

# Conclusions on Myxomycetes Compiled over Twenty-Five Years from 4793 Moist Chamber Cultures

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## Abstract

Materials for 4793 moist chamber cultures were collected from three continents, Europe, Africa and North America, and cultivated identically in petri dishes. The main results compiled from the work of the two authors over the past 25 years are as follows:

**Number of specimens:** The percentage of cultures yielding identified specimens of myxomycetes was highest, at 81%, in the Mediterranean zone, in Turkey. In tropical Africa, it was also high; 71% in Gambia and 64% in Tanzania. In the boreal zone of Europe, the percentage was only 30%, and the lowest figure, 25.5 %, corresponded with material collected from temperate to oroboreal zones in Oregon, USA.

**The species:** A total of 1678 specimens of myxomycetes belonging to 117 species were developed.

**Species richness:** Differences in species richness were estimated by counting how many species emerged on average in one moist chamber. For Turkey, Gambia and Tanzania, the figure was almost identical, 0.2; for Oregon, it was much smaller, 0.05; and for the European boreal zone, it was only 0.01.

**Substrate preferences:** Strictly corticolous species were *Paradiacheopsis fimbrata*, *P. solitaria* and *Macbrideola cornea*. *Didymium difforme* and *D. dubium* were found only on deciduous tree leaves, herbaceous material, or dung.

*Echinostelium minutum*, *Arcyria cinerea* and *A. pomiformis* seemed to be indifferent. Most of the specimens (about 75%) growing on litter or herbaceous material belonged to the order Physarales. Bark seemed to be favourable for orders Stemonitales, Trichiales, Echinosteliales, and Liceales. Many Stemonitales, Liceales and Trichiales, however, grew also on decaying wood. No representatives of Ceratiomyxales appeared in our moist chambers.

**pH of the substrates:** The pH range of the substrates on which myxomycetes were harvested was very broad (pH 2–9). The Stemonitales seemed to be most tolerant in both directions. In general, members of Physarales favoured the most basic conditions.

**About phenology:** The material collected periodically every second week indicates that myxomycetous spores have no internal rhythm which would allow them to adjust their germination period to a certain time of the year.

**Longevity of dormant phases:** The lengths of incubation time needed for germination and drying and rewetting of the moist chambers reflect a very uneven pattern of germination among the species of myxomycetes. The rewetting of ten-year-old preserved moist chambers reveals the longevity of the dormant phases.

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## Introduction

Beginning in 1974, we have conducted occasional research at the University of Helsinki on myxomycetes grown in moist chamber cultures. Some of the research has had an ecological approach, but some has merely been carried out for floristic purposes. However, the methods have been more or less consistent throughout, making it possible to draw general conclusions from the combined materials.

## Material and Methods

The material for the moist chambers originates from three continents: Europe (Finland and Norway, boreal zone, and Turkey [partly in Asia], mediterranean zone), Africa (Gambia and Tanzania, tropical zone), and North America (Oregon, temperate to oroboreal zone) (Table 1). Bark and wood from living or dead trees, fallen leaves, and some herbaceous plant remnants, such as dead grass and seeds, plus dung of herbivores was used to establish a total of 4793 cultures.

cycle. After 24 or/and 48 h of incubation, the pH of the water was measured with a pH stick (Merck Universalindikator), or with an electronic meter.

The dishes were examined under a dissecting microscope daily or every second or third day for one month. When developing myxomycetes were found, the pH was measured again, and the lid of the petri dish was left slightly ajar to let the culture dry slowly. Usually by the following day the myxomycetes were already mature and were promptly removed. In most cases, the chambers were rewetted for another one-month incubation period.

## Results

In the 4793 moist chambers prepared, a total of 1678 specimens of myxomycetes developed. They represent 117 species in 25 genera. When representatives of a species appeared twice in the same petri dish, they were counted as one specimen. Specimens which stayed as plasmodia, turned into sclerotia, or were totally immature were not included in the following countings. From the material collected for different purposes, the following papers have been published: HÄRKÖNEN (1977a, b, 1978a, b, c, 1981a, b, 1988), HÄRKÖNEN & KOPONEN (1978), HÄRKÖNEN & UOTILA (1983), UKKOLA (1998a, b), UKKOLA et al. (1996). The results of the material collected from Oregon (plus some from northern California and Washington), USA are in press (UKKOLA & RIKKINEN 2000). The methods of collecting have varied in such a way that allows for no statistical method to support our conclusions.

## Different Geographical Areas as Producers of Myxomycetes

As the moist chambers are of the same size and were prepared with the same method, one can assume that the amount of productive moist chambers reflects the amount of myxomycetous spores present in each habitat.

Figure 1 shows the percentage of specimens obtained in relation to the number of moist chamber cultures prepared in the five geogra-

**Table 1: The amount of myxomycete specimens compared to the number of moist chambers with substrates from different geographical areas.**

Geographical area	Cultures prepared	Exx determined	Productivity (%)
Finland (+ Norway) – Boreal zone	3298	983	30
U.S.A., Oregon –Temperate to oroboreal zone	787	201	25.5
Turkey –Mediterranean zone	181	147	81
Gambia –Tropical zone	117	83	71
Tanzania –Tropical zone	410	264	64
<b>Total</b>	<b>4793</b>	<b>1678</b>	<b>35</b>

Pieces of substratum were placed on filter paper in sterile petri dishes (9.5 cm in diam.). The material formed a single layer covering the bottom as entirely as possible (except in the moist chambers with cereals, where only 20 seeds were put into every petri dish). The dishes were wetted with distilled water, adjusted to pH 7.0 with KOH. The petri dishes were kept in diffuse day light in normal room conditions or in an incubator at a temperature of 24–29 °C and lighted artificially in a 12:12 h

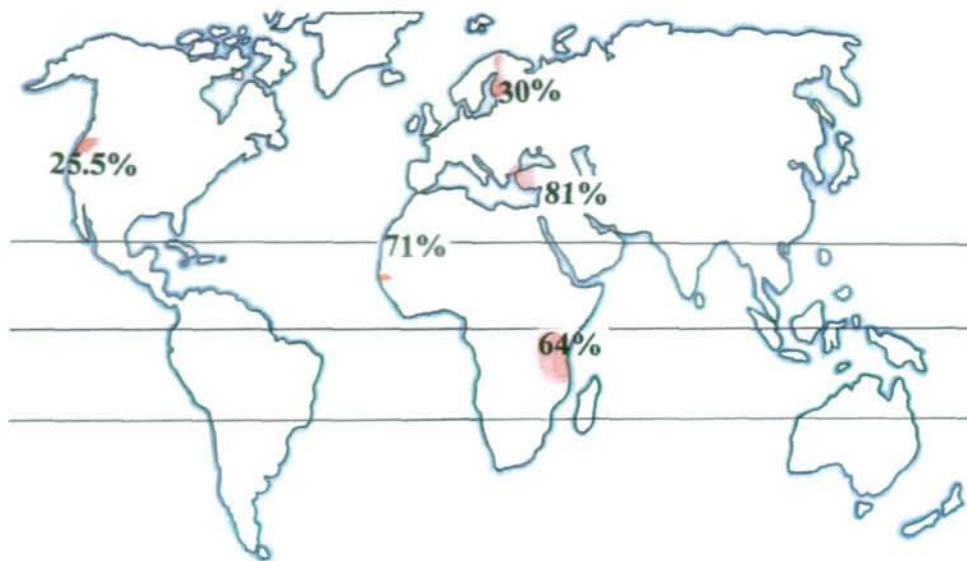


Fig. 1: The productivity of moist chamber cultures in Finland (+ Norway), Oregon (USA), Turkey, Gambia, and Tanzania.

phical regions. It reflects to some extent the abundance of myxomycetes in these areas.

The moist chamber cultures from Turkey were the most productive. The cultures prepared with material collected from Africa (Gambia and Tanzania) were also fairly productive. The habitats in the more monotonous northern boreal zone produced significantly less myxomycetes. Likewise, in the conifer-dominated temperate to oroboreal forests of western Oregon, productivity was low.

In spite of the dry and eroded vegetation in Turkey, the moist chamber cultures prepared with the Turkish material were the most productive. The tropical areas seem to be richer in myxomycetes than earlier believed. The mild and wet, highly oceanic Sitka spruce (*Picea sitchensis*) forests on the coast of Oregon were surprisingly myxomycete-poor compared to the oceanic forests of northern Norway.

STEPHENSON (1989) has used the moist chamber culture technique for studying the distribution and ecology of myxomycetes in temperate forests in southwestern Virginia, USA. In his study, the productivity of cultures prepared with bark was 90%. The corresponding figure for litter was at least 70%, and for dung 34%. The percentages for bark and litter are high even if STEPHENSON counted as "positive" all the cultures which produced some evidence of myxomycetes, whereas we counted only those which yielded mature myxomycete specimens. Another explanation for the abundant productivity in Virginia is the more luxu-

riant vegetation with the many host-tree species of the North American temperate forest compared to the boreal forests of Europe, or the conifer-dominated forests of western Oregon.

### The Species

The following widespread species were found in all five study areas: *Arcyria cinerea* (BULL.) PERS., *A. pomiformis* (LEERS) ROSTAF., and *Echinostelium minutum* DE BARY. The following species were also very common, being absent from only one of the five studied areas: *Comatricha elegans* (RACIB.) G. LISTER, *Cribraria violacea* REX, *Didymium squamulosum* (ALB. & SCHWEIN.) FR., *Enerthenema papillatum* (PERS.) ROSTAF., *Perichaena chrysosperma* (CURR.) LISTER, *P. corticalis* (BATSCH) ROSTAF., and *Physarum pusillum* (BERK. & M. A. CURTIS) G. LISTER. The following rare taxa were found in only one study area: *Arcyria* aff. *afroalpina* RAMMELOO, *Badhamia versicolor* LISTER, *Echinostelium cribrarioides* ALEXOP., *E. ladoi* PANDO, *Leptoderma iridescens* G. LISTER, *Licea bulbosa* NANN.-BREMEK. & Y. YAMAM., *L. poculiformis* UKKOLA, *L. tanzanica* UKKOLA, HÄRK. & GILERT, *Paradiacheopsis cribrata* NANN.-BREMEK., *P. longipes* HOOF & NANN.-BREMEK., *Physarum apiculosporum* HARK., *P. echinosporum* LISTER, *P. fulgens* PAT., *P. lakhapalii* NANN.-BREMEK. & Y. YAMAM., and *P. spumarioides* T. N. LAKH. & MUKERJI var. *degawaiae* NANN.-BREMEK. & Y. YAMAM.

## Species Richness

The species richness (number of species divided by the number of moist chamber cultures prepared) was almost the same in Turkey, Gambia and Tanzania, at 0.2, in Oregon it was 0.05, but in the European boreal zone only 0.01 (Fig. 2). In Turkey 33 species emerged from only 181 moist chambers. This agrees

Table 2 shows a summary of the 20 most common species in the moist chambers of substrates from different regions. There are some clearly corticolous species such as *Licea tanzanica*, *Paradiacheopsis fimbriata* (G. LISTER & GRAN) HERTEL, *P. solitaria* (NANN.-BREMKE.) NANN.-BREMKE., and *Macbrideola cornea* (G. LISTER & GRAN) ALEXOP. Similarly, *Cribraria confusa* NANN.-BREMKE. & Y. YAMAM. and *C.*

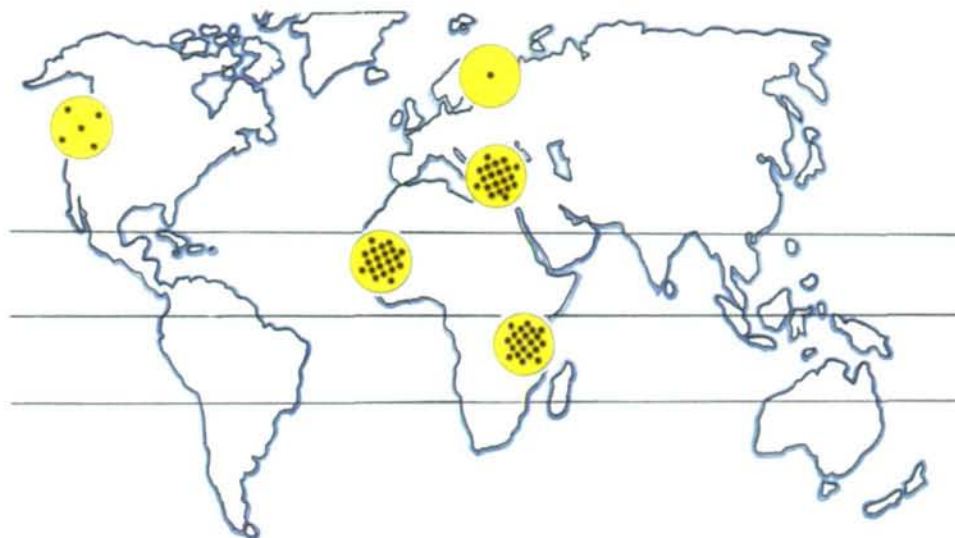


Fig. 2: Species richness: the relative amount of species in different geographical areas.

with LADO's (1993) statement that the Mediterranean region allows for the existence of one of the richest myxomycete floras in the world. The African tropical areas were as diverse as the Mediterranean ones. This is inconsistent with the results of STEPHENSON et al. (1999), who cultivated 500 litter samples collected from a forest in Puerto Rico in moist chamber cultures, resulting in 24 species of myxomycetes. This yields a species richness of 0.05, which is rather low and about the same as our yield for Oregon. The European boreal forests clearly had the lowest species richness of myxomycetes.

## Substrate Preferences

If material for moist chamber cultures is collected from the trunk of a tree and the fallen leaves directly around it, it is reasonable to assume that the selection of myxomycetous spores in those materials is about the same. If, however, different species appear on those substrata, it is reflective of a difference in their demands for the substrate.

*violacea* seem to prefer bark. In contrast, *Didymium difforme* (PERS.) S. F. GRAY and *D. dubium* ROSTAF. have appeared only on deciduous tree leaves, herbaceous material, or dung. *Echinostelium minutum* and *Arcyria cinerea* seem to be indifferent.

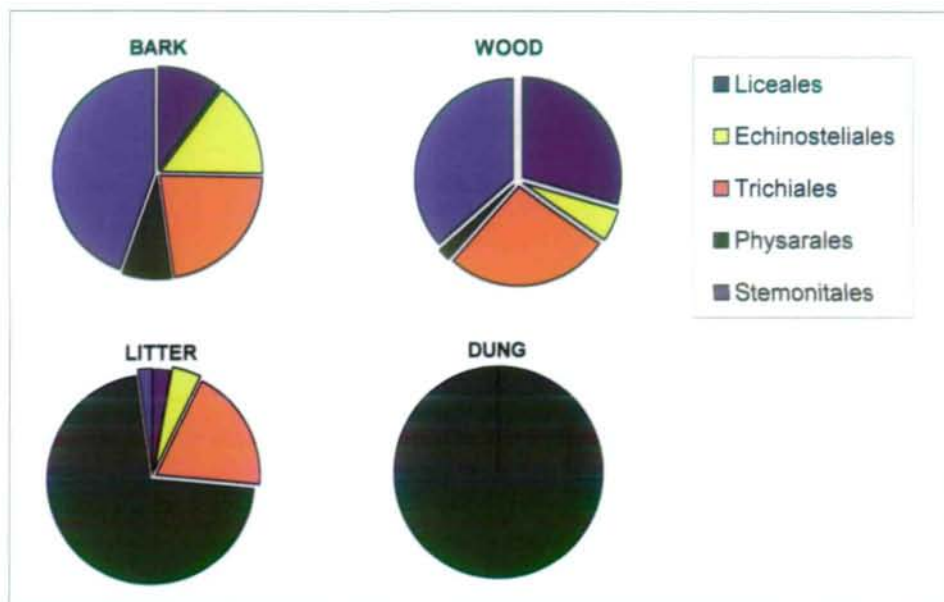
There appears to be a systematic arrangement of the myxomycetes preferring bark, wood or litter. In Figure 3 and Table 3 all the identified specimens (1678 specimens, 117 species) are arranged in orders and according to substrate. The different myxomycete orders favour different substrates. The Physarales most strongly favour litter, as also some Trichiales and Echinosteliales. The Liceales and the Stemonitales occur more rarely on litter. Bark has greater species diversity, but is favoured most by the Stemonitales. Wood is favourable to Stemonitales and some of the Liceales and Trichiales. All the eight specimens that developed on herbivore dung were members of the Physarales. All are included in ELIASSON's & KELLER's (1999) list of coprophilous myxomycetes.

**Table 2: The most common species of myxomycetes in the moist chambers and the number of specimens in different substrates. bark c = coniferous bark; bark d = deciduous bark; litt. c = coniferous litter; litt. d = deciduous litter; litt. m = mixed litter, cereal seeds, living herbaceous plants, grass and moss litter, etc.; wood = decayed or living wood; dung = dung of herbivores.**

Taxa	bark c	bark d	wood	litt. c	litt. d	litt. m	dung
<i>Paradiacheopsis fimbriata</i> 276 exx	120	155	1	–	–	–	–
<i>Didymium difforme</i> 197 exx	–	–	–	–	49	145	3
<i>Echinostelium minutum</i> 169 exx	88	59	3	4	15	–	–
<i>Arcyria cinerea</i> 120 exx	39	34	22	12	4	9	–
<i>Arcyria pomiformis</i> 118 exx	13	80	10	13	2	–	–
<i>Comatricha nigra</i> 109 exx	79	18	11	1	–	–	–
<i>Didymium squamulosum</i> 61 exx	–	5	2	2	12	38	2
<i>Enerthenema papillatum</i> 40 exx	26	7	6	1	–	–	–
<i>Perichaena corticalis</i> 28 exx	9	7	–	–	9	3	–
<i>Cribraria violacea</i> 25 exx	5	14	1	–	–	5	–
<i>Licea tanzanica</i> 21 exx	11	10	–	–	–	–	–
<i>Cribraria confusa</i> 19 exx	–	18	1	–	–	–	–
<i>Licea belmontiana</i> 16 exx	2	–	10	4	–	–	–
<i>Licea minima</i> 16 exx	6	1	8	1	–	–	–
<i>Didymium dubium</i> 14 exx	14	–	–	–	4	10	–
<i>Comatricha laxa</i> 14 exx	1	12	–	–	1	–	–
<i>Paradiacheopsis solitaria</i> 13 exx	7	6	–	–	–	–	–
<i>Physarum cinereum</i> 12 exx	1	1	–	–	5	5	–
<i>Macbrideola cornea</i> 11 exx	5	6	–	–	–	–	–
<i>Didymium quitense</i> 10 exx	3	–	–	–	–	7	–

**Table 3: The emersion of myxomycetes of different orders in moist chambers with different substrates.**

Order	No. of species	No. of exx	in bark	in wood	in litter	in dung	pH of substrate
Licheales	25	161	114	34	13	–	3.8–7.5
Echinosteliales	5	188	162	6	20	–	2.5–7.6
Trichiales	17	366	246	31	89	–	3.5–7.7
Physarales	43	424	86	2	328	8	4.0–8.5
Stemonitales	27	539	487	42	10	–	2.0–9.0
<b>Total</b>	<b>117</b>	<b>1678</b>	<b>1095</b>	<b>115</b>	<b>460</b>	<b>8</b>	



**Fig. 3: The portion of different orders of myxomycetes emerging on bark, wood, litter and dung.**

## The pH of the Substrates

Hydrogen ion concentration of the substrate is an important factor explaining the distribution of fungi. We talk, for instance, about calcicolous species. That is why pH of substrates has been measured in our cultures.

pH of the substrates tended to remain constant throughout the incubation period and was unaffected even after drying and rewetting, with a few exceptions. In general, the bark of coniferous trees was more acidic (2–7) than that of deciduous ones (2.5–9) or litter (4–7.5). In some deciduous Tanzanian trees, the pH of the bark was as low as 3.3–3.5, whereas the pH of the conifers *Juniperus* and *Cupressus* was between 6.3–7.6. In a few cases, the pH of bark or wood cultures prepared with the same tree species varied considerably. For example, the pH values of bark cultures of *Quercus garryana* (from Oregon) varied between 4.0–7.3, being, however, almost neutral in the majority of the cultures. The epiphytes growing on the bark may in part explain the variability. All the *Quercus* cultures produced myxomycetes; however, different species within different pH values.

The variation in the pH of substrates of different myxomycete orders is illustrated in Table 3. Myxomycetes appear to have a wide pH amplitude. The most tolerant in both directions seems to be the Stemonitales. The one and only species, *Paradiacheopsis fimbriata* was identified in the most acidic (pH 2) as well as the most basic substrates (pH 9 of *Ulmus* bark in a park in Helsinki). When this town-resident species is excluded, our observations support STEPHENSON'S (1989) statement that, in general, members of the Stemonitales develop under more acidic conditions than members of the Physarales or the Trichiales.

## Different Tree Species as Substrate for Myxomycetes

In the boreal forests of northern Europe, there are so few tree species that we have collected a large number of bark samples from the five most common trees: *Pinus sylvestris*, *Picea abies*, *Betula pendula* (+ *B. pubescens*), *Alnus glutinosa* (+ *A. incana*), and *Populus tre-*

*mula*. It is also easy to collect litter with leaves of only one tree species.

Of the five tree species, *Pinus sylvestris* has the most favourable bark for myxomycetes (warm and with many fissures). Among fallen leaves, *Alnus* gave rise to the greatest amount of myxomycetes. Perhaps it serves as a good substrate for the food of myxomycetes as *Alnus* sheds its leaves while still green. In both bark and litter cultures, *Betula* was the most unfavoured of the trees concerned. The low productivity of *Betula* bark can possibly be explained by its smooth and peeling surface. But why are the leaves unfavourable? In America, STEPHENSON (1989) found bark of *Betula lenta* to be one of the richest substrates for myxomycetes.

In western Oregon, the most productive tree species was *Quercus garryana*. According to MCHUGH (1998), bark cultures, which have been set up over the past 20 years in different parts of Ireland, indicate that the *Quercus* species are the most productive source of bark. The *Quercus* forests on the Iberian Peninsula are also favourable for myxomycetes (LADO 1993).

On average, no difference in productivity between coniferous or deciduous bark can be seen. It can be generalized from our material that among conifers the species of Cupressaceae were especially productive.

## About Phenology

Many fungi have an internal rhythm to adjust germination to a certain time of year. In the Finnish herbaria, most of the myxomycete specimens are collected in August or September. LADO (1993) found two peaks in the sporulation phenology of myxomycetes in the Mediterranean zone, one in spring and another in late autumn. Is that because of some internal rhythm or merely due to circumstances?

To answer this question we collected bark from living trees from three habitats for one year to establish 150 moist chamber cultures every second week. In this material, no such rhythm could be seen. The spores on bark collected during frosty and short winter days germinated as well as those collected in sum-

mer or autumn within the favourable conditions of the laboratory.

### Incubation Time

The first myxomycetes (e.g. some *Cribraria* species and *Echinostelium minutum*) began to appear in the moist chambers after two days of incubation (possibly even earlier, but this was not checked). Some appeared for the first time only after drying and rewetting after 61 days of incubation. The incubation period varied markedly within all species.

### Resistance to Drought

In the research on fungi in cereal seeds, some of the seeds were placed in moist chamber cultures (630 chambers) soon after collecting, and the other portion was first dried in a typical heated grain dryer (850 moist chamber cultures). Drying of the seeds evidently decreased the number of Eumycotina, but not that of myxomycetes. On the contrary, in most cases, there was an even greater number of myxomycetes on dried than on fresh grain. Thus, the myxomycetous spores seem to be especially tenacious.

### Longevity of the Dormant Phases

We dried and stored some incubated moist chambers containing bark from Tanzania for ten years. The petri dishes were kept since 1989 in a closed, dark laboratory cupboard. In March 1999, 71 closed petri dishes were taken out and rewetted with distilled water. To prevent any contamination, the wetting, as well as any other opening of the lids, was made in a sterile room, radiated beforehand with UV light. The moist chambers were cultivated for two months.

After some days several plasmodia appeared. Astonishingly, other parts of the miniature ecosystem also recovered and we could see small white nematodes eating the plasmodia.

The following species which we had harvested ten years ago, reappeared in the same petri dish:

*Arcyria pomiformis*, *Licea biforis* MORGAN, *L. bulbosa*, *L. tanzanica*, *Perichaena corticalis*, *P. depressa* LIB., and *Physarum crateriforme* PETCH. Furthermore, new species emerged which had not been found in the petri dish ten years ago. These are as follows, listed with the former species in the same dish in parentheses: *Arcyria cinerea* (–*Licea tanzanica* and *L. bulbosa*); *Comatricha elegans* (–*Arcyria pomiformis*); *Diderma hemisphaericum* (BULL.) HORNEM. (–*Badhamiopsis ainoae* (YAMASH.) T. E. BROOKS & H. W. KELLER and *Licea tanzanica*). *Clastoderma debaryanum* A. BLYTT and *Didymium bahiense* GOTTSB. developed in cultures that had produced no fructifications ten years ago. In addition, thirteen non-sporulating plasmodia developed. After 61 days incubation, a specimen of *Diderma* appeared with two equatorial rings in the minutely warted spores. We have not been able to identify it thus far. The species is new to Tanzania.

Overall, the productivity of the ten-year-old, rewetted, moist chambers was 26% (43%, if the unidentified plasmodia are also included), which means that the spores are long-lived and have a very uneven pattern of dormancy: some dormant phases waking immediately after wetting, some needing several wettings and dryings.

An uneven pattern of germination must be advantageous to myxomycetes. Quickly germinating individuals may have sufficient time to go through their entire life cycle after the first showers, but if the periods of rain are very short, these quickly germinating individuals will perish. Sometimes it is favourable not to germinate until the rains have continued for a longer period and it is wet all around; however, if it was just the last day of rain, these myxomycetes will probably die before they produce offspring. Thus, the circumstances must support evolution toward irregular germination.

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