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On the ecology of fungal consortia of spring sap-flows

Roland W. S. WEBER

Department of Biotechnology, University of Kaiserslautern, Erwin-Schroedinger-Str. 56, 67663 Kaiserslautern, Germany

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ABSTRACT

Spring sap-flow, *i.e.* xylem sap exuding through wounds of deciduous trees in spring, quickly becomes colonised by yeasts and filamentous fungi, of which several species occur regularly in consortia from different trees or regions. This article considers the survival, spread and population dynamics of these fungi, with particular emphasis on the substantial research by Wladyslav I. Golubev on birch sap-flows in Russia. Most fungi survive in the soil or epiphytically on tree trunks, and dispersal between trees is probably mediated by insects. Competition within and between sap-flow yeast species may be due to nutritional effects or the action of killer toxins (mycocins).

1. Introduction

In the May issue of this year's *Mycologist* I co-authored a short article describing a colourful consortium of filamentous fungi and yeasts colonising freshly cut birch stumps in Germany (Weber *et al.* 2006). Despite substantial literature searches on this topic, I remained unaware of a series of three seminal and highly relevant papers published by Wladyslav I. Golubev in the former Soviet Union (Golubev *et al.* 1977a,b, Shkidchenko & Golubev 1980). Although these articles are available in English translations, they have been cited only rarely by non-Russian scientists. It is therefore appropriate to discuss Golubev's work here, not least because it provides an interesting background and contrast to our results on red yeast consortia discovered in Germany (Weber *et al.* 2006) and subsequently in Italy (Weber & Davoli 2005).

2. Chronic exudates and spring sap-flows

Tree exudates may be of two kinds, *viz.* slow but long-lasting (chronic slime-fluxes) or vigorous but short-lived (spring sap-flows). Chronic exudates are caused by various factors, such as mechanical injury or activity of invertebrate and microbial (especially bacterial) tree pathogens. Lasting for several months or even years, they harbour their own well-characterised microbial comunities. Many yeast species have been isolated and described from invertebrates associated with slime-fluxes, such as wood-boring insects (see Phaff & Starmer 1987), flies (Phaff & Knapp 1956) and nematodes (e.g. Kerrigan *et al.* 2004). There is a high degree of host tree specificity which may be enforced by selection due to the nutrient composition of the sap or the presence of host defence compounds. Further, dispersal may be influenced by the feeding preferences of insects which are the most important vectors for microbial consortium members (Phaff & Starmer 1987; Phaff 1990). In fact, yeasts play an important role in insect nutrition, and the insect gut harbours an immense diversity of yeasts, many of them belonging to as yet undescribed species (Suh *et al.* 2005). These yeasts may be spread by defaecation, as is well-known for *Pichia* spp. (Kurtzman 1998).

In contrast to chronic slime-fluxes, spring sap-flows are confined to the period of bud break when the high root pressure of many broad-leaved trees causes sap to rise. If the bark of trunks or branches is injured, massive bleeding

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E-mail address: rwsweber@rhrk.uni-kl.de

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(sap-flow) may result. The xylem sap contains its highest concentrations of sugars and other nutrients in spring because these underground storage compounds are mobilised towards the expanding buds (Kallio & Ahtonen 1987; Ahtonen & Kallio 1989). Not surprisingly, therefore, the microbial composition of spring sap-flows differs from that found in chronic slime-fluxes. Birch trees (*Betula* spp.) are particularly prolific producers of spring sap-flow, and their sap, being rich in monosaccharides (up to 1 % w/v), is collected commercially in Finland and North America for use as a health-promoting beverage or in the production of birch syrup as an alternative to maple syrup.

3. The diversity of yeasts suspended in birch sap

Although the natural occurrence of birch trees showing spring sap-flows is limited in time and space, characteristic microbial communities of yeasts, filamentous fungi and bacteria quickly establish themselves as biofilms on solid surfaces, and as a planktonic phase of cells suspended in the sap itself. Golubev *et al.* (1977a,b), working in *Betula pendula* forests in central and northern regions of the European part of Russia, observed that a reproducible succession of yeast species developed as suspensions in spring sap-flow. Following an initial period of sterility, colonisation of the sap occurred in two phases. The first phase, associated with rising daytime temperatures but continued night-time frosts, was characterised by the emergence of a wide diversity of yeast species, each at a low cell density. Frequently encountered species are listed in Fig. 1 under their current taxonomic names according to Barnett *et al.* (2000). Most of them belong to the Basidiomycota, the most abundant yeasts in this phase being *Cryptococcus skinneri* and *C. magnus*. Ascomycete yeasts were less obvious, examples including *Nadsonia fulvescens* var. *elongata* (Fig. 1) and members of the genera *Metschnikowia*, *Candida* and *Aureobasidium* (W.I. Golubev, personal communication).

The occurrence of frost-free nights heralded the advent of a second phase in which the density of yeast cells suspended in the sap increased dramatically so that the sap became turbid. This coincided with the rise to dominance of two yeasts of low initial abundance, *Trichosporon pullulans* and *Xanthophyllomyces dendrorhous* (Fig. 1). Ludwig (1891) already noted a colour change of spring sap-flows from white to red. His red yeast ('*Rhodomyces dendrorhous*') was probably *X. dendrorhous*, and the white one ('*Endomyces vernalis*') may have been *Trichosporon pullulans* (see Kobayashi 1953).

4. The diversity of biofilm-forming fungi

Fungi have been reported in the past as growing in biofilms in spring sap-flows, but most of these older investigations are deficient in details of species identification and/or give no indication of species composition of individual consortia (see Kobayashi 1953). For this reason, Weber and Davoli (2005) and Weber *et al.* (2006) examined spring sap-flow consortia on *Betula pendula* in Kaiserslautern (Germany) and Modena (Italy), and on *Carpinus betulus* in the Upper Appennines (Italy). The most obvious overlaps with the Russian suspension consortia described by Golubev *et al.* (1977a,b) were the late-stage colonisers *X. dendrorhous* and *T. pullulans* at the species level, and *Cryptococcus* at the generic level (Fig. 1); another overlap was the red yeast *Cystofilobasidium capitatum* (W.I. Golubev, personal communication). Nonetheless, the German and Italian biofilms differed from the Russian suspensions in several important aspects, e.g. the prominence of the ascomycete yeast *Hanseniaspora uvarum* and the red basidiomycete yeast *Cystofilobasidium infirmominiatum*, and the presence of mycelial fungi, notably *Mucor hiemalis*. These are fungi associated with plant remains and the soil, but repeated records of their presence in sap-flow biofilms argue against chance contaminations.

5. Interactions between consortium members

By means of carefully executed single- and dual-culture laboratory experiments, Shkidchenko and Golubev (1980) found that *T. pullulans* and *X. dendrorhous* have faster growth rates than their main competitors, concluding that substrate competition, in addition to low freeze-thawing tolerance, is a likely explanation for the dominance of these two species in the final phase of birch sap colonisation. Nutritional interactions have also been described. Thus, *X. dendrorhous* appears capable of utilising organic acids released by *C. skinneri*, resulting in the coexistence and enhanced growth of these two species in dual-culture in the absence of other competitors (Shkidchenko & Golubev 1980).

A more intriguing case is that of infraspecific (*i.e.* strain-specific) competition in *T. pullulans*. Golubev *et al.* (2002) found that certain strains of *T. pullulans* produce a proteinaceous toxin capable of killing non-toxigenic strains of the same species from sap-flows but ineffective against other yeast species and even against *T. pullulans* strains from other habitats. Such killer toxins, called mycocins, are encoded by virus-like agents which bestow resistance upon the yeast cell containing them. Mycocins with an infraspecific action spectrum are common among ascomycete and basidiomycete yeasts (e.g. *Saccharomyces*; Magliani *et al.* 1997), whilst broader action spectra have been reported less frequently (Golubev 2006). In nature, killer strains are generally more abundant in nutrient-rich than nutrient-deficient habitats (Starmer *et al.* 1987). Assuming that mycocin production poses an significant metabolic burden with a retarding effect on growth rate, mathematical models can be developed to simulate the co-existence of killer and non-killer strains in transient and discrete habitats such as sap-flows (Czárán & Hoekstra 2003). In *T. pullulans*, the proportion of mycocin-producing cells among the total population in birch sap-flows rises steeply as the cell density of *T. pullulans* increases during spring, and mycocin production is lost again before the onset of the next growing season (Golubev *et al.* 2002). Other yeasts found in sap-flow consortia may also produce mycocins, e.g. *C. infirmominiatum* (Golubev *et al.* 2003) and *H. uvarum* (Schmitt *et al.* 1997), although their production in this habitat does not seem to have been examined as yet.