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BASIDIOSPORE GERMINATION AND CONIDIAL STAGES IN THE LIFE CYCLES OF *FOMES FOMENTARIUS* AND *FOMITOPSIS PINICOLA* (FUNGI, POLYPORALES)

VICTOR ANDREEVICH MUKHIN & ANTONINA ALEXANDROVNA VOTINTSEVA

Abstract: Basidiospore germination and the development of primary and dikaryotic mycelia in *Fomes fomentarius* (L.: Fr.) J. J. Kickx and *Fomitopsis pinicola* (Sw.: Fr.) P. Karst. were studied experimentally. This paper reports the first evidence of the conidial stages in the development of primary and dikaryotic mycelia of *Fomes fomentarius* and *Fomitopsis pinicola*, and proposes a new model of their life cycles.

Key words: *Fomes fomentarius, Fomitopsis pinicola,* basidiospore, germination, mycelium, conidial reproduction, life cycles

Victor Andreevich Mukhin, Institute of Plant and Animal Ecology, Urals Division, Russian Academy of Sciences, 8 Marta Str., Ekaterinburg, 620144, Russia; e-mail: victor.mukhin@ipae.uran.ru
Antonina Alexandrovna Votintseva, Department of Botany, Biological Faculty, Ural State University, 51 Lenina Str., Yekaterinburg, 620083, Russia; e-mail: a_votintseva@mail.ru

INTRODUCTION

Fomes fomentarius (L.: Fr.) J. J. Kickx and *Fomitopsis pinicola* (Sw.: Fr.) P. Karst. are very common, cosmopolitan, ecologically flexible polypores. For example, both of them are widespread in all habitat zones of the West Siberian Plain, whose forest vegetation was shaped mainly by climate. The ecological optimum of *F. fomentarius* fits nearly all habitat zones from forest-steppe to northern taiga, whereas that of *F. pinicola* is found only in the southern and middle taiga areas. However, the latter is found on more substrates in the forests of the West Siberian Plain than is *F. fomentarius* (Mukhin 1993).

The reasons for the flexibility of *Fomes fomentarius* and *Fomitopsis pinicola* are not clear. It could be accounted for by their biology, in particular their reproductive characteristics: basidiospore viability, and the presence or absence of asexual reproduction in the life cycle. These questions are raised in a few works (Whithey & Bohaychyk 1971; Tsuneda & Kennedy 1978; Ingold 1986, 1991; Adaskaveg & Gilbertson 1989; Hsiang *et al.* 1989), so further research into the reproduction of polypores is of interest.

These experiments focus on the viability and the dynamics of basidiospore germination, primary and dikaryotic mycelia growth, conidia development of *Fomes fomentarius* and *Fomitopsis pinicola*.

MATERIALS AND METHODS

The experiment used basidiospores obtained from spore prints of *Fomes fomentarius* and *Fomitopsis pinicola* basidiocarps from pine-birch forests of the Middle Urals in June 1999–2000 in accordance with Ryvarden & Gilbertson's (1993) recommendations. The spore prints were kept in paper envelopes at room temperature and were checked once a month for viability. The basidiospores were placed in Petri dishes with 2% malt extract agar (MAA; 20 g agar, 20 g malt extract, 1000 ml distilled water). The medium was sterilized under 0.5 kg/cm steam pressure for 30 min. Germinated spores were counted under a microscope in 10 fields of 50 spores each after 48 h.

When the dynamics of basidiospore germination were being studied the basidiospores of the two species were germinated on 2% MAA in Petri dishes (diameter 5 cm) and on glass slides in accordance with 'Methods of experimental mycology' recommendations (Dudka *et al.* 1982). In both cases the basidiospores were added to the

media as water suspensions or powders with a microbiological needle. The preparations were checked under a microscope every 3 h until the first wall in the germination tube appeared (i.e., before germination stopped and the haploid mycelium began growing).

In the experiments on the influence of water extracts of bark from *Betula pendula* Roth and *Pinus sylvestris* L on primary mycelium growth and conidia development the extracts were prepared immediately before the experimental treatments, by placing 10 g finely ground bark in 150 ml Erlenmeyer flasks, to which 40 ml distilled water was added. The mixture was kept at room temperature for 4 days. Then the liquid was filtered from the bark with paper filters and 0.3 ml extract with suspended basidiospores was put into Petri dishes with 2% MAA. In the experiments of this series as control 0.3 ml distilled water with suspended basidiospores was put into Petri dishes with 2% MAA. The extracts or distilled water were not boiled; 3 mg/l tetracycline was dissolved in the media to prevent infection. The preparations was checked under a microscope once every 24 h for 7 days.

Pure cultures of dikaryotic mycelia were obtained by a standard method (Dudka *et al.* 1982; Ryvarden & Gilbertson 1993) from basidiocarps of *F. fomentarius* and *F. pinicola*. The medium for isolating dikaryotic material and for experimenting with it was 2% MAA. When dikaryotic mycelium and conidia development were observed, the material was checked once every 24 h for 7 days. A BIOLAM light microscope, an camera lucida RA-4 (400×) and an MOB-1-15× micrometer eyepiece were used.

RESULTS

VIABILITY AND BASIDIOSPORE GERMINATION ON MAA MEDIUM WITH DISTILLED WATER

One-month-old basidiospores of *Fomes fomentarius* were found to be highly viable; nearly 98% germinated within the first 24 h. When *F. fomentarius* basidiospores were kept longer their viability markedly decreased; at 9 months only half of the basidiospores germinated, and only *ca* 5% after 13 months. They preserved their viability for up to 14 months (Fig. 1).

Fomitopsis pinicola basidiospores remained viable for more than two years (26 months) but their viability gradually decreased with age, with nearly 98% germination one month after the spore prints were taken, *ca* 55% at 13 months, and only 3% at 25 months (Fig. 1).

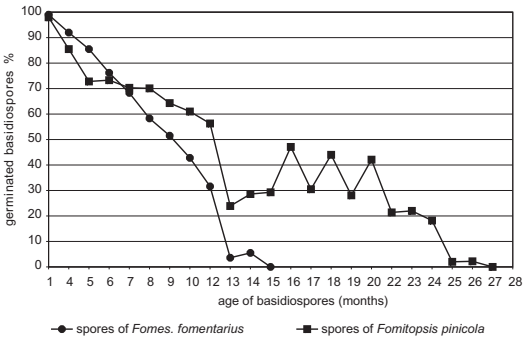

Fig. 1. Viability of *Fomes fomentarius* (L.: Fr.) J. J. Kickx and *Fomitopsis pinicola* (Sw.: Fr.) P. Karst. basidiospores over time on MAA medium with distilled water.

As seen in Fig. 2, the number of *Fomes fomentarius* basidiospores germinating with two tubes

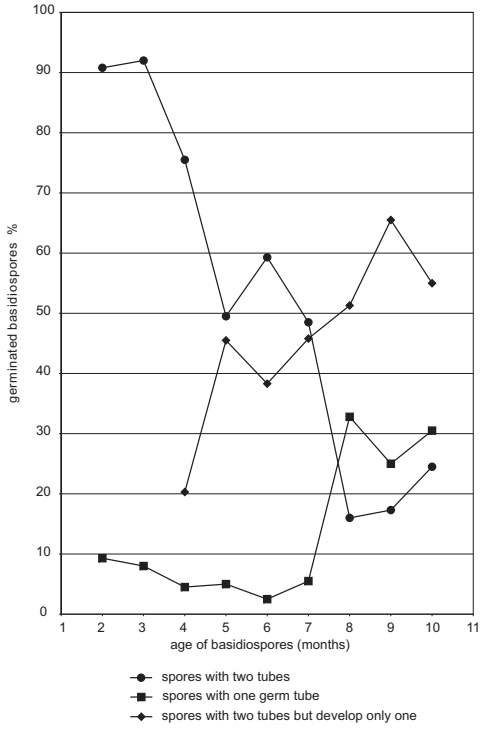

Fig. 2. Percentage of germinating basidiospores of *Fomes fomentarius* (L.: Fr.) J. J. Kickx with one and two sprout tubes on MAA medium with distilled water.

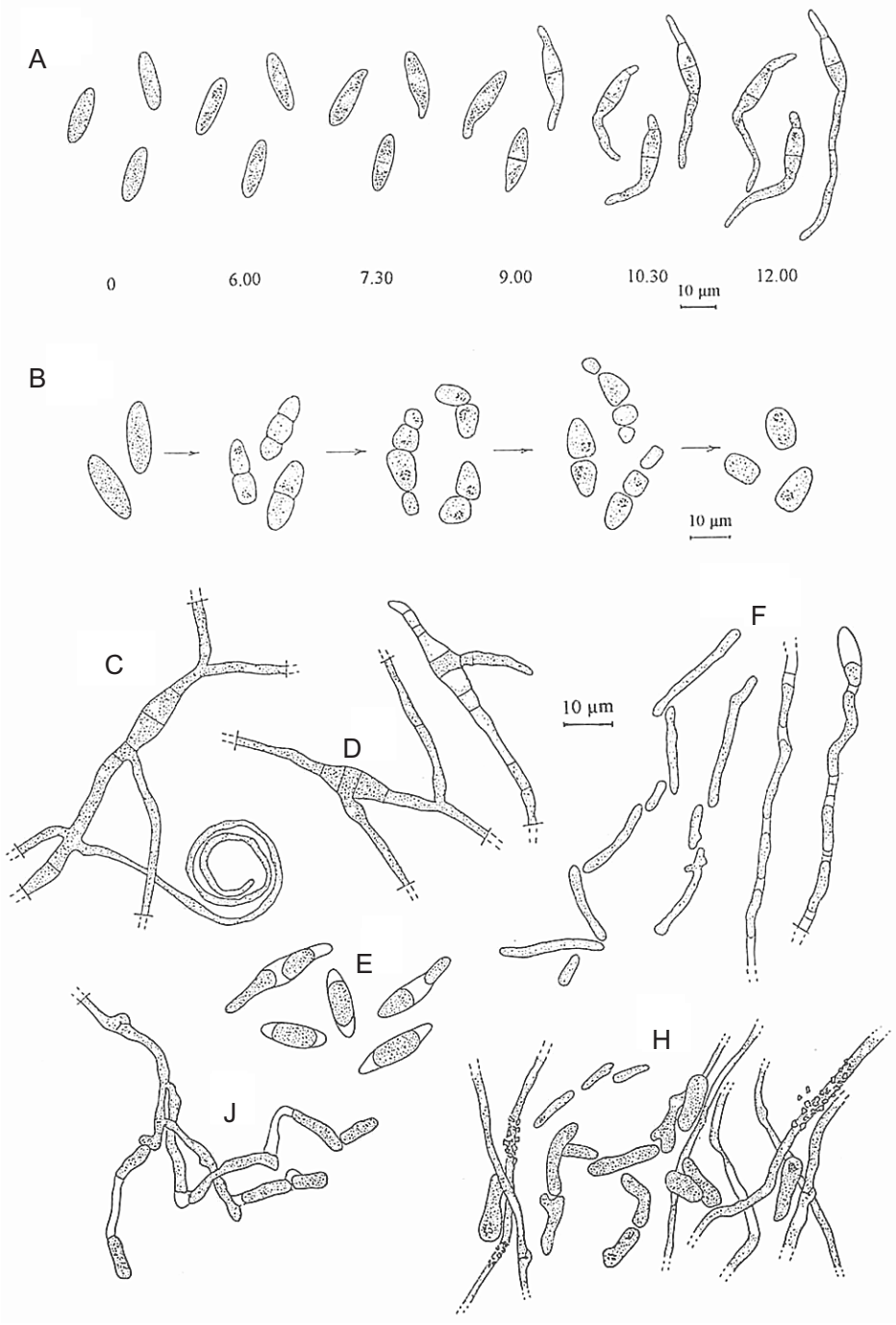

Fig. 3. *Fomes fomentarius* (L.: Fr.) J. J. Kickx (MAA medium with distilled water). A – basidiospore germination, B – basidiospore splitting, C – mycelium coils, D – basidiospores with lateral sprout tubes, E – chlamydospore (?) inside basidiospore, F – monokaryotic hyphae with retraction septa and monokaryotic conidia, J – dikaryotic hyphae with conidia separated by empty parts, H – dikaryotic conidia.

gradually decreased over time. The germinating basidiospores did not change their shape or size, and as a rule two apical germ tubes appeared, simultaneously or in turn (Fig. 3A). They were separated from the spore cavity by walls.

Fomitopsis pinicola basidiospores also germinated within the first 24 h, but unlike those of *Fomes fomentarius* they grew in size and become round or ellipsoid before forming the germ tubes. Normally two germ tubes, occasionally only one, appeared on the basidiospores, apically as a rule. Sometimes the germ tubes were separated from the basidiospore cavity by walls (Fig. 4A).

In both species under study the germinating basidiospores can become multicellular. This was especially common in *F. fomentarius*, whose spores (till 68% of the cases) contained two and less often three or five cells before forming germ tubes, due to the appearance of one, two or four walls (Fig. 3A, B). Two-cellular basidiospores in *Fomitopsis pinicola* were much less common, recorded in 2.6–7.3% of the cases (Fig. 4A, B).

Each of several cells in *Fomes fomentarius* basidiospore is capable of forming one or two germ tubes (Fig. 3D). If a basidiospore contained three or five cells, its germ tubes appeared first on apical cells and then on central ones. A certain number of multicellular basidiospores of *F. fomentarius* (5–7%) split into separate cells before the appearance of germ tubes or sometimes afterwards (Fig. 3B). This phenomenon was observed in *Fomitopsis pinicola* only rarely, and in most cases the split was not complete (Fig. 4B).

In only two cases and only in *Fomes fomentarius*, one or several round structures with dense homogeneous cytoplasm and their own walls formed inside the basidiospore and sprouted tubes. Probably they were chlamydospores (Fig. 3E).

PRIMARY MYCELIUM GROWTH AND CONIDIA DEVELOPMENT ON MAA MEDIUM WITH DISTILLED WATER AND BARK EXTRACT

The primary mycelium formed by germinating basidiospores of *Fomes fomentarius* on medium with distilled water contained hyphae 2.0 μm in diameter, whose peripheral branches can be spirally twisted (Fig. 3C). Usually the primary mycelium started forming mycelial conidia three or four days after basidiospore germination. The cytoplasm in hyphal cells divided into separate sections with additional walls between them, producing chains of thalloconidia with smooth colorless walls and dense cytoplasm (Fig. 3F). The size of the separate thalloconidia was 5–27 × 2–3 μm.

In same conditions in *Fomitopsis pinicola*, thalloconidia of the primary mycelium similarly formed after three days (Fig. 4D). In this species the primary mycelium consisted of branching hyphae 2.2 μm in diameter, sometimes with terminal circle-like shapes (Fig. 4C). However, in this species the thalloconidia are formed much less frequently and they are much smaller (4–15 × 2–3 μm) than in *Fomes fomentarius*. When the thalloconidia of both species were placed on new media, they germinated and formed mycelia identical to the primary ones.

On MAA medium with bark extract from *Pinus sylvestris* the primary mycelium of *Fomes fomentarius* and *Fomitopsis pinicola* formed both thalloconidia and blastoconidia. After two or three days the primary mycelium of both species grew short side projections, first with a septum at the base, then completely independent of the hyphae (Fig. 5A, B). The blastoconidia of *Fomes fomentarius* had smooth, colorless walls and dense cytoplasm, and their size was 6.0–7.5 × 1.0–3.0 μm. *Fomitopsis pinicola* blastoconidia were of similar size (6.0–7.5 × 1.1–2.5) μm. When germinating, the blastoconidia grew in size, were divided into two parts by a central septum, and germinated with one or two tubes (Fig. 5C, D).

On the MAA medium with bark extract from *Betula pendula* there were numerous side projections on the primary mycelia of both species, but in our experiments they never separated from the hyphae as blastoconidia.

DIKARYOTIC MYCELIA AND CONIDIA DEVELOPMENT ON MAA MEDIUM WITH DISTILLED WATER

Thalloconidia are formed on the dikaryotic mycelia in both species. For example, after five or six days on MAA medium the dikaryotic mycelium of *Fomes fomentarius* formed colonies about

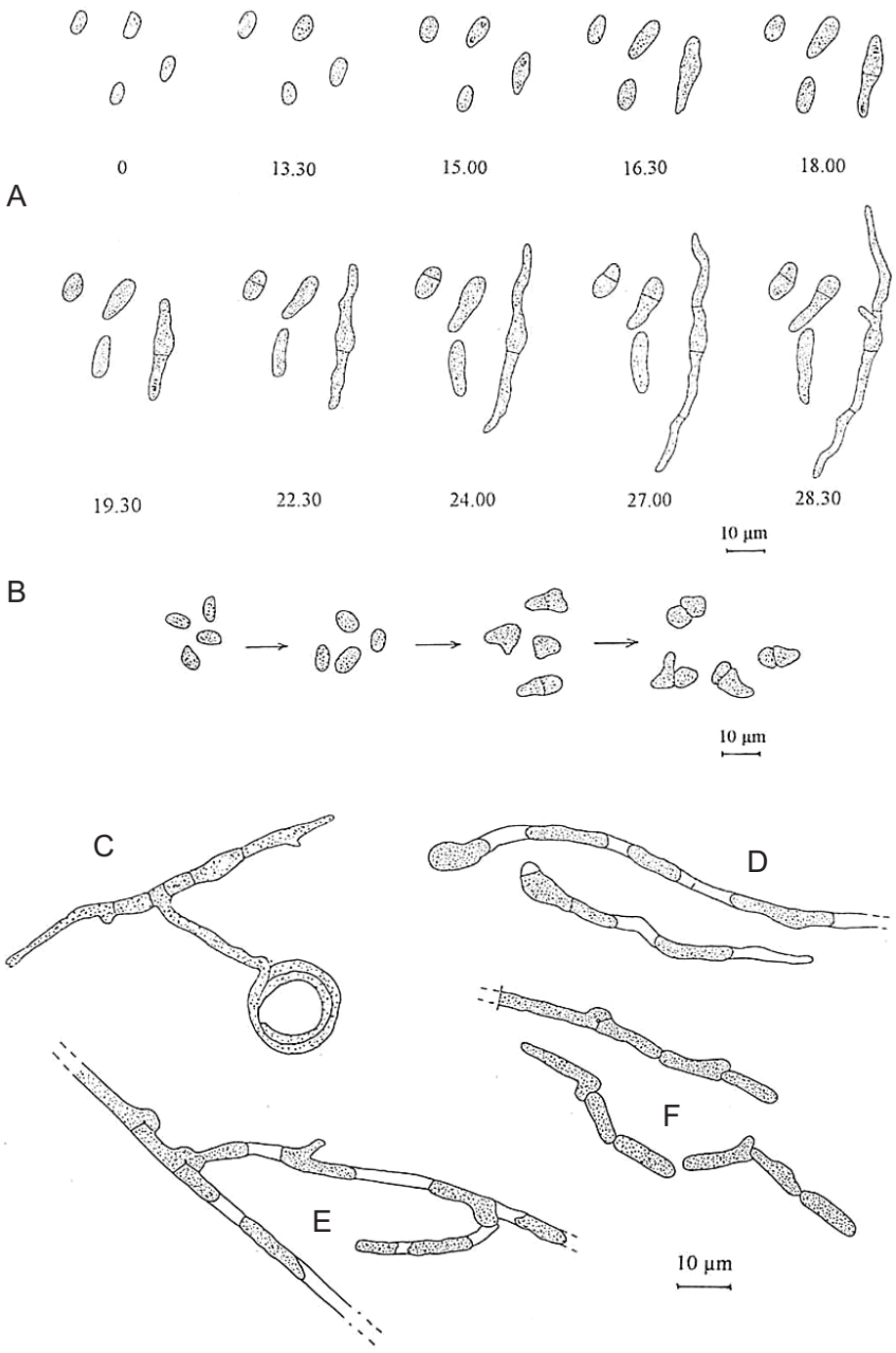

Fig. 4. *Fomitopsis pinicola* (Sw.: Fr.) P. Karst. (MAA medium with distilled water). A – basidiospore germination, B – incomplete splitting of basidiospore, C – mycelium coils, D – monokaryotic hyphae with conidia, E – dikaryotic hyphae with conidia separated by empty parts, F – dikaryotic hyphae fragmentation and dikaryotic conidia.

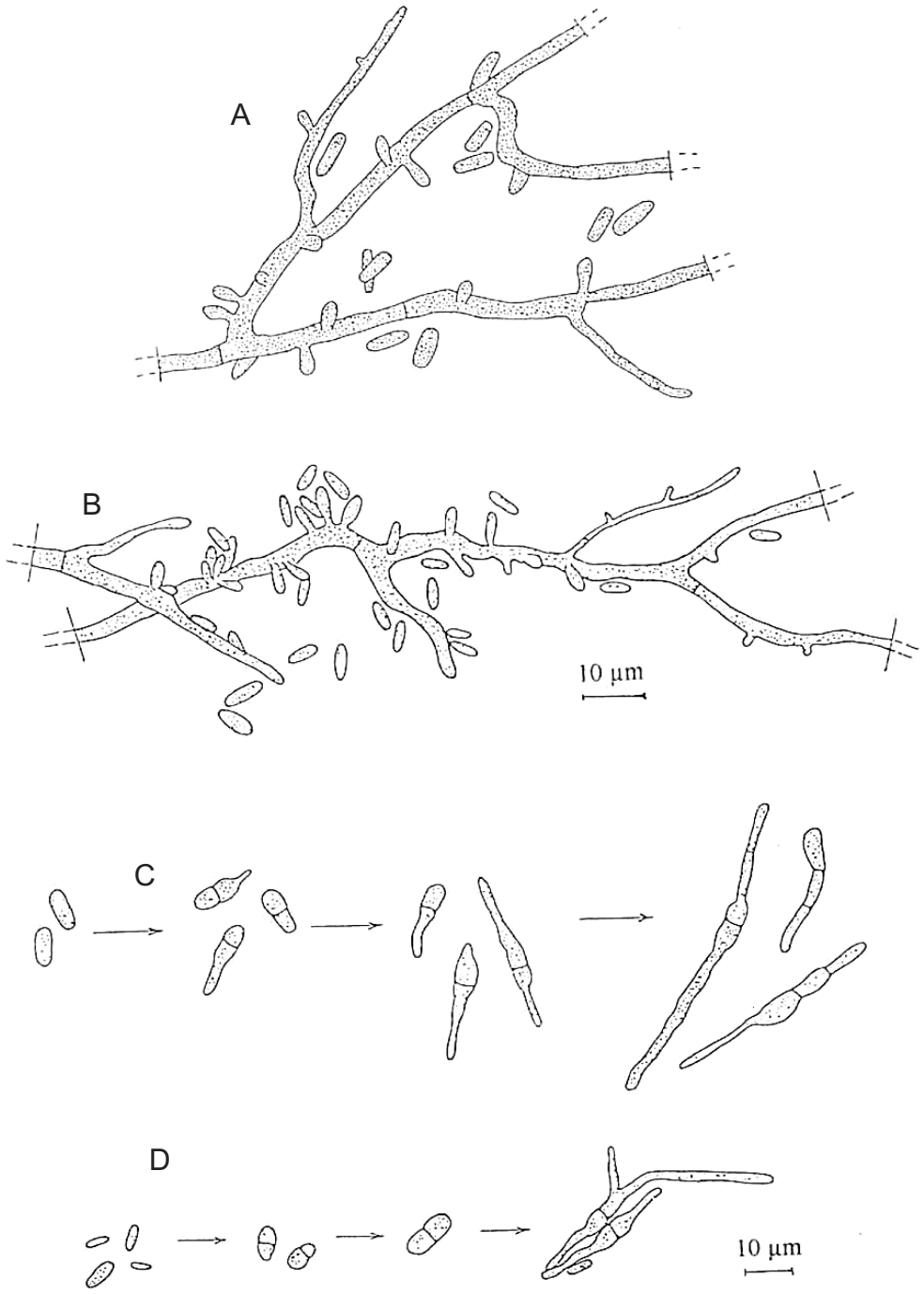

Fig. 5. Development and germination of conidia on MAA medium with *Pinus sylvestris* bark extract. A – monokaryotic hyphae of *Fomes fomentarius* (L.: Fr.) J. J. Kickx, B – *Fomitopsis pinicola* (Sw.: Fr.) P. Karst. developing conidia; *Fomes fomentarius* (C) and *Fomitopsis pinicola* (D) germinating monokaryotic conidia.

3 cm in diameter, with hyphae 2.4 μm wide with clamps and crystals in the central area of the colony. First the thalloconidia formed in the center of dikaryotic colonies, then spread to other parts. When thalloconidia formed, the cell cytoplasm first divided into separate parts, with additional walls appearing afterwards. This resulted in chains of dikaryotic conidia separated from each other by sites of hyphae without cytoplasm which then degenerate completely (Fig. 3J). Sometimes a different mechanism was involved in the formation of dikaryotic conidia in *F. fomentarius*: the cytoplasm was immediately divided by walls, making the hyphae disintegrate into thalloconidia (Fig. 3H). *F. fomentarius* dikaryotic thalloconidia were large (12.5–23.0 × 3.6–5.5 μm) and normally irregular in shape, with smooth colorless walls and dense cytoplasm.

Dikaryotic thalloconidia formed less frequently in *Fomitopsis pinicola* than in *Fomes fomentarius* but followed the same pattern: first the cytoplasm of the hypha became denser and divided into parts which were then separated by septa and areas without cytoplasm; finally the hyphae split into thalloconidia (Fig. 4E, F). Dikaryotic conidia of *F. pinicola* were irregular in shape and often had different thickenings, sometimes cylindrical, with smooth walls, 6.0–18.0 × 2.0–4.5 μm. Dikaryotic conidia germinated in both species, producing dikaryotic mycelia.

DISCUSSION AND CONCLUSIONS

In this study, *Fomes fomentarius* and *Fomitopsis pinicola* basidiospores remained viable for quite a long time – up to 14 and 26 months, respectively. Their viability gradually decreased from nearly 100% one month after gathering to 3–5% 13 and 25 months after collecting. The reasons for the differences in viability between basidiospores and the importance of the differences to their ecology are still unknown. However, it goes without saying that basidiospores effectively fulfill the role of reproducing and distributing *F. fomentarius* and *F. pinicola*, especially within the first months of their existence.

Fomes fomentarius and *Fomitopsis pinicola* basidiospores have more than one method of germination. They can germinate as monocellular organisms described by Tsuneda and Kennedy (1978), in which case two apical germ tubes usually appear. As basidiospores become less viable, one of the two germ tubes fails to grow to its full size or fails to grow at all. With age, many more basidiospores germinate as multicellular organisms; this is especially true of *F. fomentarius* basidiospores, as Tsuneda and Kennedy (1978) have demonstrated.

Both polypore species are capable of asexual reproduction. The main structure for asexual reproduction are thalloconidia that formed by both primary and secondary mycelia of *F. fomentarius* and *F. pinicola*, and are likely an obligatory part of the development scenario. Under certain conditions – on medium with bark extract from *Pinus sylvestris* and *Betula pendula* – blastoconidia are formed by primary mycelia as structure for asexual reproduction.

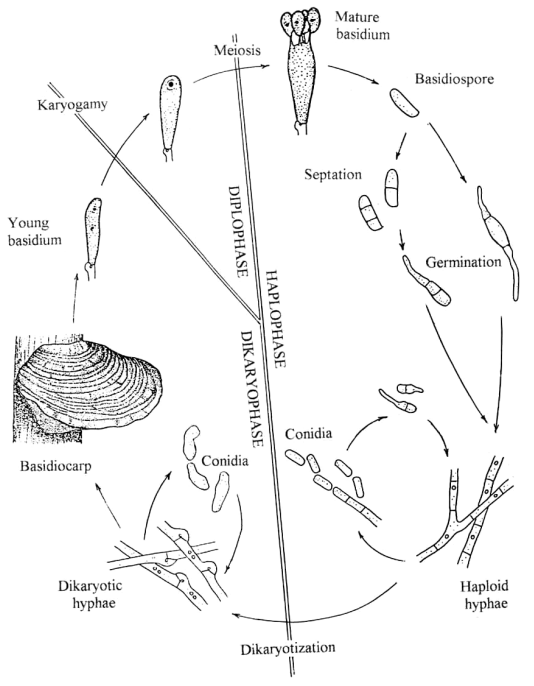

Fig. 6. Life cycles of *Fomes fomentarius* (L.: Fr.) J. J. Kickx and *Fomitopsis pinicola* (Sw.: Fr.) P. Karst.

The ability to reproduce asexually has been found in many polypores: *Polyporus badius* (Pers.) Schwein., *Polyporus squamosus* (Huds.): Fr., *Polyporus varius* (Pers.): Fr. (Ingold 1986, 1991), *Trametes gibbosa* (Pers.: Fr.) Fr., *Trametes hirsuta* (Wulfen: Fr.) Pilát, *Trametes ochracea* (Pers.) Gilb. & Ryvarden, *Trametes versicolor* (L.: Fr.) Pilát (Golumbievskaja & Votintseva 2000) and *Heterobasidion annosum* (Fr.) Bref. (Hsiang *et al*. 1989). In particular, *Polyporus squamosus* and *P. varius* reproduce asexually with both haploid and dikaryotic mycelia, and *P. badius* only with haploid mycelia. These three species are like *Fomes fomentarius* and *Fomitopsis pinicola* in their manner of forming asexual spores: the hypha disintegrates into conidial chains, with the conidia separated from each other by secondary walls and empty hyphal sites.

The ability of polypores to reproduce asexually shows that there are repeating stages in their life cycles. Figure 6 displays the possible life cycles of *Fomes fomentarius* and *Fomitopsis pinicola*, incorporating our results. We propose the term 'repetitive' for life cycles of this type. Asexual reproduction makes these species ecologically more fit. In particular it enables them to evolve as separate clones (both haploid and dikaryotic), which is especially important in colonizing new territories. Asexual reproduction is also indispensable in natural or anthropogenic conditions in which population numbers and home range areas become reduced. It may be one reason why *Fomes fomentarius* and *Fomitopsis pinicola* have a wide home range and high adaptability.

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