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Studies on the physiology of the three soft rot fungi *Allescheria terrestris, Phialophora (Margarinomyces) luteo-viridis* and *Phialophora richardsiae*

Fysiologiska studier över de tre soft rot svamparna Allescheria terrestris, Phialophora (Margarinomyces) luteo-viridis och Phialophora richardsiae

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Contents

1 Introduction

The term "soft rot" was coined by Savory (1954 a) to describe a type of fungal degradation of wood where the wood surface was extremely soft when wet and cracked across the grain when dry. Microscopially, longitudinal sections of wood decayed by soft rot show cavities with conical ends in the cell wall while transverse sections show holes in the secondary wall and erosion of the cell wall from the cell lumen or both.

The wood-destroying capacity of the soft rot fungi and their ability to form cavities have been in the forefront of physiological investigations ever since Savory (1954a, 1954b) and Findlay and Savory (1954) started studies on soft rot in the 1950s.

Duncan's work from 1960 was the first which dealt thoroughly with the physiology of the soft rot fungi, i.e. their temperature relations, oxidase production, pH preferences and tolerances to woodpreserving chemicals. That soft rot fungi were more tolerant to preservatives and their chemicals than wood-destroying Basidiomycetes was earlier reported by Savory (1955), Rennerfelt (1956), and others. Utilization of nitrogen and carbohydrates and the vitamin requirements of fungi known to cause soft rot have been studied or included in general physiological studies of fungi by Arêa Leão and Cury (1950), Brewer (1959), Levi and Cowling (1969), Käärik (1960), Omvik (1970), Tansey (1970), Takahashi and Nishimoto (1973) and others. The pH preference of soft rot fungi were especially studied by Sharp and Eggins (1970). These studies and results from Brewer (1959), and Duncan (1960) show that the soft rot fungi are capable of growing at pH 3 to 8 or 9. Temperature studies have shown that soft rot fungi can grow from about 5°C to about 60°C (Bergman and Nilsson 1966, 1967, 1968, 1971). *Allescheria terrestris*, a thermophilic fungus, for example, grows on a malt agar medium from about 20°C to about 55°C.

With regard to physiology, *Chaetomium globosum* Kunze ex Fr. is the most studied soft rot fungus. *C. globosum* is also often included in cavity studies, textile strength loss tests and wood-decaying tests. Temperature studies are on the whole the only known physiological studies of *Allescheria terrestris* and were made by Apinis (1963), Nilsson (Bergman and Nilsson 1966, 1967) and Ofosu-Asiedu and Smith (1973).

The present investigation has been focussed on *Allescheria terrestris*. *Phialophora (Margarinomyces) luteo-viridis* and *Phialophora richardsiae* have been studied for reasons of comparison.

2 Materials and methods

The fungi used in this investigation included *Allescheria terrestris* Apinis (strain Apinis and strain H63-1), *Phialophora luteo-viridis* (van Beyma) Schol-Schwarz (syn *Margarinomyces luteo-viridis* van Beyma) (strain Beyma 206.38 and strain M74-IV) and *Phialophora richardsiae* (Nannf.) Conant (strain BB40-V). Strain H63-1 was isolated from aspen chips in 1965 and strain M74-IV and strain BB40–V from birch chips in 1964. The sampling was made by Dr. T. Nilsson at The Royal College of Forestry, Stockholm, Sweden. Strain Apinis has been supplied by Prof. A.E. Apinis at the Dept. of Botany, University of Nottingham, Nottingham, England, and strain Beyma = CBS 206.38 has been obtained from "Centraalbureau voor Schimmelcultures" in Baarn, Holland.

Other fungi occasionally used are *Petriellidium boydii* (Shear) Malloch strain SP31-4 obtained from Dr. T. Nilsson and *Stereum hirsutum* (Willd. ex Fr.) Fr. strain A-255. This fungus was from the stock culture collection of the Institute of Forest Products of the Royal College of Forestry, Stockholm.

Before the experiments the fungi were kept in plastic petri dishes on malt agar for about 6 days at 45°C for *Allescheria terrestris*, 15 days at 25°C and 30°C for *Phialophora luteo-viridis*, 20 days at 25°C for *P. richardsiae*, 15 days at 25°C for *Petriellidium boydii* and 7 days at 25°C for *Stereum hirsutum*. The about 5 x 5 mm pieces of inoculum were taken from the peripheral parts of fungus cultures.

The experiments were mainly carried out in plastic petri dishes, culture tubes and 100 ml Erlenmeyer flasks. The flasks used in experiments with fluid synthetic media were carefully cleaned with dichromaticsulphuric acid solution and washed with hot tap water and redistilled water.

In the experiments with liquid nutrient media, 20 ml of nutrient solution per flask was used. After autoclaving for 20 min at 120°C, the flasks for surface cultures were inoculated with a piece from an agar culture. The mycelial dry weights of cultures in liquid media were determined by filtering off the nutrient solution and collecting the mycelia in weighed glass crucibles or on circular filter papers. After this the mycelia were washed thoroughly with distilled water and dried for about 15 hours at 105°C, then cooled in a desiccator and weighed. The reported mycelial weights usually represent the mean of four to five replicates. The pH of the nutrient solution was determined at the beginning as well as at the end of the experiments.

The following nutrient media were used:

Medium A (malt agar)	
Malt extract syrup)	25.0 g
Agar	15.0 g
Distilled water to	1000 ml
Medium B	
Malt extract (syrup)	25.0 g
Distilled water to	1000 ml
Medium C (Modified after Brewer 1959)	
Glucose	20.0 g
KH_2PO_4	1.0 g
$MgSO_4 + 7H_2O$	0.5 g
Ammonium tartrate	1.0 g
$CaCl_2 + 2H_2O$	0.3 g
NaCl	0.1 g
$ZnSO_4 + 7H_2O$ (0.5 % solu.)	0.5 ml
Ferric citrate (1 % solu.)	0.5 ml
Thiamine HCl	100 µg
Distilled water to	1000 ml
Medium C_1 ("incomplete" medium C)	
Glucose	20.0 g
KH_2PO_4	1.0 g
$MgSO_4 + 7H_2O$	0.5 g
$(NH_4)_2SO_4$	0.5 g
Ferric citrate (1 % solu.)	0.5 ml
Distilled water to	1000 ml
Medium D (Modified after Lindeberg 1944)	
Glucose	10.0 g
Ammonium tartrate	1.0 g
KH_2PO_4	0.35 g
K_2HPO_4	0.15 g

$MgSO_4 + 7H_2O$	0.5 g
$CaSO_4 + 2H_2O$	0.1 g
$MnSO_4 + 4H_2O$	0.01 g
NaCl	0.5 g
$FeCl_3 + 6H_2O$ (1 % solu.)	0.5 ml
$ZnSO_4 + 7H_2O$ (0.5 % solu.)	0.5 ml
Thiamine HCl	50 µg
Distilled water to	400 ml

Medium E (Modified after Duncan 1965)

NH_4NO_3	6.0 g
K_2HPO_4	4.0 g
KH_2PO_4	5.0 g
$MgSO_4 + 7H_2O$	4.0 g
Glucose	2.5 g
Distilled water to	1000 ml

3 Temperature and growth

Temperature is a very important external factor which influences many of the processes involved in the growth of fungi. The temperature response of fungi occurring in chip piles is especially important since the temperature in the piles may vary between several degrees below zero and +65°C to +70°C. The temperature requirements for the soft rot fungi concerned were studied for radial growth on medium A, mycelial production on medium B, capacity to survive on medium A at high temperatures and the capacity to survive in birchwood at high and low temperatures.

3.1 Radial growth on medium A

The results (Figure 1) show that the optimal temperature for radial growth on this medium is about 45°C for *Allescheria terrestris*. Other cardinal temperatures for growth are 20°C as the minimum temperature (trace of growth) and just above 50°C as the maximum temperature, but the fungus can survive at higher temperatures (see Table 1). Nilsson (Bergman and Nilsson 1966, 1967) reported 45°C as the optimal temperature for growth and trace of growth at 55°C. Ofosu-Asiedu and Smith (1973) also found 45°C to be the optimal temperature for growth. Potato-dextrose agar as the medium (Evans 1971) gave 22°C, 42–45°C, 55°C as the minimum, optimum and maximum temperatures for growth. Czapek agar as the medium (Apinis 1963) gave 28°C–48°C as the approximate minimum and maximum temperatures for growth. *A. terrestris* is listed as a thermophilic fungus by Emerson (1968).

As can be seen from the results in Figure 2, it is evident that strain Beyma grew faster than strain M74–IV of *Phialophora luteo-viridis*. The optimal growth temperature is about 30°C for both strains. Nilsson (Bergman and Nilsson 1967) found the same optimal growth temperature. Table 2 shows that this fungus can survive in temperatures higher than 40°C if the mycelium has started to grow at lower temperatures.

Optimal growth for *Phialophora richardsiae* BB40–V was obtained at 25°C (Figure 2). Nilsson (Bergman and Nilsson 1968) reported 25°C and Brewer (1959) 30°C as the optimal growth temperature. Duncan (1960) found that different strains of *P. richardsiae* can have different temperatures for optimal growth, both 28°C and 34°C. As can be seen from Table 2, this fungus can survive at temperatures higher than 35°C if the mycelium has started to grow at lower temperatures.

3.2 Mycelial production on medium B

The studied fungi were grown as floating mycelia on medium B. From Figure 3 it can be seen that *A. terrestris* had the highest mycelial production on this medium after 7 days at about 40°C. For strain H63–1 this is 5°C lower than the optimal growth on malt agar. At 25°C and 55°C there was no mycelial production.

For *P. luteo-viridis* and *P. richardsiae* (Figure 3) it is more difficult to fix the optimal temperature for mycelial production. However, for *P. luteo-viridis* the highest mycelial production appears to occur at approx. 30°C. The aerial mycelia of these fungi are poorly developed, which makes it difficult to obtain real floating cultures. Note that *P. richardsiae* grew at 35°C on medium B but not on medium A.

When the fungi are cultivated on this medium for longer periods (14–21 days) disturbances arise in the growth, possibly due to the fact that exudated substances are accumulated in the nutrient solution, perhaps disturbing growth.

3.3 The capacity of the fungi to survive on medium A at high temperatures

Mycelia from the fungi studied were allowed to grow about 4–5 mm at the optimal growth temperatures before they were placed at super-maximal temperatures during times fluctuating between one hour and 14 days. Subsequently, the fungi were again placed in the optimal growth temperatures and their growth noted after a week.

The results (Table 1) show that *Allescheria terrestris* survived at about 55°C for 14 days without difficulty. This temperature was normally too high for growth on this medium (Figure 1). Apinis (1963) held *A. terrestris* at 57°–58°C for five days and then transferred the fungus to 37.5°C. He found that *A. terrestris* did not grow on any of the five different media tested. On Table 1 it may be seen that *A. terrestris* survived two days at 60°C. The two strains of *Phialophora luteo-viridis* showed a similar pattern. They survived for 14 days at 40°C, a temperature too high for growth (Table 2). On the other hand, *Phialophora richardsiae* did not survive longer than seven days at 35°C. At this temperature no growth could be observed (Table 2). The experiment demonstrated the capacity of the three soft rot fungi studied to recover from the temperature shocks when they were again placed in optimal growth temperature. The capacity was dependent on the storage time in the super-maximal growth temperature.

3.4 The capacity of the fungi to survive in birchwood at high and low temperatures

Autoclave-sterilised blocks (2 x 0.5 x 0.5 cm) from sapwood and central wood of birch were inoculated with the fungi. The inoculation was arranged so that the birch blocks were placed on fungus cultures growing on medium A and at the optimal growth temperature for 14 days. Then the blocks were transferred to plastic petri dishes with 15 ml of medium A in two different super-maximal growth temperatures. The following temperatures were tested:

Allescheria terrestris	Apinis H63–1	55°C	60°C
Phialophora luteo-viridis	Beyma M74–IV	40°C	45°C
Phialophora richardsiae	BB40–V	35°C	40°C

The growth of the mycelia was recorded once a week. As a control the fungi were inoculated on medium A, where they were allowed to grow a few millimetres at optimal growth temperatures before they were placed in the above-mentioned temperatures.

Neither the two strains of *Phialophora luteo-viridis* Beyma and M74–IV nor *Phialophora richardsiae* BB40–V grew out from birch wood on medium A at 40°C or 35°C respectively. On the other hand, *Allescheria terrestris* grew out from birch wood at 55°C for three weeks (Table 3). At this temperature the fungus did not grow on medium A (see Figure 1) directly after inoculation.

In order to investigate survival at very low temperatures, the fungi were inoculated as spore suspensions (2 ml) on autoclave-sterilised birch blocks (2 x 0.5 x 0.5 cm). These were placed in vermiculite in 100 ml Erlenmeyer flasks, 30 ml of medium E being added to each flask. The fungi were permitted to attack the blocks for three weeks at temperatures optimal for each fungus. Then the flasks were placed in a freezer at temperatures of -28°C to -30°C. The blocks were taken up on different occasions during a period of two years, and placed on medium A at optimal growth temperature. All of the three fungi tested survived the two-year period. Apinis (1963) found that a low temperature of 1°C–2°C for five days was not injurious to *Allescheria terrestris*.

3.5 Discussion

In chip piles with a volume usually in excess of 5000 m^3, where the studied soft rot fungi live, the temperature in the central parts normally rises 1°C–2°C per day during the first month of storage. Later the temperature remains constant or decreases slowly. Temperatures as high as 65°C–70°C (piles built up in summer) and 50°C (piles built up in winter) have been recorded in the central parts of piles (Bergman 1973). In the outer parts of the pile the temperature varies in

accordance with the air temperature. Outer parts of a chip pile may freeze during winter.

The capacity of the soft rot fungi studied to survive temperatures in excess of the maximal growth temperature during the laboratory tests depends on the level and duration of the higher temperature. For a few hours the temperature can rise about 20°C–30°C above the maximum growth temperature without killing the soft rot fungi. Note that the temperature rose very quickly during the laboratory tests and that the hyphae were directly exposed to the heat. In the pile the temperature rises 1°C–2°C per day.

Hyphae in wood were better protected against temperature injuries than the naked hyphae. Investigations made by Snell (1923) and others also show that fungi can survive in wood at high temperatures.

High temperatures certainly affect the type and distribution of fungi in a chip pile. Both Savory (1955) and Duncan (1960) presume that the optimum temperature of soft rot fungi is higher than that of wood-attacking Basidiomycetes. According to Liese (1969) the temperature optimum of most of the soft rot fungi is between 25°C and 35°C. The three soft rot fungi studied here indicate that soft rot fungi grow in both high and low temperatures, viz. *Allescheria terrestris* 20°–45°–50°C, *Phialophora luteo-viridis* < 5°–30°–35°C and *Phialophora richardsiae* < 5°–25°–30°C for optimal growth on medium A. The temperature for optimal growth, mycelial production and wood-decaying is not always the same. *A. terrestris* strain H63-1 shows the following optimal temperatures: for radial growth 45°C, for wood-decaying 50°C (Lundström 1973) and for mycelial production 40°C.

The soft rot fungi studied survive freezing at -28°C to -30°C for two years, then thawing directly to the optimal growth temperature. As is known from other investigations, both freezing and thawing are critical processes for the cell of the fungi. Rapid freezing and thawing reduce cellular injury. Repeated slow freezing and thawing may be most damaging to fungal cells (Deverall 1965). Fungi living in the outer parts of the chip pile, e.g. *P. luteo-viridis* and *P. richardsiae*, may be exposed to such critical trails as repeated freezing and thawing.

Heat has a decisive influence on the survival of fungi. Moist heat is often more effective in killing cells than dry heat (Snell 1923). The moisture distribution in chip piles is irregular and great variation in the moisture content of wood chips occurs during storage. The interior of a chip pile becomes drier than the exterior, especially in piles stored during the winter (Bergman 1973). The heat will therefore be somewhat dry in the central part of the piles, which is highly advantageous to fungi such as *Allescheria terrestris* living in central parts of chip piles.

4 Effect of pH on growth

Investigations by Duncan (1960) and Sharp and Eggins (1970) concerning the pH-dependence of soft rot fungi show that they grow within a wide pH spectrum. Most favourable are the slightly acidic conditions, with optimal growth tending to occur between pH 6 and 7. Some soft rot fungi, however, are capable of growing at a pH as high as pH 9.

In the experiments presented here, *Allescheria terrestris* strain Apinis and H63-1 were grown as floating cultures at 30°C and 45°C on medium D. Ten millilitres of the doubly concentrated nutrient solution and 10 ml of the buffer solution were aseptically added to the culture flasks. The following buffer systems were used: 0.2 M KCl + 0.2 M HCl for pH 2.4, 0.1 M citric acid + 0.2 M Na_2HPO_4 for pH 3.6 to 6 and phosphate buffer for 6.6 to 7.3. Dry weight of mycelium was determined after 5, 10 and 13 days at 30°C and 3, 5, 7 and 10 days at 45°C.

The results are presented in Figures 4 a, 4 b, 5 a and 5 b. They show that the tested strains of *Allescheria terrestris* grew between the tested pH values of 2.4 to 7.3. The following pH values gave the highest dry weight of mycelium at 30°C and 45°C respectively:

Strain	Incubation temp. °C	Incubation times/days	Initial pH	Final pH	Dry weight mg
Apinis	30	13	5.0	3.8	234
H63–1	30	13	6.0	4.5	266
Apinis	45	7	5.8	4.9	210
H63–1	45	7	5.8	4.9	235

The greatest decrease of pH occurred in initial pHs of 6.6 to 6.7 after 10 to 13 days at 30°C and after five to seven days at 45°C. In these series the pH fell between 2.4 to 2.7 units. Wood-destroying fungi are known to produce acids, which acidify the medium (Henningsson 1965). The growth of mycelium at the optimal pH occurred about twice as fast at 45°C (five to seven days) as at 30°C (10 to 13 days). Marked autolysis occurred after about seven days at 45°C, as shown by the fact that growth ceases or decreases and that the pH of the medium increases.

In a nutrient solution, *Allescheria terrestris* grew with a pH about 7 at 30°C and 45°C but the growth was sparse (Figures 4 a and 5 a). Nilsson (Bergman et al. 1970) could also isolate only one strain of *Allescheria terrestris* from chips treated with sodium hydroxide. The average pH of the chips was about 6.8 after storing for eight months. Several isolations of the fungus were made from untreated chips (average pH about 5.3). The two chip piles were placed side by side and consisted of two-thirds pine (*Pinus silvestris)* and one-third spruce (*Picea abies*). Changes in the pH of chips during storage are reported to be small or none (Bergman et al. 1970, Smith and Ofosu-Asiedu 1972). Shields (1970) found that the pH of chips (of balsam fir and spruce) fell after storage. According to Smith and Ofosu-Asiedu (1972), the pH values of the chips (spruce, pine) stored in the inner parts of the chip pile were lower than those stored in the outer parts. The decrease in the pH in the inner parts of chip piles may be an effect of the fungi living there, e.g. *A. terrestris*. That *A. terrestris* grew somewhat in the nutrient solution at about pH 7 but was so uncommon in chips with approximately the same pH may be explained by the fact that the pH on the surface of the chips was probably higher than the measured pH of the ground chips, 6.8. Furthermore the fungus was inoculated as mycelium in the laboratory test but in chip piles it must grow from spores.

5 Growth on different sources of nitrogen

The utilization of nitrogen compounds by fungi known to cause soft rot has not been studied on a large scale (c.f. Brewer 1959, Omvik 1970). It is evident that the wood-decaying capacity of soft rot fungi increases if nitrogen is added to wood-decaying tests (Lundström 1973 and others).

In this test the two strains Apinis and H63-1 of *Allescheria terrestris* were used. They were grown at 45°C for seven days as floating cultures on medium C with some inorganic and organic sources of nitrogen. Three concentrations of each nitrogen compound were used where the middle concentration (N) contains 76 mg nitrogen per 1000 ml of solution (corresponding to 0.5 g ammonium tartrate per 1000 ml).

From the results in Figure 6 it is evident that both of the strains of *A. terrestris* utilized the inorganic and organic nitrogen sources tested well. With the exception of ammonium chloride, the highest concentration gave the best growth.

Ammonium compounds (especially the tartrate and the nitrate) were good nitrogen sources for *A. terrestris*. Levi et al. 1968, amongst others, have reported similar results from wood-destroying Basidiomycetes. Brewer (1959) showed that ammonium tartrate gave rise to high mycelium production by the soft rot fungi *Phialophora fastigiata* and *P. richardsiae*. Two soft rot and blueing fungi, *Ceratocystis (Ophiostoma) albida* and *C. (O.) piceae*, are also reported to utilize ammonium tartrate very well (Käärik 1960). The pH decrease when ammonium nitrate was used is due to the fact that the ammonium ion was utilized first. This has been reported in several papers (e.g. Cochrane 1958). Ammonium chloride was the most poorly utilized of the ammonium compounds tested, probably due to the increase in the concentration of the hydrogen ions by the absorption of the ammonium ions. Henningsson (1965, 1967) reported the same effect in some rot fungi.

A. terrestris utilized nitrate (KNO_3) well. The rapid rise of pH, most striking in the 5N-concentration, evidently did not reduce the growth of the mycelium. Other soft rot fungi, as for example *Chaetomium thermophile* var. *dissitum* (Tansey 1970[1]), *Chloridium chlamydosporis* (Omvik 1970), *Phialophora fastigiata* and *P. richardsiae* (Brewer 1959) and *Phialophora* sp. (Nyman 1961), also utilized potassium nitrate very well as source of nitrogen. *Sporotrichum* sp. (A) and (B) do not utilize potassium nitrate (Eveleigh and Brewer 1964). *Ceratocystis (Ophiostoma) albida* and *Graphium fragrans* utilized calcium nitrate very well as a source of nitrogen (Käärik 1960).

Access to nitrate is known from other investigations to be difficult for many fungi especially for Basidiomycetes (L. Fries 1955, Hacskaylo et al. 1954, Henningsson 1965, 1967, Jennison et al. 1955 etc.). In a collation of Lilly and Barnett (1951) concerning fungi which utilize nitrate nitrogen, most of the fungi were Ascomycetes and Fungi Imperfecti. Käärik (1960) has shown that within a species – *Ophiostoma (Ceratocystis)* – there can be variations as to whether nitrate can be utilized or not.

Of the organic nitrogen compounds tested, L-asparagine and casein hydrolysate proved to be the best ones for *A. terrestris*. This is especially clear in the 5N and N concentrations which gave high dry weight of mycelium. The growth in asparagine is reported in Figures 7 a and 7 b. The figures show that in 5N concentration mycelial production is highest between five to seven days at 45°C and that thereafter autolysis is

[1]Tansey (1972) found that *Chaetomium thermophile* var. *dissitum* produced very little growth at 60°C whereas the var. *coprophile* grew vigorously. Nilsson, who showed that a strain of *C. thermophile* could produce soft rot (Bergman & Nilsson 1971), also gives temperature data for this strain, Nilsson 1973. Since his strain only showed trace of growth at 60°C it is likely that it was the var. *dissitum*.

greater than production. The rise in pH indicated autolysis. Brewer (1959) reported for *Phialophora fastigiata* and *P. richardsiae*, Nyman (1961) for *Phialophora* sp. and Käärik (1960) for *Ceratocystis (Ophiostoma) albida* and *C. (O) piceae* that asparagine was a good source of nitrogen. For many fungi asparagine has proved to be one of the best sources of nitrogen. L. Fries (1955) has also discussed asparagine as a source of nitrogen for fungi. Jennison et al. (1955) found that casein hydrolysate was a better source of nitrogen than asparagine. Henningsson (1967) reported that only three of 15 different wood-decaying fungi grew better on casein hydrolysate than on asparagine. For a number of soft rot fungi, among others *Phialophora richardsiae*, Levi and Cowling (1969) showed that sapwood of *Populus grandidentata* was attacked more rapidly if casein hydrolysate was added.

Urea was the most poorly utilized of the organic nitrogen sources tested for *A. terrestris*.

The nitrogen content of wood is low and seldom comprises more than about 0.3 per cent of the dry weight of wood (Merrill and Cowling 1966). Henningsson (1967) reported nitrogen contents of 0.08–0.14 per cent for *Betula pubescens* (whole debarked disks) in Sweden. Birch wood contains different amounts of nitrogen, depending on whether it is sapwood or central wood (Lundström 1972). These parts of wood stem, as well as springwood, commonly contain more nitrogen than intermediate parts of the wood. The nitrogen content of wood is also different in different tree species (Cowling and Merrill 1966, Merrill and Cowling 1966). The low nitrogen content of wood is certainly a limiting factor for both soft rot attack and other rot attacks. As can be seen from laboratory tests with soft rot fungi, the weight losses of the wood, judged to be a measure of the capacity of the fungi to break down the wood, increase if nitrogen is added. The nitrogen compound has either been impregnated directly into the wood or added to the soil or the vermiculite in which the wood was placed during the decay test (Duncan 1965, Levi and Cowling 1969, Lundström 1973, Bergman and Nilsson 1967, 1968, 1971). The strongly increased decay of the wood has been considered to be a consequence of the increase in the production of the cellulolytic enzymes of the soft rot fungi when nitrogen is added (Levi and Cowling 1969). The increased decay in wood when nitrogen is added can also be explained by higher mycelium production – in other words, a greater number of cells which can produce cellulase.

6 Growth on different carbohydrates

A study was made of the growth on various carbohydrates, tested in three concentrations (20, 10, 2.5 g/l) on medium C (glucose excluded) at 30°C and 45°C. Medium C and the sugars were autoclaved separately and then mixed aseptically. To avoid the breaking-down of xylose to furfural during autoclaving (Cochrane 1958), xylose was sterilized by filtering. The solutions were buffered by adding a citrate phosphate buffer.

The results presented in Table 4, Table 5 and Figure 8 show that both strains of *A. terrestris* grow well on the carbohydrates tested, with the exception of D-arabinose. The results indicate that the carbohydrates tested at the highest concentration, 20 g/l, were principally utilized during a period of between seven and ten days at 45°C, since the dry weight production of *A. terrestris* subsequently decreased.

During growth on the carbohydrates at the highest concentrations (10 and 20 g/l) at 45°C the pH in most cases first decreased before rising. A fall in pH was expected because of the nitrogen source used. Takahashi and Nishimoto (1973) reported a similar change in pH for *Chaetomium globosum*, due to the accumulation of some acidic intermediate products. At higher temperatures the pH decrease is greater, probably as a result of more accumulated acidic products. When the carbohydrates were consumed by the fungus at 45°C, the pH increased rapidly; this was not the case at 30°C within the experimental period. The tests with D-arabinose were exceptions, since here the pH already rose after three days, but this did not seem to influence the mycelium production up to ten days at 45°C. The mycelium production was not as high with D-arabinose, as with the other carbohydrates.

D-arabinose is known to be a very poor carbohydrate for fungi in general, as also for other soft rot fungi such as *Chaetomium globosum* (Takahashi and Nishimoto 1973), *Phialophora fastigiata* and *P. richardsiae* (Brewer 1959). It is possible that *A. terrestris* utilized D-arabinose in the present investigation due to impurities in the chemicals used. It is known that minute amounts of certain carbohydrates may induce utilization of another carbohydrate which is not accessible when given as a sole source of carbon. This was for instance demonstrated by Lindberg (1963), who showed that *Ophiostoma multiannulatum* could grow on galactose only when small amounts of other sugars were added to the nutrient solution.

L-arabinose, however, was a good carbon source for *A. terrestris* (better for strain Apinis than for strain H63–1) as well as for *Chloridium chlamydosporis* (Omvik 1970), *P. fastigiata* and *P. richardsiae* (Brewer 1959). Käärik (1960) found that L-arabinose was a good carbohydrate for *Ceratocystis (Ophiostoma) piceae* and *Graphium fragrans* but not for *C. (O) albida*. Within a genus – *Cephalosporium* – there can be variations in whether L-arabinose can be utilized or not (Eveleigh and Brewer 1964). Smith and Ofosu-Asiedu (1973) found that the arabinose contents of *Pinus ponderosa* Laws. sapwood decreased after degradation by *Allescheria terrestris*.

In nature arabinose is found as the L-form (associated in hemicellulose and pectin) while most other naturally occurring sugars belong to the D-series (Neish, 1959). According to Cochrane (1958) the L-isomer of arabinose is generally more available to fungi than the D-isomer.

A. terrestris utilized both xylose and mannose, which are the main constituents of wood hemicelluloses (more xyloses and less mannose in hardwoods and the reverse in softwoods). Takahashi and Nishimoto (1973) discussed the composition of hemicellulose in hardwoods and softwoods, and stated that the rapid growth and consumption for *C. globosum* in xylose and xylan media indicate a possible reference to the greater susceptibility of hardwoods to soft rot.

7 The requirements of thiamine, biotin and other vitamins

Growth in synthetic media (medium C_1) was studied with and without the addition of thiamine and a vitamin mixture. The vitamin mixture contained: biotin 10 µg, niacin 100 µg, riboflavin 100 µg, Ca-pantothenate 100 µg, pyridoxine 100 µg, folic acid 100 µg, inositol 3 µg, P-aminobenzoic acid 50 µg, vitamin B_{12} 4 µg. Agar (medium C_1 with 15 g agar added) cultures of *Allescheria terrestris* about eight days old were taken as inocula (2x2 mm). Erlenmeyer flasks (100 ml) were used, each containing 20 ml of medium C_1 buffered with citrate phosphate buffer to pH 5.4. The flasks were placed in a dark room and maintained as standing cultures at two temperatures, 35°C and 45°C respectively.

The results in Table 6 show that *A. terrestris* may be regarded as auxoautotrophic for thiamine. The same is valid for the vitamins in the vitamin mixture.

According to Arêa Leão and Cury (1950) *Allescheria boydii* Shear strain 386 and strain 1699 were biotin dependent. In order to determine whether other *Allescheria*-species and strains were also biotin dependent, the described experiment by Arêa Leão and Cury (1950) was repeated with *A. terrestris* at 45°C and *Petriellidium (Allescheria) boydii* (Shear) Malloch SP31–4 at 25°C. Figure 9 clearly demonstrates that the fungi used in this experiment do not require biotin when this test method is used. The results of Arêa Leão and Cury and those of the present investigation indicate that biotin dependence may vary between different strains of a species as *Petriellidium boydii.*

The results of the experiments (Table 6 and Figure 9) show that the two strains of *A. terrestris* are auxoautotrophic for both thiamine and biotin.

In order to test whether *A. terrestris* synthesizes some growth substances in medium C_1, a culture filtrate was taken and added to the floating culture with *Stereum hirsutum* (Willd. ex Fr.) Fr. This fungus seems to be auxoheterotrophic for thiamine (Henningsson 1967). The test was carried out as follows: Both strains of *A. terrestris* grow on medium C_1 at 45°C for seven days. Ten millilitres of this culture filtrate was aseptically added to Erlenmeyer flasks (100 ml) with 10 ml medium C_1 (pH about 4). *Stereum hirsutum* A–255 was inoculated as a floating culture at 25°C for 14 days (final pH about 3). Inoculum pieces of *A. terrestris* and *S. hirsutum* were taken from agar cultures as described above. Results:

	Medium C_1 and:			
	Only	100 µg thiamine	10 ml filtrate of strain Apinis	10 ml filtrate of strain H63–1
Mycelial dry weight (mg)	15.8	68.3	57.3	74.4

These results show that thiamine was synthesized by *A. terrestris* and exuded into medium C_1.

A large number of fungi are reported to be auxoheterotrophic for thiamine or biotin (N. Fries 1965). Wood-destroying fungi with thiamine heterotrophy have been reported by Henningsson (1965, 1967) amongst others. Thiamine or biotin requirements of soft rot fungi are reported by e.g. Brewer (1959) for *Phialophora richardsiae* (biotin), Eveleigh and Brewer (1964) for *P. fastigiata* and *Cephalosporium* sp. (biotin), Käärik (1960) for *Ceratocystis (Ophiostoma) piceae* (biotin + B_6), *C. (O) albida* (biotin + thiamine + B_6), and *Graphium fragrans* (biotin + thiamine + B_6), Arêa Leão and Cury (1950) for species within the genera *Phialophora* (thiamine), Omvik (1970) for *Chloridium chlamydosporis* (thiamine), N. Fries (1943) for *Ceratocystis (Ophiostoma) piceae* (thiamine). Pyrimidine was for *C. clamydosporis* and *C. piceae* the part of the thiamine molecule that had to be substituted. *Chaetomium thermophile* var. *dissitum (thermo-*

philic) requires both biotin and thiamine for growth at 45°C (Tansey 1970).

A. terrestris may play some part in wood-decay within a chip pile, since the fungus can supply the wood with vitamins (growth factors) needed by other wood-destroying fungi, for example the white rot fungus *Stereum hirsutum*. This fungus is common in hardwood chips (Bergman and Nilsson 1967, 1968). Since *A. terrestris* lives in the warm parts of a chip pile, the exploitation of the possible supply of vitamins for most rot fungi can take place first after a temperature decrease in the chip pile. This usually occurs after a longer storage period (Bergman 1973) or during a decrease in the temperature of the chip pile in cold weather.

8 Production of laccase and tyrosinase

The degradation of lignin is a complicated enzymic process which is largely unknown. The structure of lignin and its degradation of microorganisms is discussed by Kirk (1971). He states that "phenol oxidases can be only a part of the enzym complex that catalyzes the complete decomposition of lignin". One of the enzymes which is believed to be involved in the degradation of lignin seems to be laccase. This enzyme appears at least to play an indirect role in the degradation of lignin (Grabbe et al., 1968).

A drop-test method has been described by Käärik (1965) in which the enzymes of the fungi such as laccase and tyrosinase can be tested. Seven of the 20 phenolic compounds described by Käärik were used in this investigation, viz: benzidine and α-naphthol (reagents on laccase), p-cresol and tyrosine (reagents on tyrosinase), gallic acid, pyrocatechol and lactophenol (reagents not specific to laccase or tyrosinase).

Allescheria terrestris was allowed to grow for three to six days at 45°C and about 15 days at 30°C. *Phialophora luteo-viridis* and *Phialophora richardsiae* were permitted to grow for 15–20 days at 30°C and 25°C respectively. The temperature was the same before and after the inoculation and during the test. The reaction can vary in intensity, depending, among other things, on the temperature at which the fungus is cultivated before and after "dropping", on the age of the mycelium, etc. A fixed temperature was therefore used.

Only α-naphthol and benzidine gave positive reactions and therefore only the results of these tests are reported. As can be seen from Table 7, α-naphthol gave a positive reaction with the three soft rot fungi tested. On the other hand, the positive reaction with benzidine failed to appear in *Allescheria terrestris* strain H63–1 and *Phialophora luteo-viridis* strain M74–IV. The incubating temperature of 45°C instead of 30°C for the two strains of *Allescheria terrestris* only has the effect of accelerating the naphthol reaction by strain H63–1. The reaction with benzidine failed to appear even at a higher temperature. Käärik (1965) also found that the cultivating temperature had a very slight effect on the phenoloxidase reactions. The results must be interpreted in the following way: polyphenol oxidases of laccase type are produced by the three soft rot fungi tested. In several investigations, chemical analyses of wood attacked by soft rot have been carried out to explain whether these fungi degrade lignin or not. Nilsson (Bergman and Nilsson, 1967) and Lundström (1973) demonstrated that lignin in birchwood is degraded or modified after an *Allescheria terrestris* attack and Lundström (1973) came to the same conclusion as regards *Phialophora richardsiae*. The analyses gave no answer as to whether *Phialophora luteo-viridis* attacks lignin in birchwood (Lundström, 1973). Nilsson (Bergman and Nilsson, 1967) reported a lignin loss of three per cent from aspen wood. The degradation of beech wood by the soft rot fungus *Chaetomium globosum* was studied by Levi and Preston (1965) and Seifert (1966). They showed that most of the decrease in lignin content in the decayed wood was due to removal of methoxyl groups from the lignin.

9 Resistance to toxic substances

Soft rot fungi probably have a greater tolerance for wood preservatives than the wood-attacking Basidiomycetes. The aim of the present tests was to determine the tolerances to five chemicals with fungicidical effects among the three soft rot fungi studied. *Stereum hirsutum* (Willd. ex Fr.) Fr. was tested to compare the concentration at which a wood-attacking Basidiomycete is inhibited. This Basidiomycete is not specially selected and there are variations among Basidiomycetes with respect to the sensitivity to wood preservatives and their components. The five chemicals tested are often included in different wood preservatives (see The Swedish Wood Preservation Committee, Report No 23 1962). They are water-soluble and have been added to medium A in the following concentrations: 0.01 %, 0.05 %, 0.1 %, 0.5 %, 1.0 %, 2.0 %, 3.0 % and 4.0 % for arsenic trioxide, boric acid, copper sulphate, zinc sulphate and 0.0001 %, 0.0005 %, 0.001 %, 0.005 %, 0.01 % for sodium pentachlorophenolate. The values reported in Table 8 are the concentrations of the chemicals at which a total inhibition of the fungi appeared.

Sodium pentachlorophenolate already gave total inhibition of the fungi at very low concentrations. Duncan (1960) also found that very low concentrations of sodium pentachlorophenolate gave very strong inhibition in the growth of soft rot fungi. During comparision with common wood-destroying Basidiomycetes, some soft rot fungi showed greater resistance to sodium pentachlorophenolate and pentachlorophenol. This was especially the case with *Chaetomium globosum* (Savory 1955, Scholles 1957). All the tested fungi were sensitive to sodium pentachlorophenolate.

The tested fungi showed lower sensitivity to copper sulphate than to sodium pentachlorophenolate. The Beyma-strain of *Phialophora luteo-viridis* was inhibited in 2 per cent copper sulphate while strain M74–IV and *P. richardsiae* were inhibited in 1 per cent. *Allescheria terrestris* was somewhat more sensitive to copper sulphate than the two other fungi tested. Duncan (1960) obtained similar responses for other soft rot fungi.

A. terrestris showed temperature dependence for arsenic trioxide and boric acid since growth was inhibited in a lower concentration of the chemicals at 30°C than at 45°C. With sodium pentachlorophenolate the situation was reversal. That a higher concentration of the chemicals was required to stop the growth of the fungus at 45°C may, perhaps, be due to the higher metabolism at this temperature, as compared with that at 30°C.

Tests in which the radial growth of the soft rot fungi on agar substrate containing different concentrations of the preservatives S25 and K33 (for the chemical composition see The Swedish Wood Preservation Committee, Report No 23 1963) was recorded showed that the soft rot fungi withstood higher concentrations (0.20 – 0.25 %) than the wood-destroying Basidiomycetes (0.01 – 0.05 %) before they were inhibited in their radial growth. *Merulius lacrymans* Wulfen ex. Fr. was inhibited at 0.15 – 0.18 per cent (Rennerfelt 1956).

In addition to chemicals, ground lichen thallus and water-soluble extracts of ground lichen thallus in malt agar inhibited the growth of some decay fungi, among others *Allescheria terrestris* (Lundström and Henningsson 1973).

The method of testing fungicidal substances with malt agar as medium is very doubtful since the fungi (for example, their enzymes) probably react differently during growth on malt agar and in wood (cf. Bier and Pentland 1964). The pH of the media is of great importance. Wessels and Adema (1968) reported activity in sodium pentachlorophenolate decreased "about 100 times going from pH 5 to pH 8". This was less pronounced for other fungicides but the

effect was still appreciable. The advantage of the test method used is that it is easy to carry out in a short time.

The higher resistance of the soft rot fungi to fungicidal substances and the problems of the fixation of these in the S_2-layer of the cell wall where the soft rot fungi ordinarily grow, make it difficult to protect the wood from soft rot. In hardwoods it is even difficult for the preservative agents to penetrate into the S_2-layer (Dickinson 1973). This may, for example, make it difficult to chemically protect hardwood chips against wood destruction by soft rot fungi.

Summary

A study was made of the general physiological aspects of three soft rot fungi commonly occurring in stored soft wood chips, namely: *Allescheria terrestris* Apinis (strain Apinis and strain H63–1),*Phialophora (Margarinomyces) luteo-viridis* (van Beyma) Schol-Schwarz (strain Beyma 206.38 and strain M74–IV) and *Phialophora richardsiae* (Nannf.) Conant (strain BB40–V). *A. terrestris* is a so-called thermophilic fungus with a temperature growth range of approximately 20°C to 55°C.

Both of the *Allescheria terrestris* strains grew on malt agar within the entire temperature range of 20°C to 50°C. If the fungus was permitted to grow out from birchwood, the maximum growth temperature increased to 55°C. The optimum temperature for radial growth of strain H63–1 was 45°C, while strain Apinis achieved optimum growth at 40°C. The production of mycelium measured as dry weight largely coincided with the respective increase or reduction in length of growth at the different temperatures. However, both strains showed optimum mycelium production at 40°C.

None of the *Allescheria terrestris* strains survived one hour at a temperature of 75°C on malt agar but survived 14 days at 55°C if the temperature was again reduced to 45°C. The two *Phialophora luteo-viridis* strains grew on malt agar at temperatures between 5°C and 35°C, with an optimum growth for both strains at 30°C. No increase in the maximum temperature for growth was achieved by permitting the mycelium to grow out from birchwood. The fungi did not survive storage at 70°C for an hour on malt agar but, on the other hand, survived 40°C for 14 days if the cultivation temperature was again reduced to 30°C. *Phialophora richardsiae* grew on malt agar between 5°C and 30°C. The maximum growth temperature was not raised if the mycelium was permitted to grow out from the birchwood. The fungi did not survive storage for one hour on malt agar at 60°C but did survive seven days at a temperature of 35°C if the cultivation temperature was again reduced to the optimum growth temperature of 25°C.

Allescheria terrestris grew within the entire pH range tested, 2.4 to 7.3 at 30°C and 45°C. An initial pH of between 5 and 6 gave the greatest mycelium production.

Both strains of *Allescheria terrestris* have been well able to utilize nitrogen from both inorganic and organic nitrogen sources; even nitrate nitrogen which is difficult of access for certain groups of fungi.

All of the carbohydrate sources employed were well utilized by *Allescheria terrestris* with the exception of D-arabinose. D-arabinose has earlier been reported to afford difficult access for certain fungi.

The tests employed to determine the vitamin requirements showed that *Allescheria terrestris* was auxoautotrophic for thiamine and biotin.

All of the three soft rot fungi studied displayed a positive reaction to α-naphthol as registered by means of a drop-test. It is therefore presumed that the fungi produce polyphenol oxidase of the laccase type.

The soft rot fungi studied demonstrated great resistance to arsenic trioxide, copper sulphate, sodium pentachlorophenolate and zinc sulphate, and an even greater resistance to boric acid – all of these being chemicals which are frequently incorporated in preservatives.

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Sammanfattning

De allmänfysiologiska kraven hos tre speciellt i lagrad lövvedflis ofta förekommande soft rot svampar (mögelrötesvampar) har studerats, nämligen *Allescheria terrestris* Apinis (stam Apinis och stam H63–1), *Phialophora (Margarinomyces) luteo-viridis* (van Beyma) Schol-Schwarz (stam Beyma 206.38 och stam M74–IV) och *Phialophora richardsiae* (Nannf.) Conant (stam BB40–V). *A. terrestris* är en s k termofil svamp med tillväxt inom temperaturintervallet ca 20°C till 55°C.

De bägge *Allescheria terrestris* stammarna har tillvuxit inom hela temperaturintervallet 20°C till 50°C på maltagar. Om svampen fick tillväxa från björkved höjdes maximumtemperaturen för tillväxt till 55°C. Vid 45°C hade stam H63–1 sin optimaltemperatur för längdtillväxt medan stam Apinis hade sin vid 40°C. Mycelproduktionen mätt som torrvikt sammanföll i stort med den ökade respektive minskade längdtillväxten vid de olika temperaturerna. Dock gav båda stammarna optimal mycelproduktion vid 40°C. De båda stammarna av *Allescheria terrestris* överlevde inte en timme vid 75°C på maltagar, men 14 dagar vid 55°C om temperaturen åter sänktes till 45°C. De båda *Phialophora luteoviridis* stammarna tillväxte mellan 5°C och 35°C på maltagar med tillväxtoptimum för bägge stammarna vid 30°C. Någon höjning av maximumtemperaturen för tillväxt genom att låta mycelet växa ut från björkved åstadkoms ej. Svampen överlevde ej förvaring vid 70°C över en timme på maltagar men däremot 40°C under 14 dagar, om odlingstemperaturen åter sänktes till 30°C. *Phialophora richardsiae* tillväxte mellan 5°C och 30°C på maltagar. Maximumtemperaturen för tillväxten höjdes inte om mycelet fick växa ut från björkved. Svampen överlevde inte förvaring vid 60°C under 1 timme på maltagar, men 7 dagar vid 35°C om odlingstemperaturen åter sänktes till den tillväxtoptimala 25°C.

Allescheria terrestris tillväxte inom hela det prövade pH-området 2.4 till 7.3 vid 30°C och 45°C. Start pH mellan 5 och 6 gav den största mycelproduktionen.

Bägge stammarna av *Allescheria terrestris* har väl kunnat nyttja kvävet både från oorganiska och organiska kvävekällor; alltså även det för vissa svampgrupper mer svårtillgängliga nitratkvävet.

Av de kolkällor som användes utnyttjades samtliga väl av *Allescheria terrestris* utom D-arabinos. D-arabinos är tidigare rapporterad som rätt svårtillgänglig för vissa svampar.

Allescheria terrestris var auxoautotrofisk för thiamin och biotin.

α-naftol gav positiv reaktion genom dropptest hos alla de tre testade soft rot svamparna. Därmed antages att svamparna producerar polyfenoloxidas av laccas-typ.

De undersökta soft rot svamparna visade stor resistens mot arseniktrioxid, kopparsulfat, natriumpentaklorfenolat och zinksulfat; ännu större mot borsyra, kemikalier som ofta ingår i impregeringsmedel.

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Tables

Table 1. Radial growth on medium A of two strains of *Allescheria terrestris* after storing at 55°C to 80°C. *A. terrestris* was allowed to grow about 4–5 mm at 45°C before it was placed in the super-maximal temperatures.

Strain	Storing time at 55°C to 80°C	Radial growth in mm/week at 45°C after storing at:					
		55°C	60°C	65°C	70°C	75°C	80°C
Apinis	14 days	u	–	–	–	–	–
	7	u	0	0	0	0	0
	3	u	0	0	0	0	0
	2	u	31	0	0	0	0
	1	u	33	0	0	0	0
	8 hours	–	34	29	0	0	0
	4	–	44	37	0	0	0
	2	–	u	41	0	0	0
	1	–	u	48	26	0	0
H63-1	14 days	u	–	–	–	–	–
	7	u	0	0	0	0	0
	3	u	0	0	0	0	0
	2	u	0	0	0	0	0
	1	u	22	0	0	0	0
	8 hours	–	25	0	0	0	0
	4	–	41	33	0	0	0
	2	–	44	39	0	0	0
	1	–	48	u	27	0	0

0 no radial growth
u mycelium grown over the whole petri dish >50 mm
– not tested

Table 2. Radial growth on medium A of two strains of *Phialophora luteo-viridis* and *Phialophora richardsiae* after storing at 35°C to 70°C. The fungi were allowed to grow about 4–5 mm at 25°C[a] and 30°C[b] before they were placed at the super-maximal temperatures.

Fungus	Strain	Storage time at 35°C to 70°C	Radial growth in mm/week at 25°C[a] and 30°C[b] after storing at:							
			35°C	40°C	45°C	50°C	55°C	60°C	65°C	70°C
Phialophora luteo-viridis	Beyma	14 days	–	10	0	0	–	–	–	–
		7	–	11	7	6	0	–	–	–
		3	–	13	7	7	0	–	–	–
		2	–	14	9	8	1	–	–	–
		1	–	14	10	10	5	0	0	–
		8 hours	–	14	12	12	7	0	0	–
		4	–	15	14	13	9	6	0	0
		2	–	16	14	14	13	10	5	0
		1	–	17	15	14	14	15	12	12
	M74–IV	14 days	–	8	0	–	–	–	–	–
		7	–	10	0	0	0	–	–	–
		3	–	11	2	0	0	–	–	–
		2	–	11	5	0	0	–	–	–
		1	–	11	9	7	0	–	–	–
		8 hours	–	11	11	11	9	0	0	–
		4	–	12	11	11	10	5	0	0
		2	–	11	12	12	11	8	0	0
		1	–	13	13	13	11	10	7	10
Phialophora richardsiae	BB40–V	14 days	0	–	–	–	–	–	–	–
		7	8	0	0	0	–	–	–	–
		3	10	0	0	0	–	–	–	–
		2	10	0	0	0	–	–	–	–
		1	10	0	0	0	–	–	–	–
		8 hours	10	5	0	0	–	–	–	–
		4	11	7	6	0	0	–	–	–
		2	12	9	11	5	0	0	–	–
		1	13	13	14	10	5	0	–	–

0 no radial growth
– not tested
a) 25°C *Phialophora richardsiae*
b) 30°C *Phialophora luteo-viridis*

Table 3. Radial growth of *Allescheria terrestris* on medium A at 55°C. The fungus grew from blocks of birch *(Betula verrucosa* Ehrh.). In the control, the fungus grew from inoculated pieces of medium A.

Strain	Section of trunk (about 20 cm thick)	Radial growth mm after:		
		1 week	2 weeks	3 weeks
Apinis	Sapwood	4	5	9
H63–1	Sapwood	4	8	9
Apinis	Central wood	3	5	6
H63–1	Central wood	3	6	7
Apinis	Control	2[a)]	2	2
H63–1	Control	3[a)]	3	3

a) Radial growth before transfer to 55°C

Table 4. Dry weight production of *Allescheria terrestris* strain Apinis and H63–1 grown as a floating culture at 45°C on medium C containing eight different carbohydrates.

Carbohydrate	Conc. g/l	Initial pH	Dry weight and final pH after 3, 5, 7 and 10 days															
			3 days				5 days				7 days				10 days			
			Apinis		H63–1		Apinis		H63–1		Apinis		H63–1		Apinis		H63–1	
			mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH
D-arabinose	20	5.1	19.7	6.0	12.6	5.5	31.1	6.6	19.3	6.3	34.7	6.5	31.5	6.5	41.2	6.3	43.0	6.1
	10	5.1	18.2	6.4	12.8	5.8	20.1	6.7	17.7	6.6	24.9	6.6	23.3	6.6	27.9	6.5	32.2	6.4
	2.5	5.1	11.4	6.3	9.2	6.1	14.6	6.7	13.2	6.7	17.5	6.7	13.8	6.7	18.1	6.7	16.0	6.7
L-arabinose	20	5.2	73.8	4.5	73.1	4.0	112.9	5.7	82.4	4.0	135.6	5.6	66.1	5.2	142.1	5.4	56.5	6.4
	10	5.2	60.3	4.5	64.4	4.1	85.2	5.7	49.7	5.8	79.5	5.8	45.3	6.6	65.8	6.0	44.2	6.5
	2.5	5.2	32.6	5.9	30.5	5.8	28.0	6.5	26.7	6.6	26.7	6.6	25.4	6.8	25.5	6.5	24.2	6.6
D-xylose	20	5.1	83.0	4.4	90.8	4.1	147.3	5.0	132.3	4.4	145.9	5.7	155.2	4.9	133.0	5.8	123.2	6.1
	10	5.1	78.1	4.5	85.7	4.3	82.3	5.7	77.5	6.1	67.0	6.3	81.1	6.2	55.8	6.4	60.7	6.4
	2.5	5.1	31.8	6.5	31.4	6.4	31.2	6.5	30.5	6.6	29.1	6.6	28.1	6.6	24.3	6.6	24.4	6.6
D-glucose	20	5.2	88.0	4.2	73.1	4.4	98.5	4.5	126.4	4.4	115.9	4.8	160.1	4.6	131.1	5.6	128.1	6.0
	10	5.2	61.2	4.3	53.9	4.5	80.9	5.5	75.9	4.8	67.1	6.1	68.0	6.2	57.9	6.5	63.4	6.5
	2.5	5.2	27.5	5.4	30.3	6.2	27.3	6.6	27.0	6.7	30.5	6.6	27.0	6.7	32.4	6.7	25.2	6.7
D-galactose	20	5.3	43.0	4.5	58.1	4.5	117.5	4.5	127.6	4.2	140.3	4.6[a]	157.4	4.4[a]	152.7	5.5	113.9	5.3
	10	5.3	30.3	4.6	27.7	4.9	65.5	4.6	74.3	4.7	75.9	5.7[a]	87.1	5.7[a]	67.7	6.2	65.0	6.5
	2.5	5.3	19.6	5.0	18.0	5.1	28.7	6.5	28.5	6.5	30.0	6.5[a]	27.1	6.5[a]	25.7	6.7	26.4	6.7
D-mannose	20	5.0	84.2	4.3[b]	89.5	4.2	132.3	4.6	121.3	4.2	115.2	4.7	132.6	4.2	124.2	5.6	115.1	5.9
	10	5.0	75.6	4.4	78.0	4.3	65.9	5.8	80.5	4.8	71.7	6.1	73.2	6.2	58.8	6.4	66.6	6.5
	2.5	5.0	33.9	6.3	32.3	6.3	30.5	6.6	27.5	6.7	30.3	6.1	28.7	6.7	29.3	6.7	21.9	6.7
D-cellobiose	20	5.1	87.4	4.2	95.4	4.1	132.0	4.4	138.1	4.1	151.0	4.7	153.5	4.2	137.0	5.4	149.3	5.0
	10	5.1	47.4	4.3	65.5	4.4	84.7	5.9	80.8	4.5	68.0	6.3	79.5	6.2	56.8	6.4	74.0	6.3
	2.5	5.1	33.1	6.1	30.0	5.7	27.7	6.6	26.9	6.6	24.8	6.7	27.6	6.7	29.7	6.6	25.2	6.7
Starch	20	5.1	25.9	5.1	20.2	5.1	79.2	5.4	51.8	5.9	73.8	5.8	72.9	5.9	65.4	6.1	68.9	6.0
	10	5.1	32.7	5.3	22.7	5.9	41.4	6.2	36.6	6.0	35.0	6.4	35.4	6.2	34.3	6.4	34.1	6.4
	2.5	5.1	17.0	6.1	11.9	6.1	18.1	6.6	18.1	6.6	19.3	6.7	18.6	6.6	21.4	6.6	18.4	6.6
None		5.1	10.3	6.3	7.5	5.9	11.7	6.7	10.1	6.6	17.8	6.8	14.1	6.8	15.2	6.8	13.1	6.8

a) Initial pH 5.1
b) Initial pH 5.2

Table 5. Dry weight production of *Allescheria terrestris* strain Apinis and H63–1 grown as a floating culture at 30°C on medium C containing four different carbohydrates.

Carbohydrate	Conc. g/l	Initial pH	Dry weight and final pH after 3, 5, 7 and 10 days														
			3 days				5 days				7 days				10 days			
			Apinis		H63–1		Apinis		H63–1		Apinis		H63–1		Apinis		H63–1	
			mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH
D-glucose	20	5.1	24.0	4.9	25.1	4.9	58.4	4.2[b]	52.5	4.2[b]	95.7	4.2[b]	93.2	4.1[b]	147.1	4.3[b]	146.7	4.1[b]
	10	5.1	16.6	5.0	16.1	5.0	52.9	4.2[b]	55.3	4.2[b]	74.5	4.3[b]	77.6	4.3[b]	83.5	4.6[b]	80.2	4.4[b]
	2.5	5.1	14.6	5.0	13.1	5.0	24.9	4.7[b]	25.0	4.6[b]	23.0	5.0[b]	23.4	4.7[b]	25.2	5.6[b]	21.1	5.2[b]
D-galactose	20	5.3	13.1	5.3	13.4	5.3	23.7	5.0	28.0	4.9	47.9	4.3[a]	97.3	4.1[a]	93.6	4.5	150.2	4.2
	10	5.3	10.8	5.3	11.2	5.3	17.4	5.1	33.8	4.6	37.3	4.4[a]	44.5	4.3[a]	65.2	4.4	82.7	4.4
	2.5	5.3	6.1	5.3	7.8	5.3	16.5	5.2	18.1	5.0	22.8	4.7[a]	25.0	4.6[a]	25.3	5.7	27.6	5.8
D-mannose	20	5.0	17.8	4.9	20.5	4.9	55.8[b]	4.3	59.6[b]	4.0	94.9	4.1	110.3	4.0	150.0	4.2	154.7	4.1
	10	5.0	15.5	5.0	15.6	4.9	48.0[b]	4.2	50.5[b]	4.0	88.3	4.3	92.7	4.2	80.6	4.6	82.7	4.4
	2.5	5.0	13.9	4.9	11.8	5.0	27.0[b]	4.5	28.7[b]	4.5	29.5	4.8	26.4	4.8	26.9	6.0	26.1	5.3
D-cellobiose	20	5.1	23.1	4.9	21.8	4.8	53.0	4.2	40.6	4.4	88.2	4.1	88.8	4.0	124.9	4.2	133.3	4.0
	10	5.1	26.3	4.8	19.7	4.8	40.5	4.3	39.4	4.3	72.7	4.2	86.4	4.1	87.7	4.4	97.2	4.2
	2.5	5.1	14.5	4.9	12.3	5.0	21.7	4.7	23.9	4.6	24.7	4.7	28.3	4.7	24.1	5.6	26.4	5.1
None		5.1	7.5	5.3	5.1	5.3	7.6	5.3	4.6	5.3	10.8	5.4	5.8	5.3	7.5	5.6	3.6	5.3

a) Initial pH 5.1
b) Initial pH 5.2

Table 6. Mycelial dry weight (mg) of *Allescheria terrestris* grown as a floating culture on medium C_1 with and without thiamine and vitamin mixture (see page 15). Initial pH 5.4.

Strain	Incubation time/days	Incubation temperature °C	Thiamine/μg per litre										Vitamin mixture/ml per litre			
			0	Final pH	1	Final pH	10	Final pH	100	Final pH	200	Final pH	0	Final pH	1	Final pH
Apinis	3	45	25.1	4.7	28.3	4.7	24.1	4.7	25.0	4.7	24.6	4.6	–	–	–	–
	7	45	45.4	4.7	47.7	4.6	39.6	4.7	41.3	4.6	50.1	4.7	55.8	4.5	54.3	4.5
	7	35	36.6	4.4	34.8	4.5	38.3	4.4	32.4	4.6	35.0	4.5	–	–	–	–
H63–1	3	45	29.1	4.4	37.5	4.2	30.7	4.4	33.3	4.4	26.4	4.6	–	–	–	–
	7	45	60.2	4.1	54.0	4.3	53.2	4.3	52.8	4.2	55.8	4.2	68.1	4.0	68.5	4.0
	7	35	38.5	4.3	46.4	4.2	40.0	4.3	50.1	4.2	55.3	4.2	–	–	–	–

– not tested

Table 7. Reagent to benzidine and α-naphthol in drop-test for *Allescheria terrestris, Phialophora luteo-viridis* and *Phialophora richardsiae.*

Fungus	Reaction time/days															
	Benzidine								α-naphthol							
	30°C[a]				45°C[a]				30°C[a]				45°C[a]			
	1	2	3	7	1	2	3	7	1	2	3	7	1	2	3	7
Allescheria terrestris strain Apinis	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
Allescheria terrestris strain H63–1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1
Phialophora luteo-viridis strain Beyma	2	2	2	–	–	–	–	–	2	2	2	–	–	–	–	–
Phialophora luteo-viridis strain M74–IV	0	0	0	0	–	–	–	–	2	2	2	–	–	–	–	–
Phialophora richardsiae strain BB40–V	1[b]	1[b]	1[b]	1[b]	–	–	–	–	1[b]	1[b]	1[b]	1[b]	–	–	–	–

0 no reaction
1 weak reaction
2 medium reaction
3 strong reaction
– not tested
a incubation temperature
b incubation temperature 25°C

Table 8. Concentrations of chemical compounds which inhibited the growth on malt agar of three soft rot fungi and one white rot fungus *Stereum hirsutum* (Willd. ex Fr.) Fr.

Fungus	Strain	Incubation temp. °C	% in malt agar which inhibited the growth for 25 days				
			Arsenic trioxide As_2O_3 pH ≈4.5 – 5	Boric acid H_3BO_3 pH ≈5	Copper sulphate $CuSO_4 (H_2O)_5$ pH ≈4	Zinc sulphate $ZnSO_4 (H_2O)_7$ pH ≈4.5 – 5	Sodium pentachlorophenolate (commercial) pH ≈5 – 5.5
Allescheria terrestris	Apinis	30	<0.1[a]	3	0.1	0.5	0.001
		45	0.5	>4	0.1	0.5	0.0005
Allescheria terrestris	H63–1	30	<0.1[a]	3	0.1	0.5	0.001
		45	0.5	>4	0.1	0.5	0.0005
Phialophora luteo-viridis	Beyma	30	1.0	2	2.0	0.5	0.01
Phialophora luteo-viridis	M74–IV	30	0.5	2	1.0	1.0	0.001
Phialophora richardsiae	BB40–V	25	0.5	2	1.0	1.0	0.01
Stereum hirsutum	A–255	25	<0.1[a]	2	0.5	0.5	<0.005[a]

a) The lowest concentrations tested.

Figures

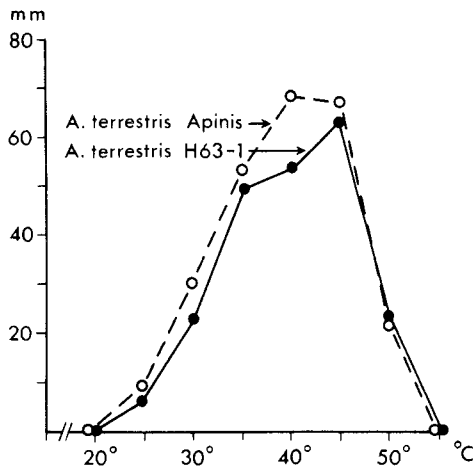


Figure 1.
Radial growth of two strains of *Allescheria terrestris* on medium A after seven days culturing at different temperatures.

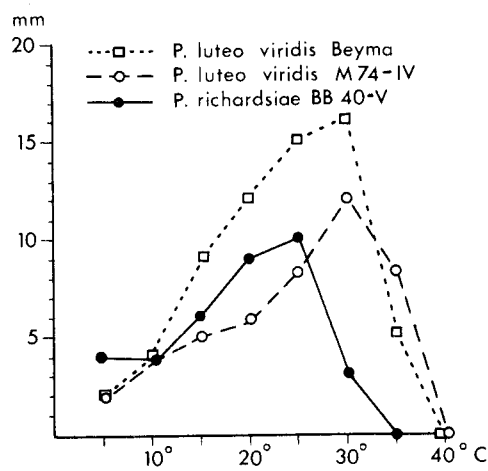


Figure 2.
Radial growth of two strains of *Phialophora luteo-viridis* and *Phialophora richardsiae* on medium A after seven days culturing at different temperatures.

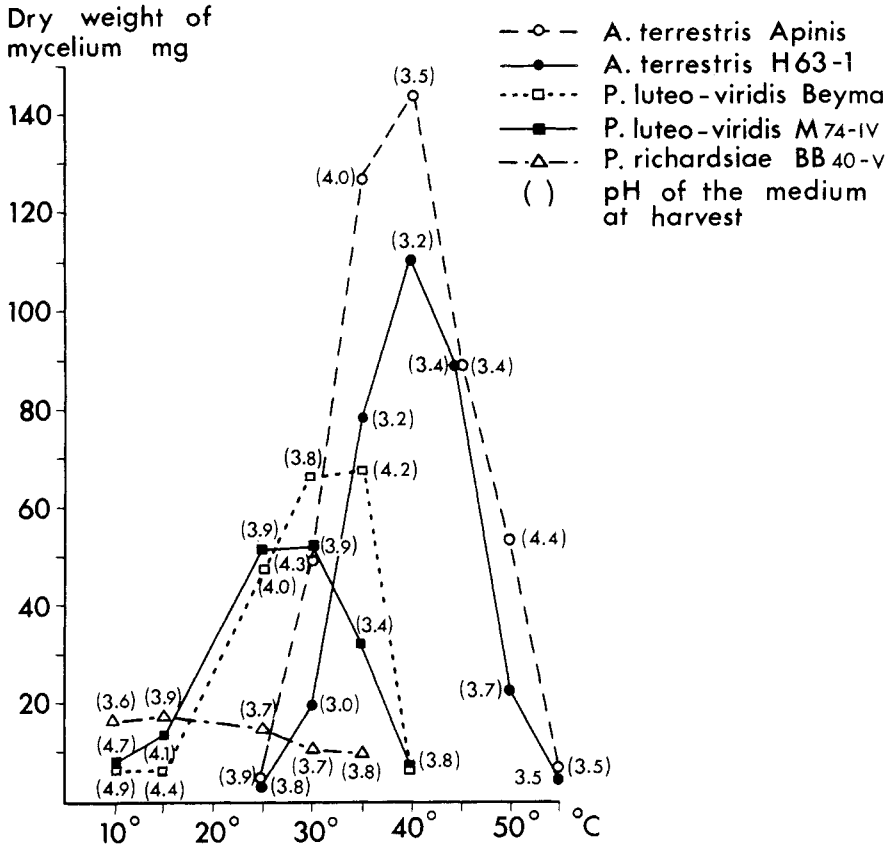


Figure 3.
Dry weight production of two different strains of *Allescheria terrestris* and *Phialophora luteo-viridis* cultured for seven days as floating cultures on medium B at different temperatures and one strain of *Phialophora richardsiae* grown under the same conditions. The initial pH of medium B was 4.6–4.7.

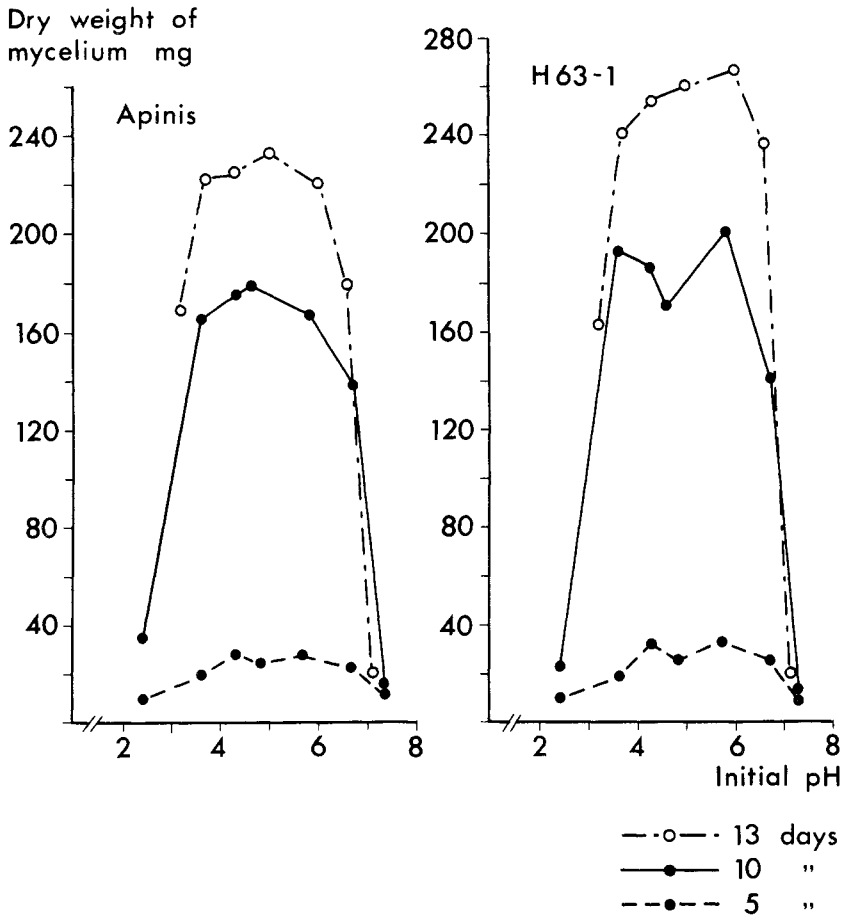


Figure 4 a.
Dry weight production of *Allescheria terrestris* strain Apinis and strain H63–1 grown as a floating culture on buffered medium D after 5, 10 and 13 days at 30°C.

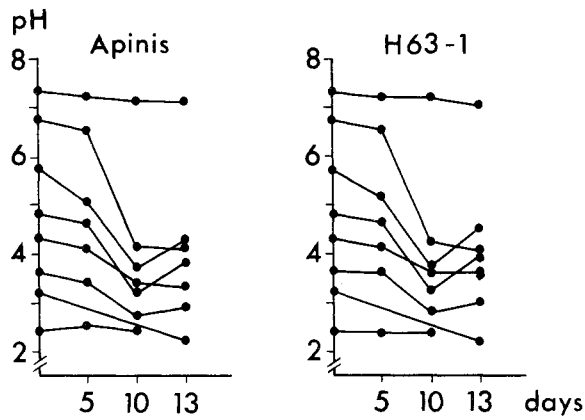


Figure 4 b.
Changes in the pH of the nutrient solutions during the growth of the mycelium.

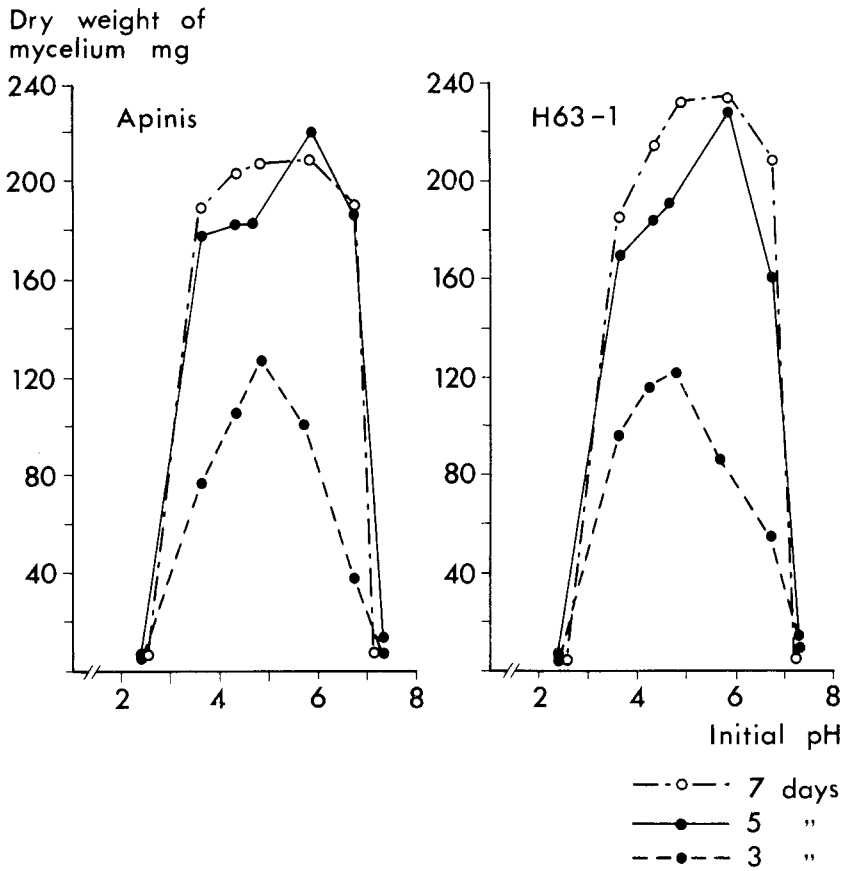


Figure 5 a.
Dry weight production of *Allescheria terrestris* strain Apinis and strain H63–1 grown as a floating culture on buffered medium D after 3, 5 and 7 days at 45°C.

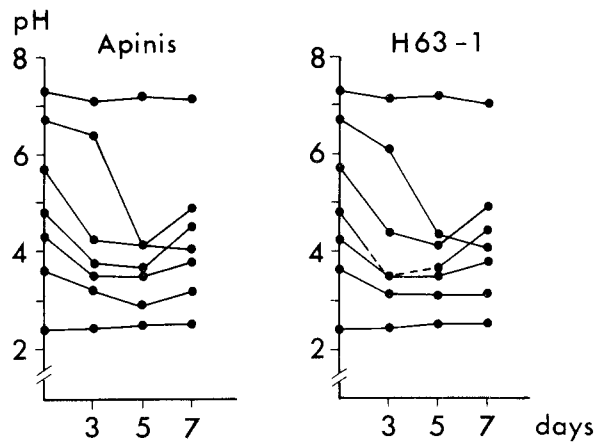


Figure 5 b.
Changes in the pH of the nutrient solutions during the growth of the mycelium.

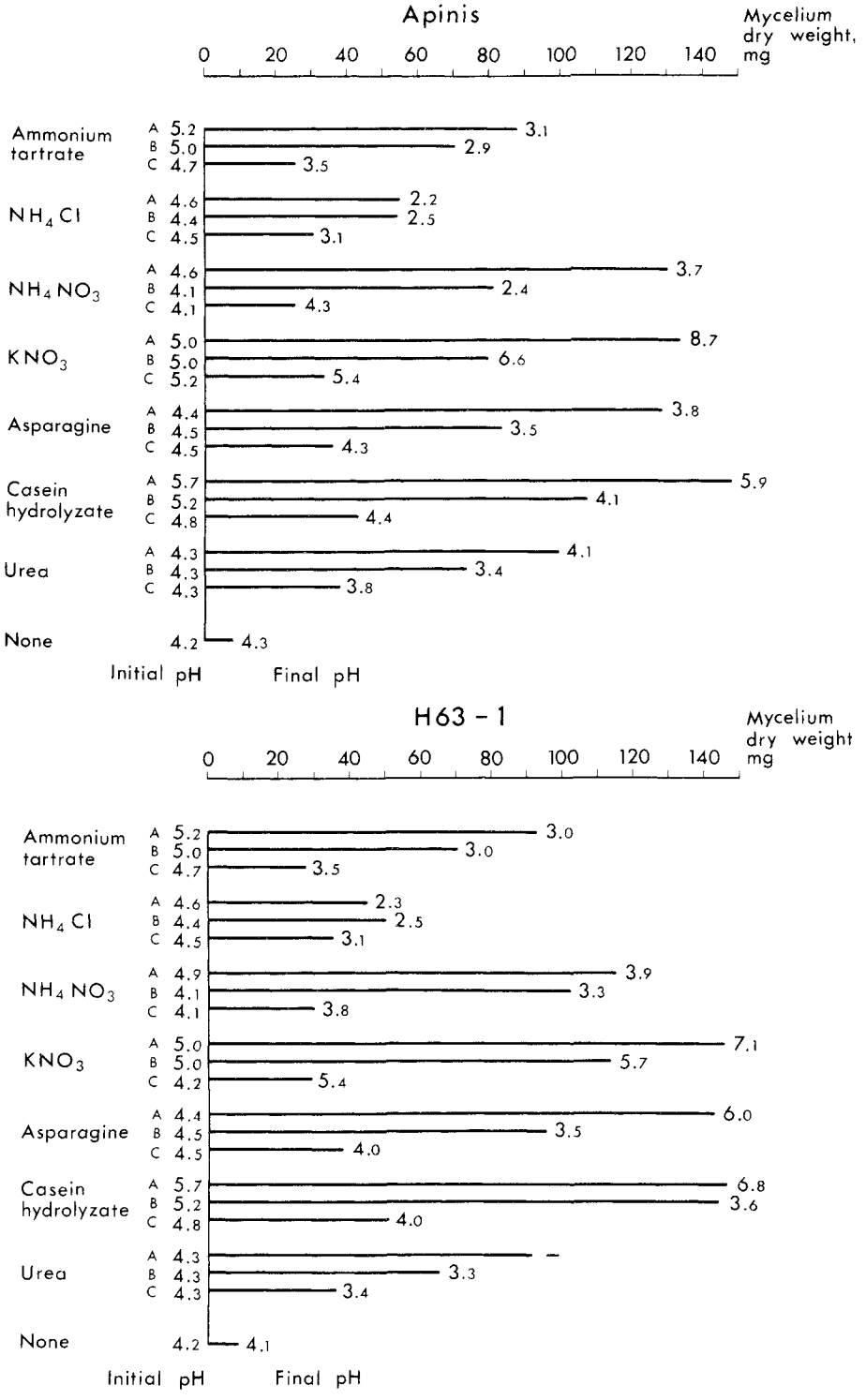


Figure 6.
Dry weight production of *Allescheria terrestris* strain Apinis and strain H63–1 grown as a floating culture on medium C at 45°C for seven days with varying nitrogen sources in three concentrations: 5N (A), N(B) and N/5 (C). N–concentration corresponding to 0.5 g ammonium tartrate per 1000 ml of solution.

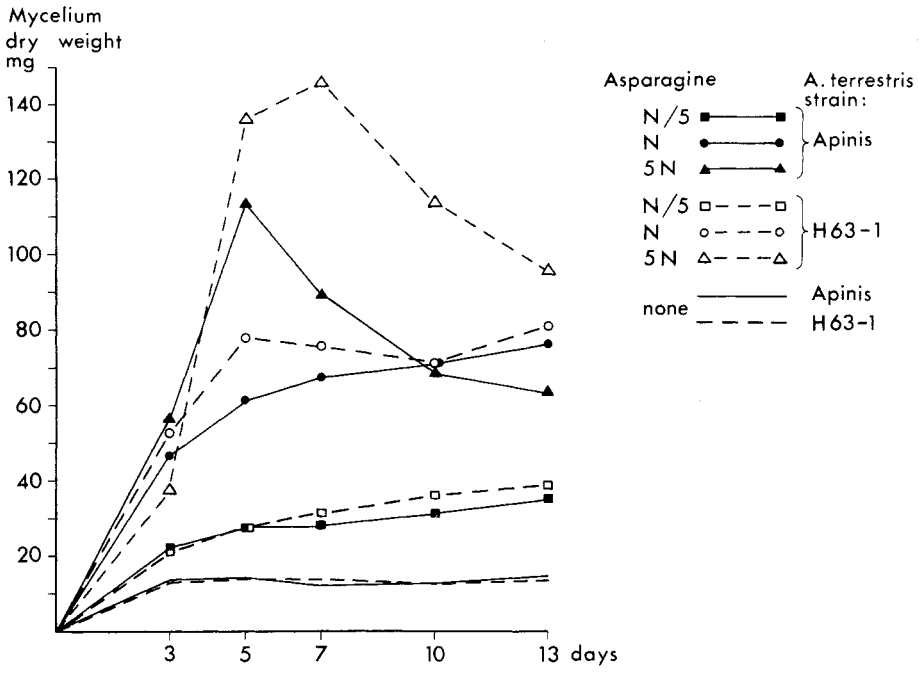


Figure 7 a.
Dry weight production of *Allescheria terrestris* strain Apinis and H63-1 grown for 13 days on medium C containing asparagine in three different concentrations at 45°C.

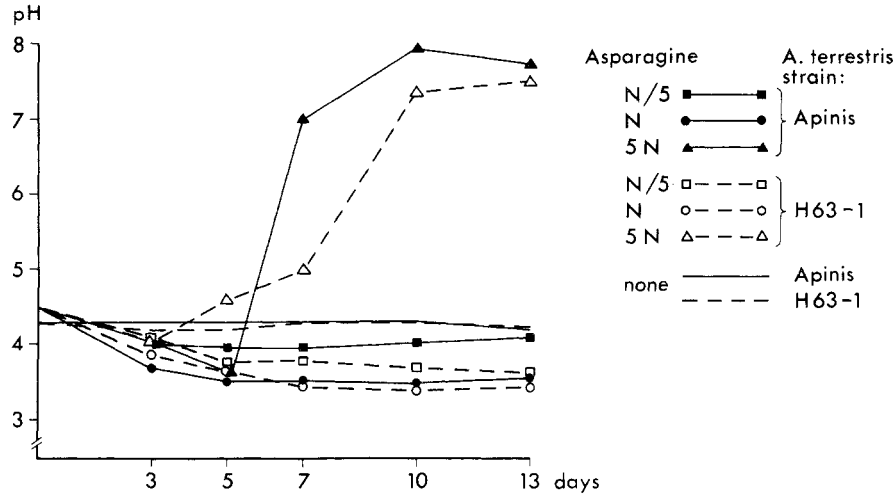
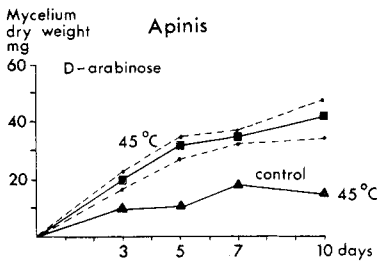
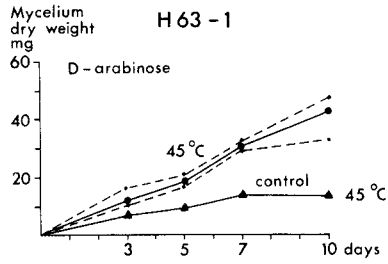
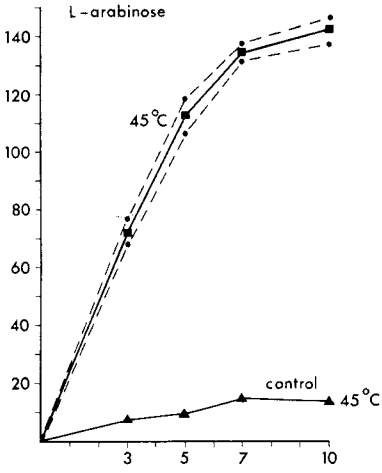


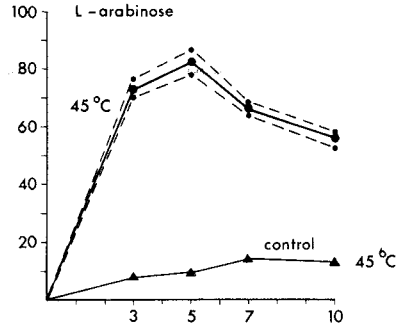
Figure 7 b.
Changes in the pH of medium C with three different concentrations of asparagine during the growth of *Allescheria terrestris.*

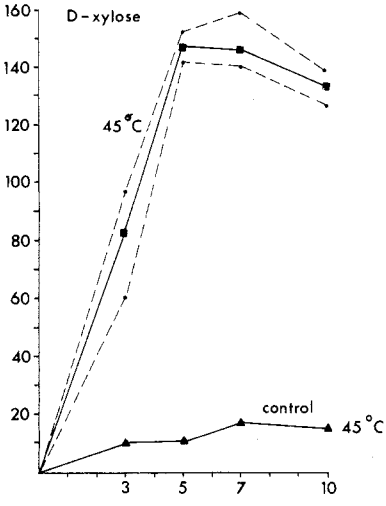
Figur 8 a

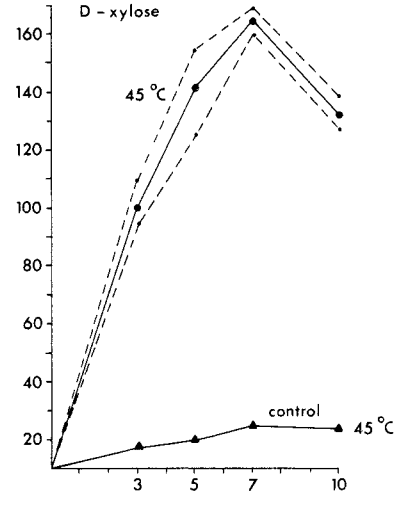

Mycelium
dry weight
mg
Apinis
D-arabinose
60
40
20
45 °C
control
45 °C
3
5
7
10 days


Mycelium
dry weight
mg
H 63 - 1
D-arabinose
60
40
20
45 °C
control
45 °C
3
5
7
10 days

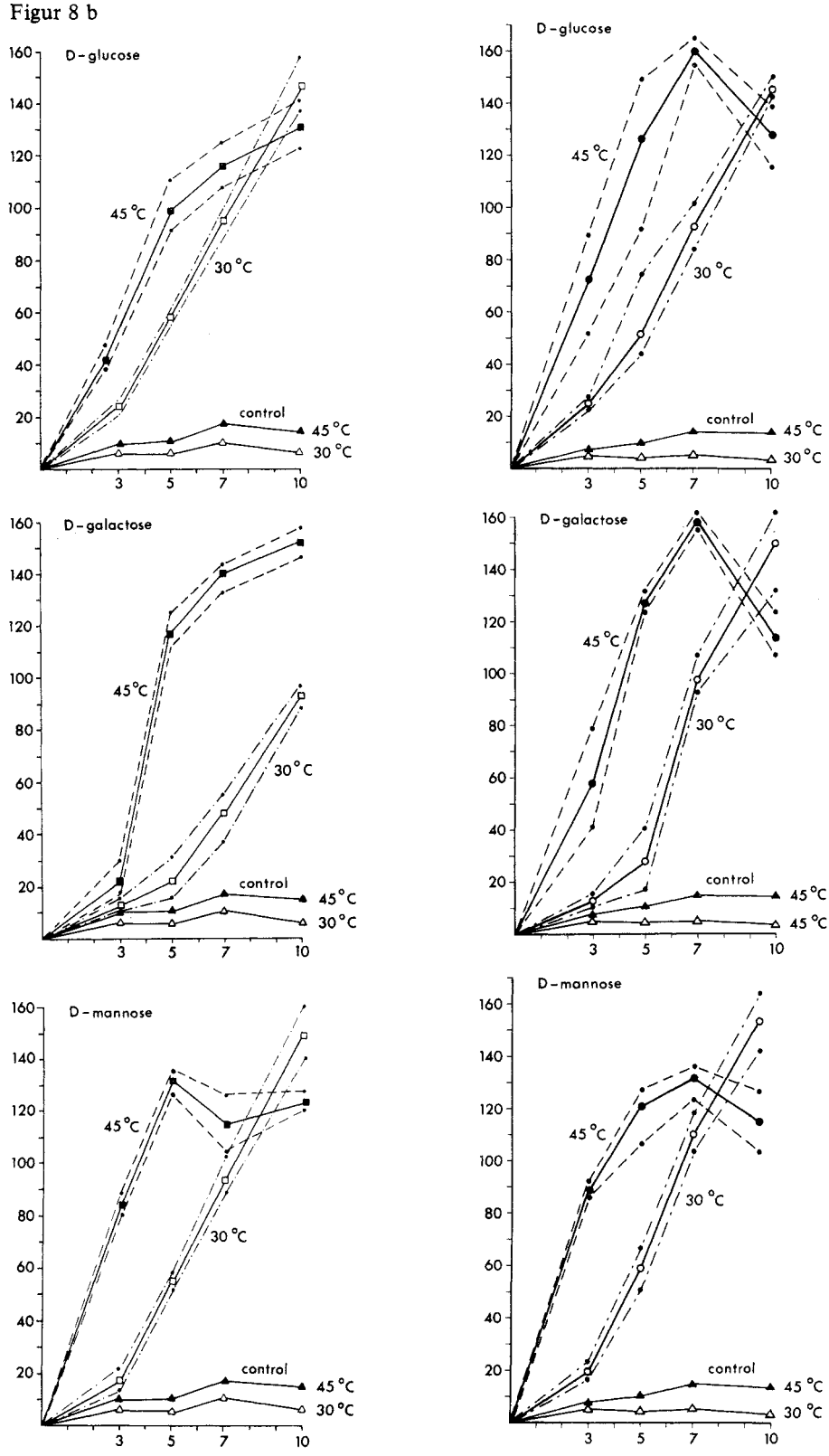

L-arabinose
140
120
100
80
60
40
20
45 °C
control
45 °C
3
5
7
10


L-arabinose
100
80
60
40
20
45 °C
control
45 °C
3
5
7
10


D-xylose
160
140
120
100
80
60
40
20
45 °C
control
45 °C
3
5
7
10


D-xylose
160
140
120
100
80
60
40
20
45 °C
control
45 °C
3
5
7
10

Figur 8 b


D-glucose
45 °C
30 °C
control
45 °C
30 °C
D-glucose
45 °C
30 °C
control
45 °C
30 °C
D-galactose
45 °C
30 °C
control
45 °C
30 °C
D-galactose
45 °C
30 °C
control
45 °C
45 °C
D-mannose
45 °C
30 °C
control
45 °C
30 °C
D-mannose
45 °C
30 °C
control
45 °C
30 °C
160
140
120
100
80
60
40
20
3
5
7
10

Figur 8 c

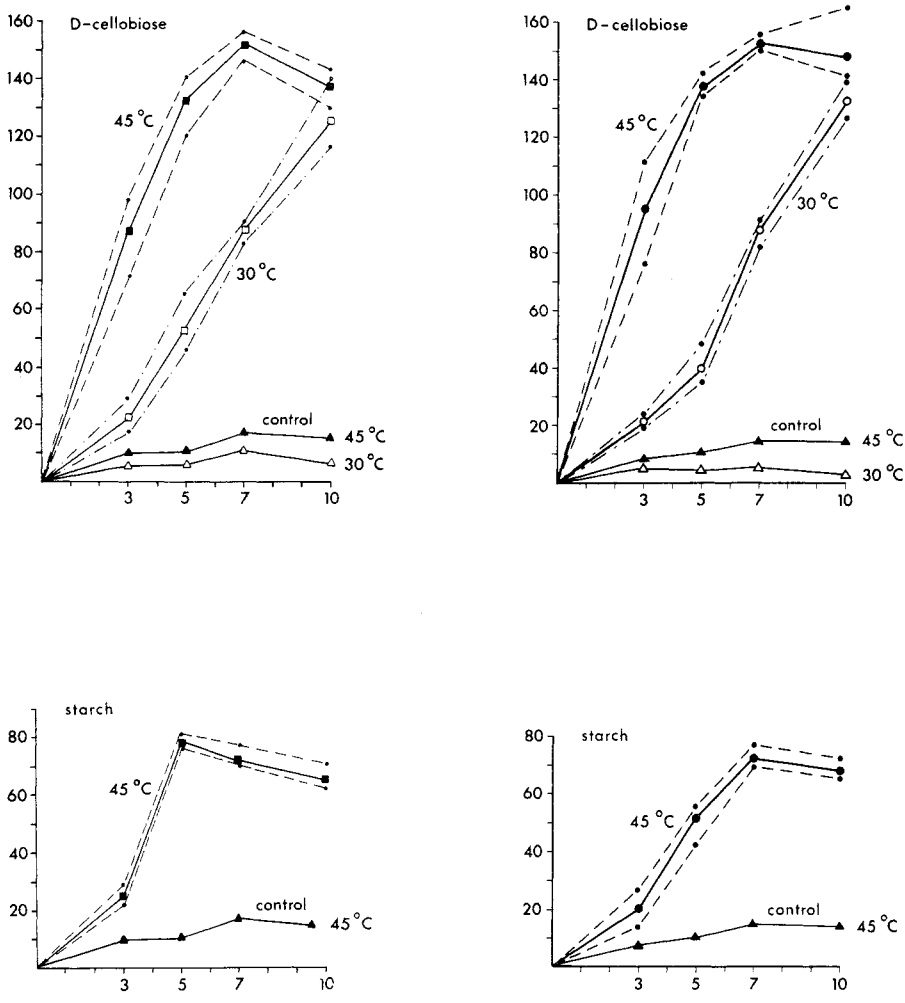


Figure 8 a – 8 c.
Dry weight production of *Allescheria terrestris* strain Apinis and strain H63–1 grown on medium C containing eight carbohydrates (20 g/l) at 30 °C and 45 °C during ten days. Broken lines represent the highest and lowest mycelium dry weight.

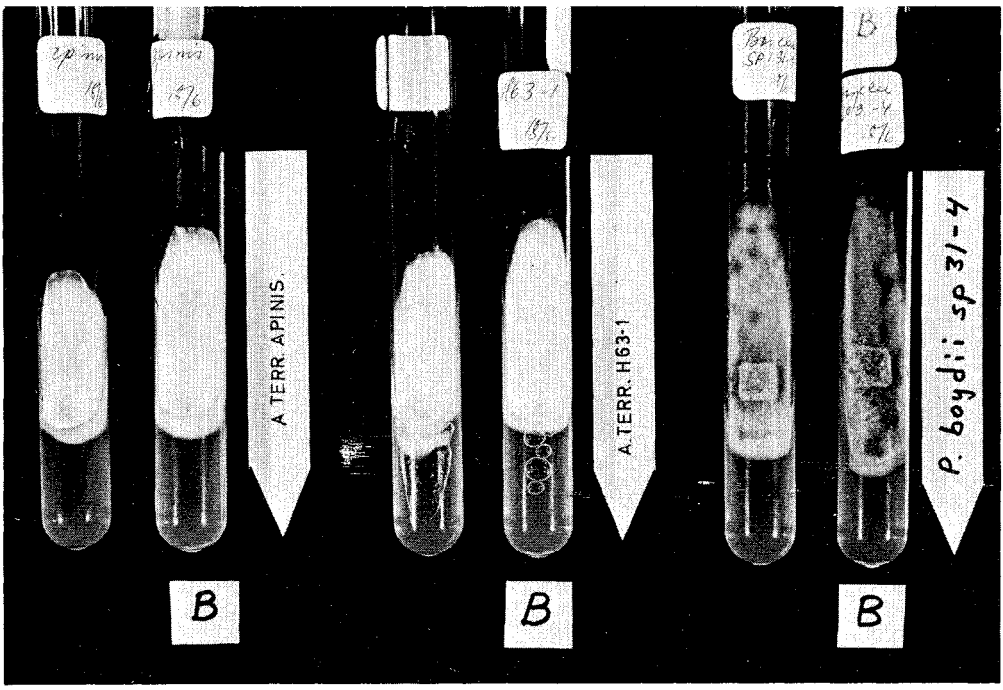


Figure 9.
Allescheria terrestris strain Apinis and strain H63–1 at 45°C and strain H63–1 at 45°C and *Petriellidium boydii* strain SP31–4 at 25°C growing on agar medium in inclined test tubes with (B) and without biotin.