CHAPTER 24

SPORULATION BY AQUATIC HYPHOMYCETES

FELIX BÄRLOCHER

63B York Street, Dept. of Biology, Mt. Allison University, Sackville, NB, Canada E4L 1G7.

1. INTRODUCTION

Fungi are instrumental in leaf decomposition in streams (Gessner & Chauvet 1994), and their biomass accumulating on leaves improves substrate palatability and nutritional value to shredders (Bärlocher 1985, Suberkropp 1992, Graça 1993, 2001). The preferred method to measure fungal biomass is based on the indicator molecule ergosterol, which occurs at a relatively constant concentration in living mycelia (Chapter 25). A very substantial proportion of fungal production, often in excess of 50% (Findlay & Arsuffi 1989, Chauvet & Suberkropp 1998, Sridhar & Bärlocher 2000), is invested in propagules that are released from leaves. Asexually produced spores (mitospores, conidia) dominate. On leaves freshly recovered from a stream, only a few conidia can be observed. However, if such leaves are incubated for 1—2 days under conditions that stimulate sporulation (low to intermediate nutrient levels, high turbulence), newly formed conidia will be released. They can be trapped on a membrane filter, stained, and counted and identified under a microscope. There is a significant correlation between maximum fungal biomass on the leaf and maximum spore production over the course of decomposition (Gessner & Chauvet 1994, Maharning & Bärlocher 1996). However, at any given point during decomposition, high sporulation rate by a species does not imply the presence of high mycelial biomass belonging to the same species on the leaf (Bermingham et al. 1997). Sporulation under laboratory conditions can be as high as 4000 spores produced day^{-1} mg^{-1} of leaf dry mass (for review, see Gessner 1997; selected values are shown in Table 24.1).

The aim of this chapter is an estimate of the reproductive potential of the mycelia present in leaves recovered from a stream, following procedures based on Bärlocher (1982). This and similar procedures are also described in Gessner et al. (2003). The data can be used to estimate the amount of conidial biomass released from leaves, or, to describe the diversity and composition of the fungal community.

M.A.S. Graça, F. Bärlocher & M.O. Gessner (eds.), Methods to Study Litter Decomposition: A Practical Guide, 185 – 188.

Table 24.1. Maximum spore production rates (no. day^{-1} mg^{-1} of leaf dry mass) reported from leaves decomposing in streams, from selected studies. Length = number of days of incubation in stream before maximum was reached.

Sporulation rate	*Length*	*Leaf species*	*Condition*	*Reference*
75	93	*Liriodendron tulipifera*	Softwater	1
425	21	*Liriodendron tulipifera*	Hardwater	1
1500	28	*Alnus glutinosa*	Softwater, 10 ºC	2
4000	14	*Alnus glutinosa*	15 ºC	3
7000	28	*Eucalyptus globulus*	15 ºC	3

1 = Suberkropp (2001); 2 = Hieber & Gessner (2002); 3 = Bärlocher et al. (1995)

2. EQUIPMENT, CHEMICALS AND SOLUTIONS

2.1. Equipment and Material

- Autumn-shed leaves, air dried
- Litter bags (10 × 10 cm, 10, 1 or 0.5 mm mesh size).
- Erlenmeyer flasks, 250 ml, with 150 ml of deionized, sterile water
- Membrane filters, 5 µm pore size, and filtering apparatus
- Supply of pressurized air (e.g. aquarium pumps), tubing and Pasteur pipettes, or shaker
- Drying oven (40—50 °C)
- Balance (±1 mg precision)
- Microscope (with 16, 40 and 100× objectives)

2.2. Chemicals and Solutions

- Lactic acid
- Deionized water
- Phenol and glycerol for long-term storage
- 0.1% Trypan Blue or Cotton Blue in 60% lactic acid (Trypan Blue in lactophenol is preferable for long-term storage: 10 ml phenol, 10 ml lactic acid, 20 ml glycerol, 10 ml H_2O)

3. EXPERIMENTAL PROCEDURES

1. Prepare litter bags to be placed in a stream as indicated in Chapters 5 and 6.
2. Anchor leaf bags to stream bed by means of bricks, steel pegs or other devices. Be careful not to place too many bags close to each other, because this may greatly change flow patterns and thereby affect fungal colonization of leaves.
3. Recover bags at appropriate intervals (fungal colonization proceeds faster on leaf species that are more rapidly decomposed; see Chapter 6).
4. Rinse leaves to remove silt, sand and invertebrates.
5. Place some leaf material (ca. 9 cm^2) in an Erlenmeyer flask with sterile, deionized water or filtered stream water.

6. Induce turbulence by placing the flask on a shaker (100—150 rpm) or by aerating it (connect Pasteur pipettes with tubing to source of pressurized air and adjust air-flow to approx. 1 ml s^{-1}).
7. After 24—48 h, remove leaf material and determine its dry mass (40—50 °C, 2 days or until constant weight is reached).
8. Filter supernatant through membrane filter.
9. Add a few drops of Trypan Blue solution to filter; incubate for 30—60 min at 40—50 °C.
10. Scan the surface of the filter under the light microscope. Count and identify all conidia, or, if they are very numerous, all conidia in 20—30 randomly chosen microscope fields, or, on a defined fraction of the filter (cf. Gönczöl et al. 2001).
11. Express the number of spores produced during laboratory incubations per leaf dry mass or ash-free dry mass.
12. To estimate biomass of the spores, determine total volume of spores, and assume a density of 500 fg μm^{-3} (Findlay and Arsuffi 1989). Volumes of selected species are listed in Bärlocher & Schweizer (1983). Chauvet & Charcosset (2000) provide average spore masses of additional species. Or, assume an average conidial biomass of 200 pg (conservative estimate; Gessner 1997).
13. Analyze fungal community structure as described in Chapter 42 or by other means (e.g. multivariate analyses).

4. REFERENCES

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