated growing-rooms usually were not optimal, since the aim was primarily to establish false truffle.

Effect of amount of inoculum. Inoculation at spawning in Trial 1 in 8 replicates with 50 g per tray of compost and casing soil from mushroom crops with mycelium and ascocarps of *D. microsporus* caused an average yield of 11.4% (1.4 kg/m^2) of the control (100% = 12.3 kg/m^2), whereas 2 g per tray of similar inoculum resulted in a yield of 13% (1.6 kg/m^2) of the same control. In Trial 2, however, 2 g resulted in a much higher yield, viz. 5.1 kg/m^2 or 40.2% of the control (Table 2). About the same loss in yield was caused by 2 g of mycelium of *D. microsporus* on autoclaved sorghum grains. False truffle was detected in almost every inoculated tray. Yield decreases of strain Somycel 2.017 caused by *D. microsporus* were highly variable but always significant in all trials. In Trial 3 in 20 replicates, control trays yielded 13.5 kg/m^2 (100%) on average, trays inoculated with 2 g mycelium on autoclaved sorghum grains yielded 5.0 kg/m^2 (37%) and trays inoculated with an ascospore suspension (ca. 10^7 spores/m^2) yielded 7.6 kg/m^2 (56.3%). False truffle was present in every inoculated tray.

Inoculation with mycelium often resulted in the immediate appearance of ascocarps

Fig. 3. Incidence of *D. microsporus* in percentage (●.....●; 100% = 10 trays with false truffle) and yields in percentage of uninoculated control (●——●; 100% = 5.7 kg/m^2) after inoculation with 10^1 to 10^8 ascospores per m^2. The trial was in 10 replicates.

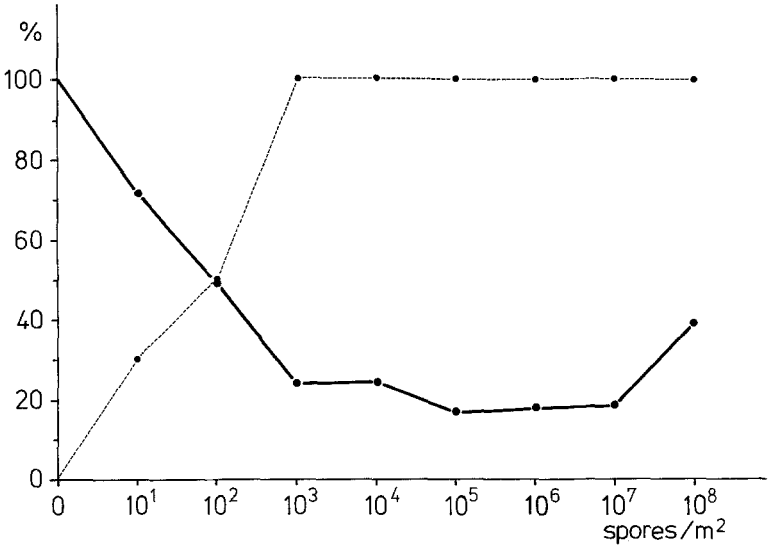

Fig. 3. Aanwezigheid van D. microsporus in procenten (●.....●; 100% = 10 kisten met valse truffel) en opbrengsten in percentage van die van de niet-geïnoculeerde controle (●——●; 100% = 5,7 kg/m^2) na inoculatie met 10^1 tot 10^8 ascosporen per m^2.

during the first flush of mushrooms. Later in the crop the ascocarps could not be found any more because of disintegration.

The results of Trial 4 on the required amount of ascospores are presented in Fig. 3. Even ten spores per m^2 at spawning resulted in the incidence of false truffle in 3 out of 10 trays. From 10^3 spores per m^2 all trays contained *D. microsporus*. The increase in yield at the rate of 10^8 spores per m^2 was surprising. A similar trend related to stimulation of pinhead formation by large amounts of ascospores was noted in several other trials. A stimulative effect on the mushroom mycelium (Lambert, 1932) might be indicated.

Effect of time of inoculation

A. Inoculation with mycelium and ascocarps. In Trial 2, two g per tray of inoculum a, compost and casing soil from a mushroom crop containing mycelium and ascocarps with spores of false truffle, was compared with 2 g of inoculum b, mycelium on autoclaved sorghum grains. Inoculation was on the day of spawning (t_0), 12 days later (t_{12}) or 26 days later (t_{26}). The results are given in Table 2. The time of inoculation seemed to be more important than the kind of inoculum. Infection during or shortly after spawning and casing was most serious.

B. Inoculation with ascospores. In Trial 5, trays were inoculated 1, 8, 15, 22 and 29 days after spawning with ascospore suspensions giving 10^7 spores/m^2. The results are presented in Fig. 4. Although inoculation 22 days after spawning (= 9 days after casing) resulted in *D. microsporus* in 9 out of 10 trays in the last week of picking, yield was not decreased. Again, infection during or shortly after spawning and casing was most serious.

Table 2. Average yields of *A. bitorquis* (strain Somycel 2.017) and incidence of false truffle[1] after various times of inoculation with inocula a[2] and b[3] (9 replicates).

Time from spawning to inoculation (days)	Yields (kg/m^2) and number of trays with ascocarps		
	untreated	inoculated with	
		inoculum a[4]	inoculum b[4]
0 (t_0) (= at spawning)	12.7 (0)	5.1** (9)	4.6** (9)
12 (t_{12}) (= at casing)		2.8** (9)	6.7** (9)
26 (t_{26})		12.9 (0)	12.5 (1)

[1] Figures in parentheses represent number of trays with ascocarps.
[2] Inoculum a consisted of 2 g of compost and casing soil from a mushroom crop invaded by *D. microsporus*.
[3] Inoculum b consisted of 2 g of mycelium of *D. microsporus* on autoclaved sorghum grains.
[4] Double asterisks indicate values significantly different from untreated control at the 1% level.

Tabel 2. Gemiddelde opbrengsten van A. bitorquis (ras Somycel 2.017) en aanwezigheid van valse truffel na verschillende tijdstippen van inoculatie met de inocula a en b.

Fig. 4. Incidence of *D. microsporus* in percentage (●– – –●; 100% = 10 trays with false truffle) and yields in percentage of uninoculated control (▲——▲; 100% = 10.3 kg/m²) after inoculation with 10^7 ascospores per m² at various times.

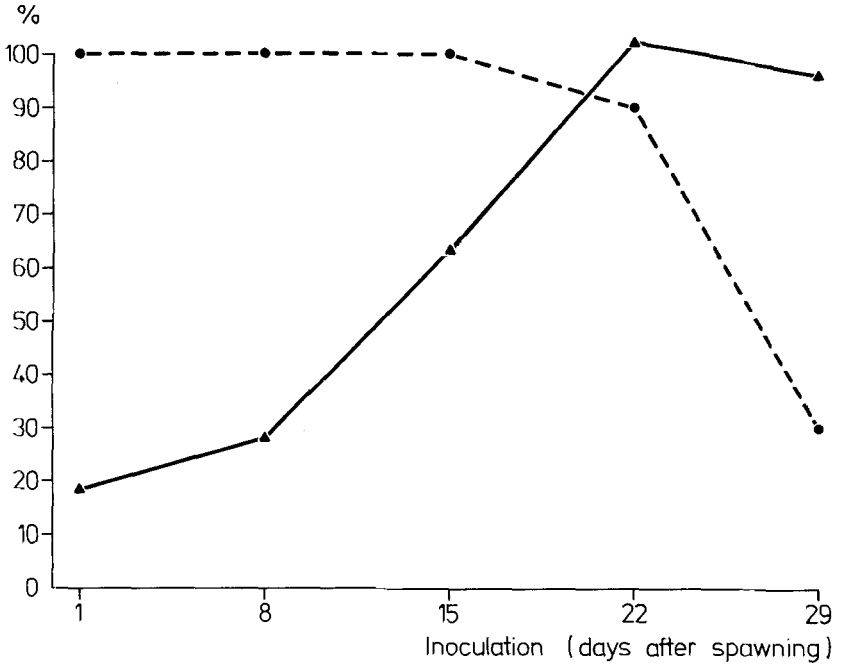

Fig. 4. Aanwezigheid van D. microsporus in procenten (●– – –●; 100% = 10 kisten met valse truffel) en opbrengsten in percentage van die van de niet-geïnoculeerde controle (▲——▲; 100% = 10,3 kg/m²) na inoculatie met 10^7 ascosporen per m² op verschillende tijdstippen.

Thermal sensitivity of ascocarps and ascospores. In Trials 6, 1 and 3, inoculation at filling with 50 g per tray of compost and casing soil from mushroom crops with mycelium and ascocarps containing spores of *D. microsporus* did not result in yield loss or presence of false truffle. In these trials, inoculation at spawning with similar, stored inoculum yielded on average 1.4, 1.4 and 8.8 kg/m², respectively, and ascocarps in all trays. The uninoculated controls yielded 7.8, 12.3 and 14.4 kg/m². The trials were in 10, 8 and 10 replicates, respectively. Ascospores apparently could not survive peak-heating. Similar inoculum was used in Trials 3 and 7 with a short pasteurization time (viz. maximum air temperature 3 h at 57°C). Trays inoculated at filling yielded on average 12.8 and 11.5 kg/m², respectively, trays inoculated at spawning yielded 7.9 and 7.3 kg/m² and the uninoculated controls 12.5 and 11.1 kg/m². False truffle only occurred in all trays inoculated at spawning.

The results on thermal sensitivity of ascospores in suspension are presented in Table 3. The thermal death-point was between 80°C and 85°C in these trials. Yields of the controls in Trials 8, 9 and 10 were very low due to the large number of successful inoculations, creating a most unfavourable climate for a good mushroom crop on uninoculated trays. Ascospores were not able to survive treatments for 1 h at 80°C and 3 h at 70°C. Although temperature treatments of ascospore suspensions and subsequent inoculation of trays were most carefully done, spontaneous incidence of false

Table 3. Trials on thermal sensitivity of ascospores. Average yields (in percentages of uninoculated controls) of *A. bitorquis* (strain Somycel 2.017) and incidence of false truffle[1]. Trays inoculated just after spawning with heat-treated ascospore suspensions[2] of *D. microsporus* (9 replicates in Trials 8 and 9, 10 replicates in Trials 10–12).

Treatment	Trial[3]									
	8		9		10		11		12	
– (control)	100	(0)	100	(0)	100	(0)	100	(0)	100	(1)
inoculated with untreated spores	18.1	(9)	10.5	(9)	53.6	(10)	78.2	(10)	44.8	(10)
40°C, 0.5 h	3.4	(9)	7.4	(9)	–		–		–	
50°C, 0.5 h	6.9	(9)	5.5	(9)	–		–		–	
60°C, 0.5 h	68.8	(8)	23.7	(7)	–		–		–	
65°C, 0.5 h	–		65.2	(2)	–		95.9	(3)	–	
70°C, 0.5 h	69.7	(2)	57.5	(3)	98.3	(0)	90.7	(6)	–	
1 h	–		–		95.4	(0)	94.3	(3)	–	
2 h	–		–		89.2	(0)	89.5	(3)	104	(0)
3 h	–		–		–		–		106	(0)
4 h	–		–		93.6	(0)	–		106	(0)
75°C, 0.5 h	–		48.9	(5)	57.8	(4)	92.7	(4)	–	
1 h	–		–		72.3	(1)	100.1	(1)	106	(0)
80°C, 0.5 h	80.3	(0)	80.0	(0)	–		94.1	(2)	104	(0)
1 h	–		–		–		–		108	(0)
2 h	76.3	(0)	–		–		–		–	
4 h	96.9	(0)	–		–		–		–	
85°C, 0.5 h	–		–		–		–		108	(0)

[1] Figures in parentheses represent number of trays with ascocarps.

[2] Suspensions with 9.7×10^5 to 1.5×10^6 ascospores per ml were treated in vitro in 20 ml aliquots. Final concentration at inoculation: 7 to 11×10^7 spores/m^2.

[3] 100% equals 3.2 kg/m^2 in Trial 8; 6.9 kg/m^2 in Trial 9; 5.2 kg/m^2 in Trial 10; 13.1 kg/m^2 in Trial 11 and 16.1 kg/m^2 in Trial 12.

Tabel 3. Proeven over de temperatuurgevoeligheid van ascosporen. Gemiddelde opbrengsten (in percentages van die van de niet-geïnoculeerde controle) van A. bitorquis (ras Somycel 2.017) en aanwezigheid van valse truffel. Kisten werden juist na het enten geïnoculeerd met warmte-behandelde ascosporensuspensies van D. microsporus.

truffle – possibly caused by escape of a few spores – interfered with the results of these trials as can be seen in Trial 12 (control). Therefore, truffle spores probably cannot resist treatments for 2 h at 70°C and 0.5 h at 80°C.

In Trials 8 and 9, inoculation with ascospores treated for 0.5 h at 40°C and 50°C resulted in stronger yield losses and earlier formation of ascocarps than inoculation with untreated spore suspensions, indicating a stimulative effect on spore germination. Both stimulative temperatures are far below the lethal temperature.

Inoculation at spawning of 10 trays with 50 g of compost and casing soil with mycelium and ascocarps of *D. microsporus* from trays of a previous trial, that had been cooked out for 12 h at 70°C (compost temperature), did not result in yield loss or presence of false truffle.

In the same trial (13), similar inoculum had been treated for 0, 1, 2, 4 and 8 h at 70°C after a heating up period of 8 h. Temperature in the compost was checked with thermocouples. Trays were inoculated with 50 g, in nine replicates per treatment.

Trays inoculated with the untreated material yielded on average 6.1 kg/m² and ascocarps were present in all nine trays. The uninoculated control and the four other treatments yielded 10.2 to 10.8 kg/m² on average and false truffle was not observed in any of the trays.

Survival of mycelium and ascospores in wood. In Trial 14 in 9 replicates, trays that had contained false truffle in a previous trial and were refilled with spawned compost without being cooked out or treated with SPCP, yielded 3.0 kg/m² on average. Ascocarps occurred in each tray. Similar trays that had been cooked out and treated with 4% SPCP before being refilled yielded 11.3 kg/m² and no false truffle. Trays that had only been cooked out, compost temperature exactly 12 h at 70°C, yielded 9.2 kg/m² with ascocarps observed in two trays. The results prove the assumption of Lambert (1932) that *D. microsporus* can survive from one crop to another in the wood of trays and shelves.

Sensitivity to competition by D. microsporus of various strains of A. bitorquis. Table 4 presents the results of Trials 1, 15 and 16 on the sensitivity of strains of *A. bitorquis*. The rate of decrease in yield and the amount of trays with ascocarps clearly indicate that Somycel 2.017 is more sensitive to the competition by *D. microsporus* than the other strains tested. Although all decreases in yield following inoculation were significant except that of Horst K32 in Trial 15, yield of Somycel 2.017 was always reduced by inoculation more than that of the other strains. In most inoculated trays spawned with Somycel 2.017 and even in some uninoculated trays, ascocarps were found. In inoculated trays spawned with other strains false truffle was occasionally present.

Table 4. Average yields (in kg/m²) of various strains of *A. bitorquis* and incidence of false truffle[1] after inoculation, at spawning, with *D. microsporus*[2] (8 replicates in Trial 1, 10 replicates in Trials 15 and 16).

Strain of *A. bitorquis*	Trial 1		Trial 15		Trial 16	
	untreated	inoculated[3]	untreated	inoculated[3]	untreated	inoculated[3]
Somycel 2.017[4]	12.3 (1)	1.6**(7)	4.8 (0)	2.1**(8)	13.2 (1)	5.0**(8)
Les Miz 444[4]	–	–	11.2 (0)	8.7**(5)	–	–
Horst B_{30}[5]	13.8 (0)	7.2**(4)	–	–	–	–
Horst K_{26}[5]	–	–	18.2 (0)	13.9**(6)	14.5 (0)	12.4**(3)
Horst K_{32}[5]	–	–	14.0 (0)	13.9 (4)	16.7 (0)	14.6**(3)

[1] Figures in parentheses represent number of trays with ascocarps.
[2] Inoculum in Trial 1 was 2 g of compost and casing soil from a mushroom crop with mycelium and ascocarps of *D. microsporus*, in Trial 15: 2 g of mycelium and ascocarps on autoclaved sorghum grains and in Trial 16: ascospore suspensions giving 5×10^6 spores/m².
[3] Double asterisks indicate values significantly different from uninoculated controls at the 1% level.
[4] Commercial spawn.
[5] Selection and breeding products of the Mushroom Experimental Station, Horst (L). Horst K_{26} and Horst K_{32} are commercially available.

Tabel 4. Gemiddelde opbrengsten (kg/m²) van verschillende rassen van A. bitorquis en aanwezigheid van valse truffel na inoculatie, bij het enten, met D. microsporus.

Table 5. Average yields (in kg/m²) of strains of *A. bitorquis* and incidence of false truffle on composts with three different moisture contents[1]. Trays inoculated just after spawning with mycelium[2] or ascospores[3] of *D. microsporus* (6 replicates).

Strain of *A. bitorquis*	Moisture content of compost (%)	Yields and number of trays with ascocarps[4]		
		Untreated	inoculated with	
			mycelium[5]	ascospores[5]
Somycel 2.017	63	8.1 (0)	7.2 (2)	3.9** (6)
	69	13.7 (0)	8.9** (4)	4.0** (6)
	73	12.2 (0)	4.5** (6)	10.7 (6)
Horst K_{26}	63	13.2 (0)	10.0* (4)	7.2** (6)
	69	19.4 (0)	17.5 (2)	18.4 (0)
	73	17.5 (0)	16.8 (3)	18.9 (0)
Horst K_{32}	63	7.1 (0)	7.7 (0)	7.0 (4)
	69	14.5 (0)	13.3 (1)	13.8 (0)
	73	15.1 (0)	9.2** (4)	14.0 (0)

[1] Moisture contents in percentage of the total weight, at filling.
[2] 2 g of mycelium on autoclaved sorghum grains.
[3] 10^6 ascospores/m².
[4] Figures in parentheses represent number of trays with ascocarps.
[5] One asterisk indicates values significantly different from uninoculated controls at the 5% level, double asterisks at the 1% level.

Tabel 5. Gemiddelde opbrengsten (kg/m²) van rassen van A. bitorquis op composten met drie verschillende vochtgehalten. Kisten werden juist na het enten geïnoculeerd met mycelium of ascosporen van D. microsporus.

Influence of moisture content of the compost and kind of inoculum on sensitivity of strains. Table 5 shows the results of Trial 17 in six replicates with three strains of *A. bitorquis* on composts with moisture contents at filling of 63%, 69% and 73% of the total weight (69–70% is optimal) and with two kinds of inoculum. Again, yields of Somycel 2.017 were most seriously reduced by inoculation. Ascocarps were found in all trays spawned with this strain and inoculated with ascospores. In trays spawned with Horst K26 and Horst K32 and inoculated with ascospores, *D. microsporus* was only found if the compost was very dry (moisture content at filling 63%).

The success of inoculation with mycelium of false truffle was enhanced by increased moisture content of the compost, as indicated by the response of Somycel 2.017 and by the significant decrease in yield of Horst K32 after inoculation, on compost with a moisture content of 73%.

The preference of spores of *D. microsporus* for dry conditions and of mycelium for humid conditions was already recognized by Kligman (1944) and Beach (1937), respectively.

In normal and humid composts, strains Horst K26 and Horst K32 may be disturbed by the incidence of mycelium of false truffle rather than by that of ascospores.

Fungicides. In vitro sensitivity to some fungicides of mycelial growth of *D. microsporus* on malt agar is shown in Table 6. As benomyl and captafol were the most

Table 6. Sensitivity to some fungicides of mycelial growth of *D. microsporus*. Radial growth on malt agar was measured after 5 and 13 days incubation at 22°C in each of 5 replicates.

Fungicide	ED50 (µg a.i./ml)
chlorothalonil	> 500
captafol	ca. 10
vinclozolin	10–100
benomyl	< 1

Tabel 6. Gevoeligheid voor enkele fungiciden van de myceliumgroei van D. microsporus. De radiale groei op moutagar werd gemeten in 5 herhalingen na 5 en 13 dagen incubatie bij 22°C.

promising, these fungicides were selected for use in mushroom growing Trials 6, 1 and 14. In addition the chemicals mancozeb and copper sulphate (Gandy et al., 1953) were tested. Table 7 presents the results. Captafol, mancozeb and copper sulphate had no effect on false truffle. Only in Trial 1 did application of benomyl result in significantly higher yields than that of the inoculated control. Yields were still reduced to about 50% of the uninoculated control with a significant difference,

Table 7. Effect of fungicides on yield of *A. bitorquis* (strain Somycel 2.017) and on incidence of false truffle, after inoculation[1] at spawning with *D. microsporus*.

Fungicide	Concentration of fungicide (g a.i./m²)	Application	Average[2] yields[3] and number of trays with ascocarps[4]		
			Trial 6	Trial 1[5]	Trial 14
– (uninoculated)	–	–	100 (0)	100 (0)	100 (0)
– (inoculated)	–	–	17.3 (10)	13.4 (7)	45.8 (9)
benomyl[6]	0.75	at spawning	23.5 (10)	44.7** (7)	60.2 (7)
benomyl[6]	0.75	at spawning and casing	18.5 (10)	55.6** (6)	49.1 (9)
mancozeb	0.3	at spawning and casing	11.2 (10)	–	–
copper sulphate	20	at filling	19.7 (10)	–	–
copper sulphate	40	at filling	26.8 (10)	2.4 (8)	–
captafol	2.4	at spawning	–	–	44.6 (8)
captafol	2.4	at spawning and casing	–	–	41.3 (9)

[1] Inoculum was 50 g of compost and casing soil from a mushroom crop with mycelium and ascocarps (Trial 6), 2 g of similar material (Trial 1) and 2 g of mycelium and ascocarps on autoclaved sorghum grains (Trial 14).

[2] Trial 6 was in 10 replicates, Trial 1 in 8 and Trial 14 in 9 replicates.

[3] Yield in percentage of uninoculated control. 100% equals 7.8 kg/m² in Trial 6; 12.3 kg/m² in Trial 1 and 10.5 kg/m² in Trial 14.

[4] Figures in parentheses represent number of trays with ascocarps.

[5] Double asterisks indicate values significantly different from untreated, inoculated control at the 1% level.

[6] At spawning, benomyl was incorporated in the compost in Trials 6 and 1, and sprayed on top of the compost in Trial 14.

Tabel 7. Invloed van fungiciden op de opbrengst van A. bitorquis (ras Somycel 2.017) en op de aanwezigheid van valse truffel na inoculatie bij het enten met D. microsporus.

however, and ascocarps occurred in most of the trays. Results with benomyl in the other trials were not promising.

To determine whether the discrepancy between the effect of benomyl in vitro and in mushroom growing trials might be caused by adsorption of the fungicide to the mushroom substrate (Sanderson, 1973), mycelium of *D. microsporus* was transferred in vitro to casing soil, peak-heated compost or ground peak-heated compost, that had been sterilized by γ-irradiation at 2.5 Mrad after addition of various amounts (0 to 20 mg/l) of benomyl. Mycelial growth on ground compost was completely inhibited at the rate of 0.5 mg a.i. benomyl per litre of substrate, and on casing soil and compost at 5 mg benomyl per litre. The amount of benomyl commonly used in practice is 20 mg a.i./litre (= 0.75 g/m^2). The discrepancy between the effect of benomyl in vitro and in mushroom growing trials probably cannot be attributed to adsorption only.

Discussion

Inoculation with mycelium or ascospores of *D. microsporus* usually succeeded in these trials by using a sensitive strain (Somycel 2.017) of *Agaricus bitorquis*, a mushroom species that prefers a warm climate. To prevent spontaneous contamination of control trays, certain precautions even had to be kept. Various authors (Beach, 1937; Kligman, 1944; Gandy et al., 1953) mentioned unsatisfactory and varying results for inoculation experiments with *A. bisporus*. In these trials with *A. bitorquis* only yield losses due to the competition of *D. microsporus* varied, with high decreases in yield in one trial and lower decreases in other trials after inoculation, with all kinds of inocula. This variability may be caused by a number of unknown factors. The amount of inoculum was not decisive for the appearance of false truffle: 10 spores per m^2 were already sufficient. Consequently about one spore per tray may result in the presence of *D. microsporus*. No author has yet reported on the amount of inoculum. Assumptions and even trials were made on the source of the fungus. Most authors blamed the (casing) soil and to a less extent the survival of *D. microsporus* in the wood. Our results with the required amount of ascospores suggest that if only a few spores escape the cooking out procedure at the end of a crop other growing-rooms can be contaminated when environmental conditions are suitable. Moreover, cooking out was not a general procedure in earlier years.

The time of inoculation did influence yield loss and appearance of false truffle. Irrespective of the kind of inoculum (mycelium, ascospores or both) inoculation only resulted in yield loss from spawning (t_0) until about three days after casing (t_{15}), when the mushroom mycelium had not yet permeated the casing layer. This behaviour clearly indicates the competitive nature of *D. microsporus*. Its competitive nature also appeared from the different sensitivity of various strains of *A. bitorquis:* for instance mycelial growth of Horst K26 and K32 in the compost is much more vigorous and rapid than that of Somycel 2.017, which apparently offers a poor chance to *D. microsporus*. Only mycelium of the fungus can cause a decrease in yield of the less sensitive strains, for instance by survival in the wood, when false truffle is first. Strains of *A. bisporus* also show a different response to false truffle: brown varieties are less sensitive to competition than white varieties (Lambert, 1932). Since Somycel 2.017, the first commercially available and not very productive strain of *A. bitorquis*, is most sensitive to the competition of *D. microsporus*, the panic among mushroom growers

was understandable. From mushroom virus disease to which strains of *A. bitorquis* are immune (Van Zaayen, 1976) they became concerned with a seemingly heat-resistant organism. Spores have been reported to withstand 82°C for 5 h (Lambert, 1932); 82°C for 4 h (Glasscock and Ware, 1941); 82°C for 7 h and 93°C for 3 h (Kligman, 1944). Lambert (1932) did not give complete details of his tests but mentioned the use of test tubes. Lambert and Ayers (1957) referred to earlier papers. Glasscock and Ware (1941) and Kligman (1944) judged the rate of germination of heat-treated truffle spores in mushroom spawn in bottles. Data on the heating-up periods were not given. Temperatures in the bottles most probably were not checked. Baker and Cook (1974, p. 105) recommended that the thermal death-point of spores of *D. microsporus* should be investigated further. This has been done in the present study and our results did not agree with the earlier data.

Temperature treatments in vitro were most carefully done, since only one spore sticking to the glass wall of a test tube might escape the temperature treatment of the suspension and cause false truffle after inoculation of the spawned trays. The results of most trials corresponded, however. Spores of false truffle in compost and casing soil were readily killed when the temperature was kept at 70°C for rather short periods, after a prolonged heating-up period. Spores were always killed – except in the wood – by cooking out (compost 12 h at 70°C after a heating-up period of the growing-room of 12 to 24 h). During trials with false truffle lasting several years in isolated growing-rooms of the Experimental Station, not one of the other growing-rooms became contaminated with *D. microsporus*, nor did spontaneous contaminations from previous trials occur in the isolated growing-rooms. In addition to cooking out, the wood of the trays was treated with SPCP.

The results of the trials with ascospores treated at different temperatures varied slightly and were not conclusive: at higher temperatures sometimes more spores seemed to survive than at a lower temperature (see for instance Table 3, Trial 10, 0.5 h 70°C and 75°C). The trials on thermal sensitivity need to be continued in vitro. In vitro germination of ascospores, however, has not been possible so far. Treatment for 0.5 h at 40°C or 50°C stimulated germination in mushroom trays, perhaps by breaking the dormancy. Retarded and problematic germination was also reported by Kligman (1944). Most authors used pure-culture manure spawn (with mushroom mycelium) to germinate ascospores of *D. microsporus*. Germination at the optimum temperature of 30.5°C on manure spawn, autoclaved or not, took about 11 days (Kligman, 1944). Optimum mycelial growth occurred at 26°C and 32°C. In crops of *A. bisporus*, false truffle can be prevented by temperature control. In recent years *D. microsporus* was hardly noticed in crops of *A. bisporus*, most probably also because a more selective, fermented (peak-heated) substrate and more vigorously growing spawn varieties were used. In crops of *A. bitorquis*, however, mycelial growth requires a temperature of 30°C in the compost, about the optimum temperature for germination of truffle spores. Mycelial growth of false truffle shows a depression at 30°C compared with 26°C and 32°C, but growth is still rather good. Raising or lowering the temperature slightly during mycelial growth of *A. bitorquis* will not affect *D. microsporus*. Greater variations in temperature may damage *A. bitorquis*.

As for *A. bitorquis*, prevention of false truffle must be searched for by other means. The fungicides tested so far did not work. Most probably the reported effects of copper sulphate (Gandy et al., 1953) and benomyl (Sanderson, 1973) were caused by

failure to turn up of false truffle. To understand the discrepancy between the in vitro effects of several fungicides and their effect in inoculated mushroom trays, the rate of adsorption of benomyl in the substrate and probably the interrelationships between antagonists and *D. microsporus* require further research. Other strains of *A. bitorquis* than Somycel 2.017 appeared to be less sensitive to the competition. Among these, highly productive strains Horst K26 and Horst K32 will not be hindered by *D. microsporus* if the following precautions are exercised: cooking out at the end of a crop (compost temperature 70°C for 12 hours), followed by treatment of the wood with SPCP; protection by hygiene early in the crop, i.e. covering of the compost by a thin plastic sheet during mycelial growth followed by a quick execution of casing.

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Samenvatting

Warmtegevoeligheid, biologie en mogelijkheden ter voorkoming van Diehliomyces microsporus in teelten van Agaricus-soorten

De teelt van de warmteminnende champignonsoort *Agaricus bitorquis*, die immuun is voor virusziekte, werd al spoedig na introductie bedreigd door de eveneens warmteminnende concurrent *Diehliomyces microsporus*, valse truffel. Deze schimmel kwam vroeger voor in teelten van *A. bisporus;* de sporen zouden een temperatuur van 82°C gedurende 5 uur kunnen overleven (Lambert, 1932). Tabel 1 geeft de myceliumgroei op verschillende voedingsbodems en de vorming van vruchtlichamen (Fig. 1A, B) weer. De optimale temperaturen voor myceliumgroei waren 26°C en 32°C, met een lichte depressie bij 30°C (Fig. 2). Proeven in geïsoleerde teeltruimten werden voornamelijk uitgevoerd met Somycel 2.017, een ras van *A. bitorquis*. Inoculatie met mycelium, vruchtlichamen en/of ascosporen van *D. microsporus*, al of niet in reincultuur gekweekt, leidde vrijwel steeds tot de aanwezigheid van de concurrent in de geïnoculeerde teeltkisten (Fig. 1C, D), waarbij vruchtlichamen met ascosporen (Fig. 1E) gevormd werden en tot een reductie van het aantal champignons. Tien sporen per m^2 waren al voldoende om *D. microsporus* te doen aanslaan (Fig. 3). Het tijdstip van inoculatie bleek van groot belang te zijn: onafhankelijk van de aard van het inoculum leverde dit slechts zowel valse truffel als oogstreductie op, indien het werd aangebracht in de periode vanaf enten tot enkele dagen na het afdekken (Tabel 2 en Fig. 4). Inoculatie op latere tijdstippen kon wel tot valse truffel leiden, maar niet tot oogstreductie.

Aangezien de kieming van ascosporen in vitro slechte resultaten opleverde, ook na toevoeging van diverse stimulantia, werden ascosporensuspensies in vitro geduren-

de verschillende tijden bij verschillende temperaturen behandeld; vervolgens werden teeltkisten met de behandelde suspensies geïnoculeerd (7 tot 11 $\times$ 10^7 sporen/m^2). De kisten waren tevoren geënt met Somycel 2.017. Een aantal proeven wees uit, dat de ascosporen $\frac{1}{2}$ uur 85°C, 1 uur 80°C en 3 uur 70°C niet overleefden (Tabel 3). Het spontaan optreden van valse truffel kon echter niet altijd worden voorkomen en beïnvloedde de uitkomsten van deze proeven. Daarom is het mogelijk, dat de sporen al bij een lagere temperatuur worden gedood. Vruchtlichamen en ascosporen werden gedood door het 'uitzweten' aan het begin van een teelt en door het 'doodstomen' aan het einde van een teelt (composttemperatuur 12 uur 70°C), maar de schimmel bleek in het laatste geval wel over te kunnen blijven in het hout van teeltkisten als er vervolgens geen houtontsmettingsmiddel werd toegepast.

Somycel 2.017 leed verhoudingsgewijs meer schade door concurrentie van *D. microsporus* dan enkele andere rassen (Tabel 4 en 5). Inoculatie met ascosporen bleek bij de minst gevoelige en meest produktieve rassen Horst K26 en Horst K32 slechts te gelukken in extreem droge compost; bij Somycel 2.017 daarentegen zowel in compost met een laag als met een hoog vochtgehalte. Inoculatie met mycelium veroorzaakte meer valse truffel en meer schade naarmate de compost natter was (Tabel 5).

De werking van een aantal fungiciden in vitro (Tabel 6) en in teeltkisten (Tabel 7) stemde niet overeen. Aangezien de tot nu toe getoetste fungiciden *D. microsporus* niet kunnen voorkomen of bestrijden, moet preventie van deze concurrent worden gezocht in het telen van weinig gevoelige rassen van *A. bitorquis* in combinatie met hygiënische maatregelen vroeg in en aan het eind van de teelt.

References

Baker, K. F. & Cook, R. J., 1974. Biological control of plant pathogens. W. H. Freeman Co., San Francisco, 433 pp.

Beach, W. S., 1937. Control of mushroom diseases and weed fungi. Bull. Penna agric. Exp. Stn 351, 32 pp.

Bels-Koning, H. C. & Bels, P. J., 1958. Handleiding voor de champignoncultuur. Proefstation voor de Champignoncultuur, Horst (L.), 295 pp.

Bollen, G. J. & Zaayen, A. van, 1975. Resistance to benzimidazole fungicides in pathogenic strains of *Verticillium fungicola*. Neth. J. Pl. Path. 81: 157–167.

Diehl, W. W. & Lambert, E. B., 1930. A new truffle in beds of cultivated mushrooms. Mycologia 22: 223–226.

Dieleman-van Zaayen, A., 1972. Spread, prevention and control of mushroom virus disease. Mushr. Sci. 8: 131–154.

Emerson, M. R., 1948. Chemical activation of ascospore germination in *Neurospora crassa*. J. Bact. 55: 327–330.

Ferguson, M. C., 1902. A preliminary study of the germination of the spores of *Agaricus campestris* and other Basidiomycetous fungi. Bull. Bur. Pl. Ind. U.S. Dep. Agric. (Washington) 16, 43 pp.

Fritsche, G., 1976. Welche Möglichkeiten eröffnet der viersporige Champignon *Agaricus bitorquis* (Quél.) Sacc. dem Züchter? Theor. appl. Genet. 47: 125–131.

Fritsche, G. & Sengbusch, R. von, 1962. Die züchterische Bearbeitung des Kulturchampignons (*Psalliota bispora* Lge.). Probleme und erste eigene Ergebnisse. Züchter 32: 189–199.

Gandy, D. G., Duncan, C. W. & Edwards, R. L., 1953. The use of copper sulphate for control of 'truffle' (*Pseudobalsamia microspora*). Mushr. Sci. 2: 167–174.

Gerrits, J. P. G., 1977. The supplementation of horse manure compost and synthetic compost with chicken manure and other nitrogen sources. Mushr. Sci. 9[II], Taiwan: 77–98.

Gilkey, H. M., 1954. Taxonomic notes on Tuberales. Mycologia 46: 783–793.

Glasscock, H. H. & Ware, W. M., 1941. Investigations on the invasion of mushroom beds by *Pseudobalsamia microspora*. Ann. appl. Biol. 28: 85–90.

Hasselbach, O. E. & Mutsers, P., 1971. *Agaricus bitorquis* (Quél.) Sacc. een warmteminnend familielid van de champignon. Champignoncultuur 15: 211–219.
Kligman, A. M., 1944. Control of the truffle in beds of the cultivated mushroom. Phytopathology 34: 376–384.
Lambert, E. B., 1930. Two new diseases of cultivated mushrooms. Phytopathology 20: 917–919.
Lambert, E. B., 1932. The truffle disease of cultivated mushrooms. News Letter, Div. Mycol. and Dis. Sur. B.P.I., U.S. Dep. Agric. (neostyled), 4 pp.
Lambert, E. B. & Ayers, T. T., 1957. Thermal death times for some pests of cultivated mushroom (*Agaricus campestris* L.). Pl. Dis. Reptr 41: 348–353.
Olivier, J.-M. & Guillaumes, J., 1975. Trois nouvelles maladies dans les champignonnières françaises. Annls Phytopath. 7: 357–358.
Pompen, Th. G. M., 1975. De teeltwijze van K26 en K32. Champignoncultuur 19: 283–289.
Poppe, J. A., 1972. Un excellent *Agaricus* tétra-sporique cultivable commercialement avec succès. Mushr. Sci. 8: 517–525.
Sanderson, F. R., 1973. Benomyl for the control of false truffle. Mushr. J. 12: 546–547.
Schisler, L. C., Sinden, J. W. & Sigel, E. M., 1968. Etiology of mummy disease of cultivated mushrooms. Phytopathology 58: 944–948.
Stoller, B. B., 1962. Some practical aspects of making mushroom spawn. Mushr. Sci. 5: 170–184.
Sussman, A. S. & Halvorson, H. O., 1966. Spores. Their dormancy and germination. Harper & Row, New York, London, 354 pp.
Zaayen, A. van, 1976. Immunity of strains of *Agaricus bitorquis* to mushroom virus disease. Neth. J. Pl. Path. 82: 121–131.

Addresses

Annemarie van Zaayen: Proefstation voor de Champignoncultuur, Peelheideweg 1, Horst-America (L), the Netherlands.
Bernarda van der Pol-Luiten: Laboratorium voor Fytopathologie, Binnenhaven 9, Wageningen 6140, the Netherlands.