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ORIGINAL PAPER

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The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and the content of carbohydrates and nitrogen storage bodies of the fungi

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Abstract In a water-exclusion experiment, five different ecotypes of beech (*Fagus sylvatica* L.; representing regions of different environmental and climatic conditions in Baden-Württemberg, Germany) were subjected to drought conditions of different severity between July and September of two consecutive years. Drought stress as characterised by the water content and the pre-dawn water potential of the leaves was related to the degree of mycorrhization, the type of ectomycorrhiza, and the physiological properties of individual fungus/plant interactions at the fine roots of different beech ecotypes. Our data show that decreased soil water availability did not significantly change either the degree of fungal colonisation of beech roots (measured by the amount of ergosterol) or the number of ectomycorrhizal types per root system. Drought did, however, have an influence on the composition of the ectomycorrhizal community, and different mycorrhizal types responded to drought differently in terms of their patterns of occurrence/abundance. While the abundance of the dominant mycorrhizal types, formed with *Byssocorticium atrovirens* and *Lactarius subdulcis*, was not affected, drought increased the abundance of mycorrhiza formed between beech and *Xerocomus chrysenteron*. A detailed analysis of plant and fungal carbohydrates in mycorrhizas indicated that different drought intensities led to distinguishable responses. In plants exhibiting a pre-dawn water potential of down to –1.96 MPa, drought caused the accumulation of sucrose, glucose and fructose, and of fungus-specific compounds such as mannitol and arabitol in mycorrhizal roots at the expense of, e.g. trehalose. The accumulation of sugar alcohols, which constitute compatible solutes known to counteract drought stress, was species-specific. Mycorrhizas with *X. chrysenteron* formed large amounts of arabitol, while those with *L. subdulcis* accumulated mannitol. Sustained partitioning of carbon towards the mycorrhizal fungi under drought was also reflected by an increase of nitrogen storage in the fungal vacuoles. In treatments where the pre-dawn water potential reached values of as low as –2.4 MPa, such alterations were no longer found. In such plants, the starch and soluble sugars content was generally reduced, which also resulted in a lack of increase in protective, fungus-specific sugar alcohols. In summary, the data show that, within certain limits, an increase in drought causes a shift in plant/fungus communities. The shift in the pattern of fungus-specific compounds could possibly be used as a sensitive measure of physiological stress imposed on this symbiosis.

Keywords Beech ecotypes · Ectomycorrhiza diversity · Fungal carbohydrates · Drought resistance · Nitrogen storage

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Introduction

Beech (*Fagus sylvatica*) covers more than 20% of the forest surface in south-western Germany (Baden-Württemberg) and is one of the most important tree species in this area (Anonymous 1996). Different ecotypes, adapted to local environments, have evolved naturally (Müller-Stark 1997). In central Europe, the optimal conditions for the growth of beech are 8°C annual mean temperature and more than 800 mm mean annual precipitation. Water availability is one of the main limiting factors for beech productivity and vitality (Ebert 1996). Increasing average temperatures and decreasing total precipitation thus threaten the growth of beech. Due to the large contact area between fungal hyphae and soil particles, mycorrhiza formation can improve water availability for the host plant. This is documented by the work of Duddridge

et al. (1980), who showed that water taken up by the fungus is transported to the plant through the mycelia of the ectomycorrhizal fungus. Mycorrhiza-facilitated water uptake can also support acclimatisation of woody plants to drought stress (Davies et al. 1996). It can be assumed that under conditions of limited water supply such plants perform net photosynthesis for a longer period, and thus are able to deliver photoassimilates to their fungal partners for a longer time. We know that the fungal partner can regulate carbon transfer by increasing the capacity for the uptake of host-derived hexoses (Nehls et al. 1998), which in turn leads to the accumulation of fungus-specific sugars and sugar alcohols (Hampp and Schaeffer 1999). The latter are "compatible solutes" that can increase the drought resistance of fungal cells. Such solutes do not interfere with cellular structure and function. Sugars and sugar alcohols are osmotically active and can structurally and functionally preserve proteins and membranes below a certain limit of water content (0.3 g water/g DW) by water replacement (Hoekstra et al. 2001).

We thus hypothesise that, due to the dependency of the metabolism of ectomycorrhizal fungi on the supply of carbohydrates from the host plant, drought tolerance of ectomycorrhizas could be mutually dependent on both improved water supply by the fungus and (as a consequence) extended carbon supply by the host. While we can only speculate about the latter, the field study reported here, in which the drought tolerance of different ecotypes of beech was investigated (Wilpert et al. 1996), shows that drought stress results in a shift to fungal mycorrhiza partners, which accumulate significant amounts of compatible solutes.

Materials and methods

Experimental site

The research site is located at the "Conventwald" in the Black Forest, about 10 km north-east of Freiburg, Germany. The experimental plot (780–800 m above sea level) was a clear-cut area located within a stand of beech and scattered fir with an age of about 160–190 years. The annual precipitation was 1,216 mm in 1997 and 1,387 mm in 1998. The relative water content of the soil at a depth of 30 cm fluctuated between 24 and 35% (for details see Schraml and Rennenberg 2000).

Plant material

Five ecotypes of beech trees representing different local climates (precipitation between approximately 800 and 1,400 mm year^{-1}) were investigated. Four of these originated from different areas of Baden-Württemberg (Zwiefalten, Überlingen, Forbach, Ravensburg; local gene resource program), and one was a local type (Conventwald; Volkmer 1999; Schraml and Rennenberg 2000). All plants were grown from specified seed material. In 1996, about 100 seedlings (2–4 years old) of each of the ecotypes from a nursery and the local type from Conventwald were transplanted randomly in multiple plots in the experimental area. The distance between individual plants was about 1.5 m. Roots from mature trees did not interfere.

Design of the experiment

After 1 year of acclimatisation, artificial drought was induced in two consecutive years (1997 and 1998) for a duration of 3 months (from July to September) by covering half the trees with plastic roofs with good permeability for UV light (less than 20% absorption between 300 and 380 nm, 1.5–2 m above ground; BP Chemicals, PasTec, Pfullingen, Germany). Tree ecotypes growing under natural conditions served as controls (Schraml and Rennenberg 2000). Drought was verified by measuring pre-dawn water potentials of the leaves. With values of –1.18 (control) and down to –2.4 MPa (water-exclusion), the water potential was significantly different (Schraml and Rennenberg 2000).

Sample acquisition and morphotyping

For sampling (at the end of treatment; beginning of October), the whole root system of up to four individual trees of each ecotype and treatment, together with the adhering soil core, was carefully removed from the plots. The samples were stored individually in plastic bags in ice. Before processing, all root samples were soaked in ice-cold water for about 20 min. The soil debris was washed away cautiously and gradually, using fine forceps and paintbrushes. Then, all the fine roots supporting ectomycorrhizas were cut from the main root system and kept in an ice slurry for morphotyping using a dissecting microscope. Short roots with mycorrhizas were separated, photographed and classified. Based on the morphology (Agerer 1987–1998) and sequence analyses of internal transcribed spacer and a partial fragment of the large subunit of rRNA genes (Shi 2000), 11 ectomycorrhizal types were identified to species level: *Byssocorticium atrovirens*, *Cenococcum geophilum*, *Cantharellus tubaeformis* (*Fagirhiza oleifera*), *Laccaria amethystina*, *Lactarius subdulcis*, *Russula ochroleuca*, *Sphaerozone ostiolatum*, *Tomentella badia*, *Tomentella ramosissima* (*Fagirhiza setifera*), *Tomentella sublilacina* and *Xerocomus chrysenteron*. Seven species were identified down to the genus level: *Lactarius* spp. (3), *Melanogaster* sp. (1), *Tricholoma* spp. (2) and *Tomentella* sp. (1) (*Fagirhiza spinulosa*) (Agerer 1987–1998). In order to compare the relative abundance of the different morphotypes, the relative percentage of abundance of a given type was estimated by comparing the number and amount of mycorrhizas of this type with all other mycorrhizal tips from the whole root system. All members of a single morphotype from a single tree were placed in a Petri dish, quick frozen in liquid nitrogen, and kept at –80°C until freeze-drying and subsequent analysis. Together with the known types, aliquots of unknown types were taken and kept in Eppendorf tubes at –20°C for molecular identification (Shi 2000).

Determination of metabolites

Only freeze-dried root systems were used for analysis (Hampp et al. 1998). Preparation of mycorrhizas from these samples was under controlled conditions (20°C, <40% relative humidity) using a dissecting microscope. Mycorrhizas collected this way were pooled for each type and individual root system and homogenised under liquid nitrogen, using a Microdismembrator (Braun, Germany; Hampp et al. 1998). The resulting dry powder was used for all types of analysis, except for nitrogen-containing bodies (see below).

Soluble sugars and sugar alcohols

Extraction of soluble sugars and sugar alcohols from 2 mg of powdered mycorrhizas and analysis by high performance anion exchange chromatography [Dionex high performance liquid chromatography system (DX 500) with electrochemical detector (ED 40)] was performed according to Schaeffer et al. (1995). The samples were separated on a Carbon-Pac PA1-column (4×250 mm, Dionex)

equipped with two guard columns (Ion-Pac, NG1, 4×35 mm and Carbo-Pac, PA1, 4×35 mm, Dionex). A Dionex eluent degassing system (EQ1 eluent organiser) was employed to sparge the eluents with helium. Eluent 1 was 300 mM NaOH, eluent 2 was high-purity deionised water (Seral). After equilibration, 10 µl sample extract was injected manually and sugars and sugar alcohols were separated at a flow rate of 1 ml/min. After 5 min of isocratic separation (eluent 1, 0.5%), the concentration of eluent 1 was increased by addition of eluent 2 (99.5%) to 50% in 30 min and kept stable for the following 5 min. A post column system (PC 10 Pneumatic controller; Dionex) was installed between the separation column and the detector cell to deliver eluent 1 and thus to minimise the drift of the baseline. Identification and quantification of peaks was according to standards.

Glycogen and starch

Extraction of glycogen and starch was performed according to Wallenda (1996). About 1–2 mg freeze-dried material or dried pellets from the extraction of ergosterol or sugars were suspended in 30 µl 0.5 M NaOH, and incubated under sonication in a water bath at room temperature for 1 h. The extract solution was then kept at 95°C for 5 min to selectively destroy free glucose (Mendicino 1960), and an equal volume of 1 M acetic acid was added to the extracts to adjust the pH to 4.7, which is the optimal pH for the starch- and glycogen-degrading amyloglucosidase. Finally, the extract was centrifuged at 11,000 rpm for 10 min, and the supernatant was used for the enzymatic determination of glycogen and starch (Outlaw and Manchester 1979) using a microplate reader (SLT, Salzburg, Austria).

Ergosterol

Ergosterol, a membrane component of fungi, which is used for the quantitation of fungal biomass (Salmanowicz and Nylund 1988), was analysed by high performance liquid chromatography according to Martin et al. (1990). About 2 mg freeze-dried material was suspended in 1 ml 99% ice-cold ethanol, incubated on ice under continuous shaking for 30 min, and centrifuged at 4°C and 11,000 rpm for 15 min. The supernatant was used for analysis (Wallenda 1996).

Vacuolar nitrogen storage pool

Mycorrhizas formed by *Xerocomus badius*, *L. subdulcis* and *B. atrovirens* sampled from all the ecotypes (in October 1997) as described above were examined for the amounts of nitrogen-containing bodies in fungal vacuoles (Kottke et al. 1995). Aliquots were fixed in glutaraldehyde/formaldehyde (Karnovsky 1965) and embedded in Spurr's resin using acetone for dehydration (Kottke and Oberwinkler 1988). Longitudinal semi-thin sections were stained by crystal violet to visualise the material in the fungal vacuoles. Numbers of bodies were determined per area of hyphal sheath and Hartig net using a video system and analySIS software (SIS-Soft-Imaging, Münster; version 3.0). Ten mycorrhizas of each type of mycorrhiza, beech ecotype, and treatment were analysed. Box plots and Student's t-test were applied as statistical means (SPSS version 8.0). Identification of nitrogen in the vacuolar bodies was by electron energy-loss spectroscopy (Kottke et al. 1995).

Statistical treatment of data

A two-way ANOVA test (MACANOVA, DOS version 4.12; http://www.stat.umn.edu/macanova) was used to evaluate differences in the mean concentrations of the compounds analyzed. Due to the natural differences in the abundance of the different types of mycorrhizas, the data were computed as an unbalanced ANOVA. The sum of squares was computed sequentially and as SAS type III (option marginal: T of MacAnova) including an interaction term (which was never significant). According to these calculations we arrived at the following conclusions: in all but one case (*Lactarius*, arabitol), the effects of drought treatment are significant (P =<5% and P =<1%); with regard to the beech ecotype, all but one of the drought treatments (*Xerocomus*, mannitol: here even at P =<1%) were not significant.

Results

Effect of drought on the abundance of ectomycorrhizal types

In order to establish possible differences between the ectomycorrhizal fungal community under the two water regimes, we documented the abundance of ectomycorrhizal root tips from up to four individual trees of each ecotype under drought and control conditions. Reduced water availability had no effect on the number of ectomycorrhizal types that could be identified. On average, each individual root system exhibited 5.0±2.6 (control) and 4.6±2.4 (drought; mean ± SD) distinguishable mycorrhizal types, with numbers ranging from 3 to 10. In most cases, each individual root system was colonised by one to four dominant species (abundance >10%), and the occurrence of these dominant types differed considerably between individual trees, showing a substantial spatial variation. Species such as *X. chrysenteron*, *B. atrovirens* and *L. subdulcis*, occurred in more than 50% of the samples studied. These were followed in frequency by *Cantharellus tubaeformis*, *Laccaria amethystina*, and *Melanogaster* sp.. With regard to drought, the mycorrhizal types responded differently in terms of their frequency of occurrence. For example, drought had no effect on mycorrhizas formed with *L. subdulcis* and *B. atrovirens*, whereas it increased the abundance of mycorrhiza with *X. chrysenteron* from 23.8% to 40.1% in 1997 (Table 1). However, when ergosterol was used to determine the total amount of living fungal material associated with roots, we were not able to detect any effect of drought (data not shown).

Table 1 Effects of drought on the percentage of the occurrence of three dominant ectomycorrhizal types (%; mean ± SD). In order to compare the relative abundance of the different morphotypes, the relative percentage of abundance of a given type was estimated by comparing the number and amount of mycorrhizas of this type with all other mycorrhizal tips from the whole root system. As several types of mycorrhizas coexist within the same root system, numbers exceed 100%. Only ectomycorrhizas with an abundance >10% were documented. Data were from samples taken in 1997

Mycorrhizal types	Control	Drought
Lactarius subdulcis	43.3±36.7 (n =6)	50.0±25.7 (n =5)
Byssocorticium atrovirens	33.6±25.0 (n =7)	31.7±19.7 (n =6)
Xerocomus chrysenteron	23.8±12.5 (n =4)	40.7±29.6 (n =7)

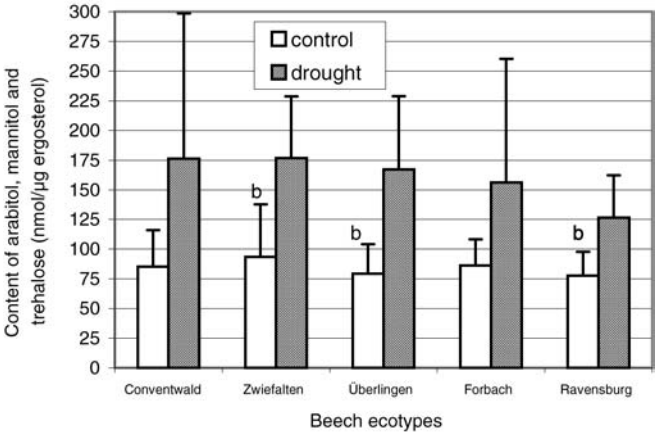

Fig. 1 Effects of drought on the pool sizes of major soluble fungus-specific compounds in ectomycorrhizas. Data are expressed as mean ± SD (n =6–8 pooled individual mycorrhizas). *b* Significant difference between treatments at P <1% level. Data are from the sampling in 1998

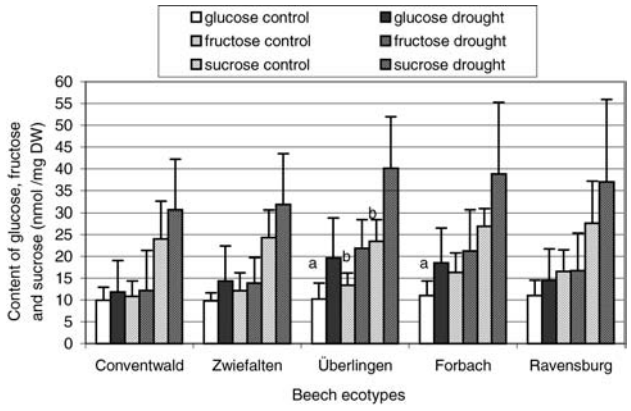

Fig. 2 Effects of drought on the glucose, fructose and sucrose content in fine roots and mycorrhizas. Data are expressed as mean ± SD (n =6–8 pooled individual mycorrhizas); *a*, *b* Significant differences between treatments at P <5% and <1% level, respectively. Data are from the sampling in 1998

Effect of drought on the content of major soluble sugars and sugar alcohols

High performance anion exchange chromatography of extracts of pooled individual mycorrhizas showed that the main soluble carbohydrates in ectomycorrhizas of beech are arabitol, mannitol, trehalose, glucose, fructose and sucrose (Figs. 1, 2). Arabitol, mannitol and trehalose are fungus-specific, sucrose is host-specific, while glucose and fructose occur in both partners. Our data show that fungus-specific compounds vary according to the type of mycorrhiza. In mycorrhizas with *X. chrysenteron*, the main fungus-specific compounds were arabitol, mannitol and trehalose, whereas in mycorrhizas with *L. subdulcis*, mannitol and trehalose dominated, and arabitol was less abundant.

In the two consecutive experimental periods (1997 and 1998), we found different patterns of fungus-specific compounds in response to drought. In 1997, when drought was severe according to the very negative pre-dawn water potential of beech leaves (–2.4 MPa; Schraml and Rennenberg 2000), there was no obvious effect on the content of these compounds in all beech ecotypes, except Zwiefalten, where they were strongly reduced (not shown). In contrast, in 1998, when the drought effect was less severe (–1.9 MPa), fungus-specific compounds were increased in all ecotypes, especially in Zwiefalten, Überlingen, and Ravensburg (Fig. 1). This difference is also reflected by the contents of soluble sugars. In 1997, drought had no significant effect on the content of glucose, fructose and sucrose (not shown), whereas in 1998, there was a tendency toward increasing levels of all these sugars, especially for sucrose. According to two-way ANOVA, differences between beech ecotypes were not significant (Fig. 2).

When individual mycorrhizas were analysed, the pool sizes of the respective compounds responded to differences in water supply in a more specific manner. Mycorrhizas formed between *X. chrysenteron* and the five beech ecotypes responded to drought in a similar way (Fig. 3). In controls, mannitol was dominant (about 100 nmol/µg ergosterol) while the content of arabitol and trehalose was very low (40 nmol/µg ergosterol, in total). Drought significantly increased the amount of arabitol (150 nmol/µg ergosterol; from 10% to around 70% of the sum of fungus-specific compounds assayed) while decreasing that of mannitol and trehalose (about 60 nmol/µg ergosterol, in total).

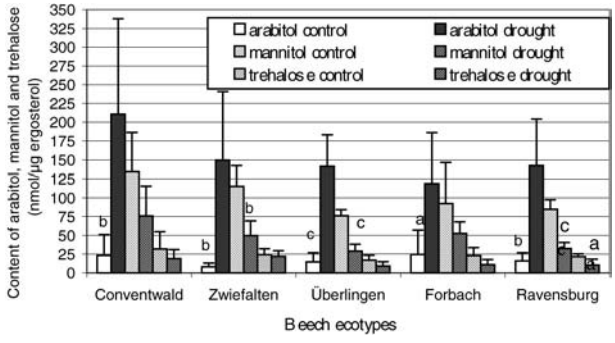

Fig. 3 Effects of drought on the main soluble fungus-specific compounds in mycorrhizas with *Xerocomus chrysenteron*. Data are expressed as the mean ± SD (n =4–6); *a*, *b*, *c* Significant differences between treatments at P <5%, <1% and <0.1% level, respectively. Data are from the sampling in 1998

In mycorrhizas with *L. subdulcis*, arabitol was of minor importance, while mannitol and trehalose were dominating. In this case, trehalose was dominant in the controls (with the exception of the ecotype Conventwald). Drought induced an accumulation of mannitol, together with a simultaneous significant decrease of trehalose (Fig. 4). The percentage of mannitol as part of all fungus-specific compounds assayed increased from about 30% (control) to about 87% (drought).

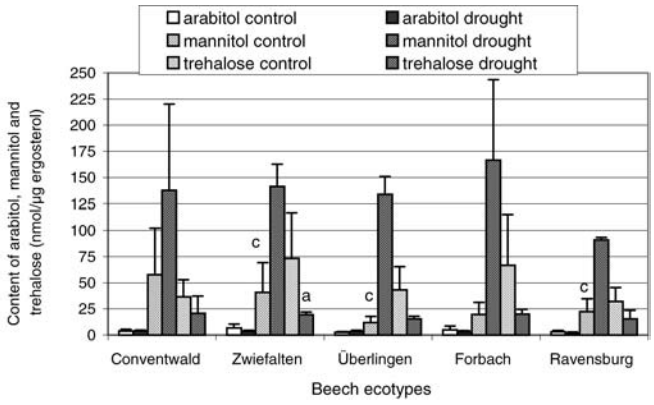

Fig. 4 Effects of drought on fungus-specific carbon compounds in mycorrhizas with *Lactarius subdulcis*. Data are expressed as mean ± SD (n =2–5); *a*, *b*, *c* Significant differences between treatments at P <5%, <1% and <0.1% level, respectively. Data are from the sampling in 1998

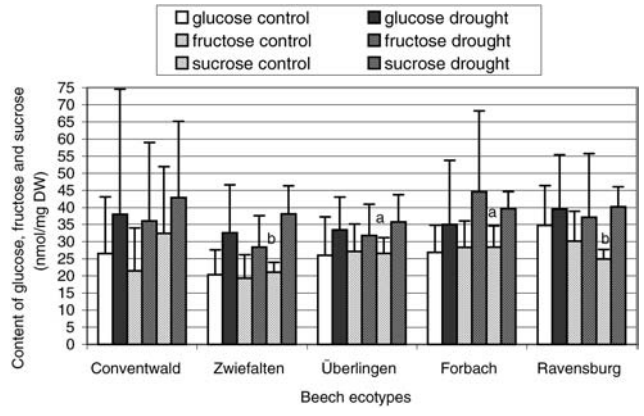

Fig. 5 Effects of drought on the glucose, fructose, and sucrose content in mycorrhizas with *X. chrysenteron*. Data are expressed as the mean ± SD (n =4–6). *a*, *b* Significant differences between treatments at P <5% and <1% level, respectively. Data were from the sampling in 1998

With regard to glucose, fructose and sucrose, the two mycorrhizal types responded to drought in a similar way. Sucrose was significantly increased in four of the five ecotypes [from 25 to 40 nmol/mg DW in mycorrhizas with *X. chrysenteron* (Fig. 5), and from 40 to about 83 nmol/mg DW in mycorrhizas with *L. subdulcis* (Fig. 6)]. There was also a tendency toward increased amounts of glucose and fructose in both mycorrhizal types (Figs. 5, 6).

Effect of drought on the amount of insoluble carbohydrates

During the 1997 sampling period, drought decreased the content of glycogen and starch in pooled mycorrhizas from the beech ecotype Conventwald (Fig. 7). This was not the case in 1998 samples (data not shown). There were differences, however, when individual mycorrhizas were investigated. In mycorrhizas formed with *L. subdulcis*, drought did not affect the content of glycogen and starch, whereas in mycorrhizas with *X. chrysenteron*, drought generally decreased the amount of these storage compounds (Fig. 8).

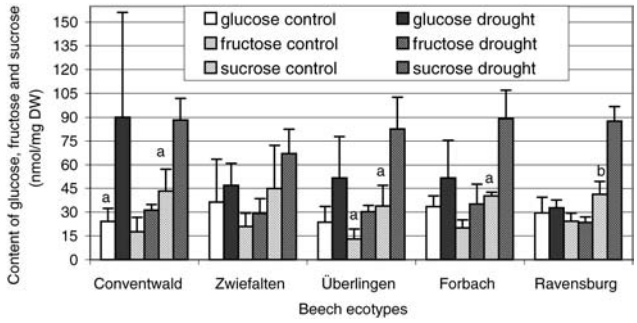

Fig. 6 Effects of drought on the glucose, fructose, and sucrose content in mycorrhizas with *L. subdulcis*. Data are expressed as the mean ± SD (n =2–5); *a*, *b* Significant differences between treatments at P <5% and <1% level, respectively. Data were from the sampling in 1998

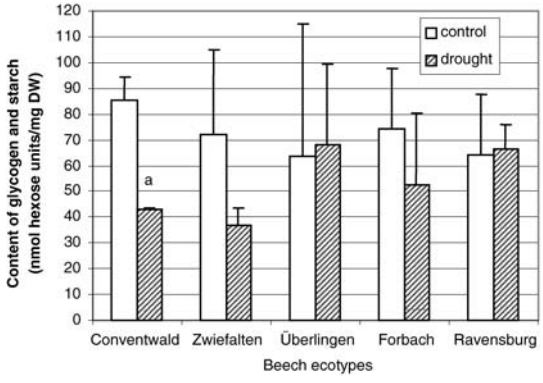

Fig. 7 Effects of drought on the glycogen and starch content in pooled mycorrhizas (samples from 1997). Data are expressed as the mean ± SD (n =2–4). *a* Significant difference between treatments at P <5%. Data are from the sampling in 1997

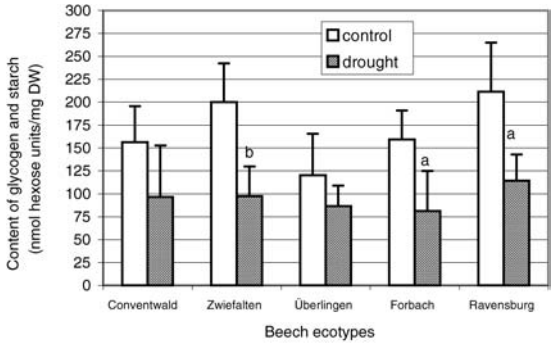

Fig. 8 Effects of drought on the glycogen and starch content in mycorrhizas with *X. chrysenteron*. Data are expressed as the mean ± SD (n =4–6); *a*, *b* Significant differences between treatments at P <5% and <1%, respectively. Data are from the sampling in 1998

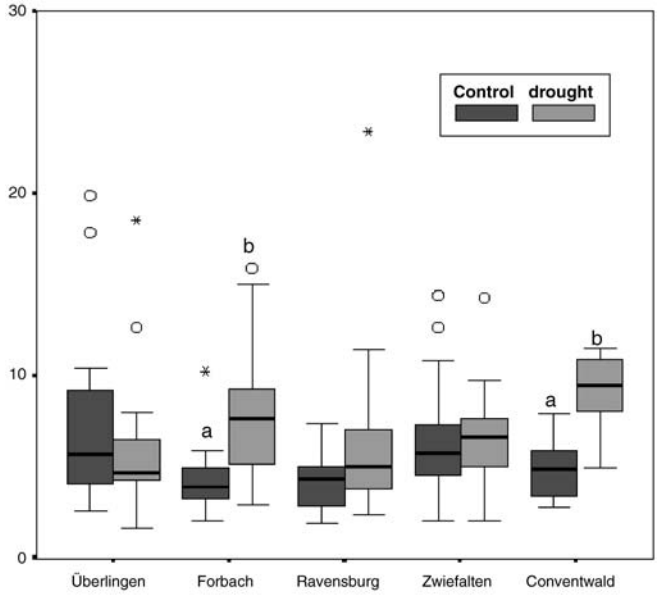

Fig. 9 Effects of drought on the amount of nitrogen-containing bodies in the fungal hyphae of pooled mycorrhizas, sampled in October 1997. The *boxplots* represent the median, the first and the third quartile (25 and 75 percentile), smallest and largest values, and extremes (*asterisks* and *circles*). *a*, *b* Significant differences between treatments at $P = 1\%$ ($n = 15–27$)

Effect of drought on the nitrogen storage pool

Drought increased the amounts of nitrogen-containing bodies in the fungal vacuoles, but no significant difference was found between the mycorrhizal types (not shown). Within the pooled samples, only a small and insignificant increase was found in the mycorrhizas of the beech ecotypes Ravensburg and Zwiefalten (Fig. 9). No change could be detected in mycorrhizas from the Überlingen ecotype (Fig. 9). In pooled mycorrhizas obtained from the ecotypes Forbach and Conventwald, the increase of vacuolar N-containing bodies was well visible and statistically significant ($P = 1\%$; Fig. 9).

Discussion

The relevance of ectomycorrhiza-forming fungi for improving the water status of their host plant has not yet been clearly shown (see Hampp and Schaeffer 1999), although some evidence does exist (Nardini et al. 2000). In endomycorrhiza, a symbiosis-dependent increase in the hydraulic conductivity of the roots, connected to a reduced stomatal resistance and thus increased rates of photosynthesis, was shown (Andersen and Rygiewicz 1991; but see Bryla and Duniway 1997, 1998). In this context, the aims of the present field study were to identify potentially more drought-tolerant fungal partners and to relate improved drought tolerance to fungal physiology.

Generally, our data show that the number of types of mycorrhizas identified was not affected by the drought treatment, suggesting that ectomycorrhizas may to some degree be protected against environmental changes by the intimate association with their host plant (Gehring et al. 1998). Drought, however, affected the abundance of single mycorrhizal types as shown by the highly variable spatial and tree-specific distribution. Under drought conditions, mycorrhizas formed with *X. chrysenteron* increased in abundance from 23% to 46%, indicating an increased drought resistance/survival rate in comparison to the other types of mycorrhizas. This possibly also had an impact on the corresponding host plant. Ecotypes of beech such as Conventwald which did not predominantly form this type of mycorrhiza suffered most from drought conditions as a biometric analysis showed (Volkmer et al. 1998; Schraml and Rennenberg 2000).

Differences in drought resistance of ectomycorrhizal fungi are well known. Drought-tolerant species, such as *Rhizopogon vinicolor* or *C. geophilum*, can grow in pure culture at water potentials of –3 MPa, whereas drought sensitive ones, such as *Laccaria laccata*, stop growing at a water potential of –1 MPa (Coleman et al. 1989). Fungal drought resistance is obviously of advantage to the host plant. For example, *R. vinicolor* decreased the effects of drought on Douglas-fir seedlings more efficiently than any drought-sensitive species (e.g. *L. laccata*) and helped seedlings recover from water stress more quickly (Parke et al. 1983). Boyle and Hellenbrand (1990) assessed the effects of mycorrhizal fungi on drought tolerance of conifer seedlings and found that fungi which grew well in pure culture under more negative water potentials, generally also showed better potential for improving performance of black spruce when compared to drought-intolerant ones. With regard to community structure, several studies have shown that water stress can result in a shift in taxa from basidiomycetes to ascomycetes (Danielson and Pruden 1989; Gehring et al. 1998; Nilson et al. 1998). Such a general shift did not occur in our study but this might be due to the relatively short experimental period (3 months).

Trehalose and mannitol are important fungus-specific compounds in ectomycorrhizal fungi (Hampp and Schaeffer 1999). Trehalose dominates in fungi belonging to basidiomycetes, whereas mannitol appears to be the preferential intermediate storage form of carbohydrates in ascomycetes (Wallenda 1996; Schaeffer et al. 1997). These two compounds also exist in ectomycorrhizas, and the same pattern of preference has been found (Wallenda 1996; Schaeffer et al. 1997). In addition, other sugar alcohols, such as glycerol, arabitol, and erythritol have been found in ectomycorrhizal fungi and ectomycorrhizas (Ineichen and Wiemken 1992; Martin et al. 1998). In our study, drought had a significant effect on the pattern of distribution of these compounds. In mycorrhizas with *X. chrysenteron* from non-covered trees, mannitol was dominant (up to 70% of all compounds analysed; Fig. 3). Drought caused a significant switch from mannitol to arabitol, the latter comprising 70% of the total pool. In mycorrhizas formed with *L. subdulcis*, controls exhibited

a dominance of trehalose, which nearly disappeared under drought, being substituted by mannitol (Fig. 4). These data indicate that there is a specific relationship between taxa and the compatible solute under stress conditions. *Pisolithus tinctorius*, belonging to the Boletales-like *Xerocomus*, also forms high amounts of arabitol (Azemar-Lorentz 1998; Martin et al. 1998).

Due to the inverse changes in pool sizes, we assume that in mycorrhizas with *L. subdulcis* the accumulation of mannitol was partly from trehalose and possibly another source. In this case mannitol could, however, also originate from the ascomycete *Leucoscypha leucotricha*. A close association of this fungus with mycorrhizas formed between beech and *L. subdulcis* has been shown by Brand (1992). Abundant ascomycete hyphae were also visible in electron micrographs of the *L. subdulcis* mycorrhizas from the different beech ecotypes (not shown). In mycorrhiza formed with *X. chrysenteron*, due to stoichiometry, arabitol could originate from mannitol, trehalose, or from glycogen (Fig. 8).

In summary, these data indicate that: (1) mycorrhizas can be distinguished according to the preferential accumulation of sugars and sugar alcohols, (2) the pattern of these fungus-specific compounds can change significantly under drought conditions, and (3) the shift in the pattern of these fungus-specific compounds could possibly be used as a sensitive measure of physiological stress imposed on this symbiosis.

In the mycorrhizal association, the host provides the fungal partner with carbohydrates, preferentially sucrose, for the formation of fungal biomass, metabolic energy and the building up of intermediate storage pools in the form of sugar alcohols or trehalose. Since the host plant has no direct access to these compounds, it is assumed that these compounds are part of a biochemical valve that could stimulate the translocation of assimilates from the host to the fungal partner (e.g. Smith et al. 1969; Hampp et al. 1995). In addition, these compounds may play an important role as compatible solutes (Lewis and Smith 1967; Jennings and Burke 1990). It has been reported that polyol accumulation correlates with resistance to stress caused by temperature, salinity, or drought in higher plants, salt stress in fungi, as well as heat, drought, or osmotic stress in bacteria and fungi (e.g. Smith et al. 1983; Jennings and Burke 1990; Kelly and Budd 1991; Adler et al. 1982; Guicherd et al. 1997). Under the conditions investigated in this study, drought induced the accumulation of mannitol and arabitol, i.e. sugar alcohols that may play a role in the compensation of drought stress, either by decreasing the osmotic potential of the cell or by protecting cellular structures (Hoekstra et al. 2001).

The disappearance of trehalose under drought could further serve to decrease the osmotic potential because, when hydrolysed, one molecule of trehalose produces two molecules of glucose, which will double the osmotic strength. However, there are also reports on trehalose accumulation in organisms exposed to drought or other stress conditions (Wiemken 1990; Müller et al. 1995), where it is thought to play a role in the protection of membranes and proteins (Crowe et al. 1984).

Independent of the type of fungus-specific compound synthesised, a prerequisite for their formation is a supply of carbohydrates from the host plant. The supply of carbohydrates is in the form of sucrose that is hydrolysed by host-derived acid invertase (for a review see Hampp and Schaeffer 1999); the resulting hexoses are then taken up by the fungus. Obviously, the supply of sucrose to mycorrhizal roots was decreased under severe drought (samples taken in 1997), but increased under less severe drought stress (samples taken in 1998; difference in predawn water potential between control and drought beeches being –0.86 MPa compared to –1 MPa in 1997; Volkmer 1999). We did not analyse sugar contents of leaves under these conditions, but Schellenbaum et al. (1998, 1999) reported that the accumulation of sucrose and trehalose in the roots of mycorrhized tobacco plants under drought stress was at the expense of hexose levels in leaves. This is probably caused by a shift in carbon partitioning toward the mycorrhized root system under stress conditions where photosynthesis still can cope with the carbon requirements.

In beech, combinations with *X. chrysenteron* and *L. subdulcis* were able to cope with drought stress better than others, i.e. this postulated sharing of protective photoassimilate was obviously of mutual benefit. Here, photoassimilates invested in the fungus possibly allowed for better protection of the fungus from drought. In parallel, plants with these types of mycorrhiza dominating showed an increased water use efficiency of photosynthesis under drought (see report by Buschmann et al. 1999).

Sustained partitioning of carbon towards the mycorrhizal fungi under drought was well reflected by the increase of nitrogen storage in the fungal vacuoles. In addition, amino acids may be synthesised at the expense of glycogen under these circumstances. This would explain in part the decrease in glycogen in the mycorrhizas of the ecotypes Forbach, Conventwald and Zwiefalten. In 1997, the larger N-storage pool of two of these ecotypes, Forbach and Conventwald, occurred in parallel with a higher water use efficiency of these ecotypes under drought stress. The potential surplus in carbon allocation to the root system could have been used for fungal amino acid synthesis. With regard to their mycorrhizas, the ecotypes Forbach and Conventwald, both from the Black Forest, were best adapted to drought stress. Although beech plants originating from the Conventwald were small and suffered most from the transplantation shock, mycorrhizas functioned well and were able to take up nutrients from the soil efficiently (as concluded from the data on N-storage). We suggest that the amount of nitrogen-containing bodies in the fungal vacuoles could be of bio-indicative value, as it should reflect the sustained ability of the host plant to allocate carbon to its fine roots. This is in line with earlier findings (e.g. Kottke et al. 1995; Turnau et al. 2001).

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