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Differential Longevities in Desiccated Anhydrobiotic Plant Systems[1]

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SYNOPSIS. Desiccation tolerance is a wide-spread phenomenon in the plant kingdom, particularly in small propagules lacking own root or rhizome system, such as seeds, pollen, spores of spore plants, and whole moss plants, but rare in whole, vascular plants. Longevities in the desiccated state vary from a few days in some pollen and spore types to many decades in some seeds and moss spores, green vegetative tissues being intermediate in that respect. Therefore, small size of a propagule does not appear to be a factor limiting life span. The formation of a glassy state in the cytoplasm upon water loss considerably increases viscosity and slows deteriorative chemical reactions. Intermolecular hydrogen bonding strength and length in the glassy cytoplasm have been suggested to play a role in desiccation tolerance and longevity. To further explore this, a comparative Fourier transform IR study among dried anhydrobiotic plant propagules belonging to different phyla was conducted. This study indicated that strong hydrogen bonding does not correlate with long life span, but rather depends on the composition of the glass forming compounds. By contrast, a large number of double bonds in the acyl chains of the polar lipids correlated with short life span. This result suggests that deteriorative processes in membranes rather than in the glassy cytoplasm determine the rate of aging of dried anhydrobiotic propagules. This would agree with the view that lipids form the only fluid or semi-fluid phase in the dried propagules, which renders them comparatively susceptible to free radical attack.

INTRODUCTION

Many plant propagules that are intended for survival/dispersal or for genetic recombination lack organs for the active uptake of water. This means that they lack the ability to actively control their own hydration status. It is, therefore, not surprising that many such propagules are endowed with mechanisms enabling them to survive periods of drought or desiccation. Particularly the microscopic propagules that are effectively dispersed via the air, such as pollen of higher plants and spores of spore-bearing plants, dry out much faster under similar environmental conditions than, for example, the much larger seeds. But even seeds are generally desiccation tolerant. Only about 10% of the seeds of all plant species are expected to experience problems as a result of water loss (Hong *et al.*, 1996). Also, green whole plants can be desiccation tolerant. This property may be particularly wide-spread in mosses, but is extremely rare in whole green plants having a developed xylem vessel system (Alpert, 2000; Alpert and Oliver, 2002; Proctor and Pence, 2002). Until now, approximately two hundred fern species and their allies, and approximately one hundred angiosperm species have been identified as being able to survive the air-dry state (Alpert and Oliver, 2002; Porembski and Barthlott, 2000). It is assumed that the appropriate rehydration via the vessel system requires specialized adaptation to avoid embolisms (Schneider *et al.*, 2003).

Desiccation tolerance is understood to include not only the ability of cells to become air-dry without loss of viability, but also to successfully rehydrate. The period of anhydrobiosis in between these two events is referred to as longevity or life span in the dried state. The definition of desiccation tolerance is rather vague with respect to the length of the dry period. However, the maximum duration of the dry period at a given temperature can be expected to be ecologically important. Anhydrobiotic specimens have been found to differ considerably with respect to their life spans in the dried state (Table 1). From a few days or weeks in some pollens (Hoekstra, 1995) and horsetail spores (Lloyd and Klekowski, 1970; Lebkuecher, 1997), survival periods can cover a few decades as in some seeds and bryophyte spores, vegetative tissues being intermediate in that respect. In the special case of *Nelumbo nucifera* seeds, longevity has been found to even exceed a 1,000 years (Shen-Miller *et al.*, 1995). However, the average seed longevity under laboratory conditions ranges from 2–10 years (Priestley, 1986). The fact that moss spores can be long-lived indicates that small size does not appear to be a factor limiting life span.

Longevity of an organism is likely to depend on the length of the unfavourable period that has to be bridged in nature. In pollen, the time from dehiscence to reaching the target is generally short. There is a specific and highly specialised target for the pollen to land on—the stigma in the case of angiosperms, or a pollination droplet or pollen collecting apparatus in the case of gymnosperms. In contrast, the propagules of the spore-bearing plants are less restricted as to their initial target site, usually the soil. Those pollen grains that fail to land on the proper site are lost, because

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TABLE 1. *Range of life spans for different dried plant propagules.*

Specimens	Life spans (approx. 25°C and 40–50% RH)
Mosses (green parts)	3 months–2 years
Mosses (spores)	up to 10 years
Ferns (spores)	few months–few decades
Horsetails (spores)	few days–2 weeks
Clubmosses (spores)	unknown
Angiosperms (green parts)	3–12 months
Angiosperms (pollens)	few days–5 months
Angiosperm (seeds)	2 years–few decades

they have little chance again to meet the appropriate vehicle for transport. After dehiscence, rainwater is generally detrimental to pollen, as it causes loss of viability, bursting, or germination at inappropriate sites (Lidforss, 1896). This extreme sensitivity to rain water is associated with the absence of dormancy, which could explain the generally short life span in pollen. By contrast, the longer lived spores of spore-bearing plants often have dormancy mechanisms similar to those encountered in seeds. They accumulate in the soil, where germination occurs only after triggering evoked by conditions that usually guarantee further growth and development.

LONGEVITY CONSIDERATIONS

Life span is the result of environmental conditions in combination with intrinsic properties of the dried anhydrobiote. It must be clear that the average longevities reported in Table 1 particularly refer to these intrinsic properties. A long life in the dried state depends on long-term structural and chemical intactness. Aging is thought to be mainly the result of free radical damage (Kranner and Lutzoni, 1999; Kranner *et al.*, 2002). The external factors temperature and water are of major importance in the production rate of free radicals and the associated rate of aging. Drying and cooling seem the obvious routes to slow down aging rate. However, the dried state prevents the possibility of repair processes, which is thought to occur in nature when the water content occasionally rises. Longevity of fern spores has thus been reported to be far better in the hydrated state than in the dried state, reaching decades (Lindsay *et al.*, 1992).

The effects of temperature and residual water content of a dried anhydrobiote become manifest in the molecular mobility of the cytoplasmic matrix (Buitink *et al.*, 1998*a*, 1999). Molecular mobility has been found to inversely correlate with longevity. On the basis of molecular mobility, longevity can be predicted for low temperatures at which determination of longevity is practically impossible (Buitink *et al.*, 2000).

In relation to the intrinsic longevity properties of anhydrobiotes, there may be several ways by which aging can be held up. One such way is curtailing the production of free radicals. This may be achieved by down regulation of the metabolism before desiccation, because the mitochondrial system is a major source of free radicals, even at low water contents (Leprince *et al.*, 2000). There is some evidence that free radical production in the dried state is less in systems that had reduced rates of respiration prior to desiccation than in systems with elevated rates (Leprince *et al.*, 1994). In this respect, pollen differs considerably from seeds. Hydrated pollens have, on average, respiration rates that are 10–60 times higher than those of hydrated seeds (*cf.*, Hoekstra and Bruinsma, 1975; Hoekstra, 1979; Miller *et al.*, 1983; Leprince *et al.*, 1999). This much higher metabolic activity might result from the high energy demand associated with rapid pollen tube growth.

Another way to control free radical production may be through slowing down chemical reaction rates and molecular diffusion in the cytoplasm by a glassy state. A glass is an amorphous, solid state with an extremely high viscosity and low molecular mobility. It melts at its glass transition temperature (*T*g). Aging has been shown to progress faster above *T*g than below (Sun, 1997; Buitink *et al.*, 1998*b*). Many cytoplasmic constituents, such as sugars, biopolymers and simple metabolites can form glasses. On drying, a glass will be formed in any cell, either anhydrobiotic or not, be it that *T*g may differ. However, it might be that there are some particular properties of a glass that allow for long survival times. We focus on this aspect in greater detail in the paragraph on hydrogen bonding length and strength.

Once free radicals have been formed, the interception of their action may constitute an additional way to free radical management. Anhydrobiotic organisms are generally loaded with antioxidants and enzymatic free radical processing systems (reviewed in Buitink *et al.*, 2002). One approach, not often considered could be the avoidance, in anhydrobiotic systems, of compounds that are extremely sensitive to free radical attack. Thus, linolenic acid content in phospholipids is generally low in seeds (Bewley and Black, 1994), but can be high in pollen (Hoekstra, 1986). The possible consequence for longevity is discussed in the paragraph on polar lipid acyl chain unsaturation. There are many other factors that are thought to relate to (macro) molecular stability in the dried state, such as Lea and heat shock proteins (reviewed in Hoekstra *et al.*, 2001; Buitink *et al.*, 2002), which are thought also to be important in the acquisition of desiccation tolerance. However, I will restrict my attention here on the two above-mentioned aspects possibly related to long-term stability in the dried state in a comparative study among anhydrobiotic specimens from widely different groups in the plant kingdom.

INTERMOLECULAR HYDROGEN BONDING LENGTH AND STRENGTH

Anhydrobiotic organisms generally are loaded with soluble carbohydrates, mainly trehalose in the case of invertebrates, and sucrose, raffinose, and stachyose in the case of plants. These carbohydrates are thought to replace the OH bonding function of water and to play

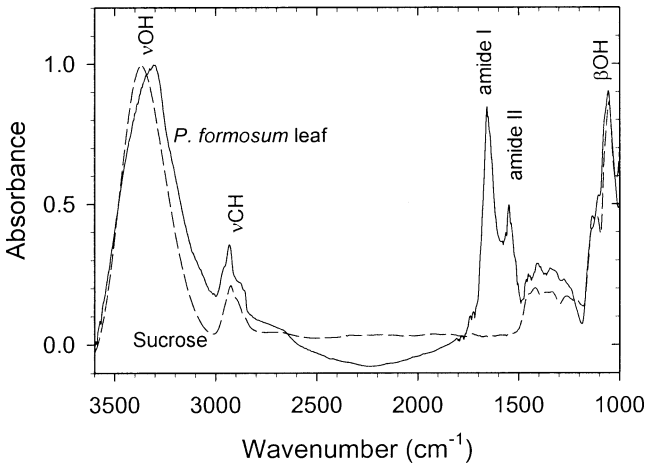

FIG. 1. FT-IR absorption spectra at 25°C (3,600 − 1,000 cm^{-1} region) of a sucrose glass and a dried moss (*Polytrichum formosum*) leaf. Five μL of a solution containing 100μg sucrose was exposed to a stream of dry air (3% relative humidity) on a circular CaF_2 window for 5 days, before hermetic closure for spectrum recording. Air-dried moss was exposed to an atmosphere of 3% relative humidity for one week before mounting a leaf between two diamond windows for spectrum recording. The OH stretching (*ν*OH) and bending (ßOH) vibration bands as well as the amide I and II bands are indicated in the figure. IR-spectra were recorded as described earlier (Wolker *et al.*, 1998*a*)

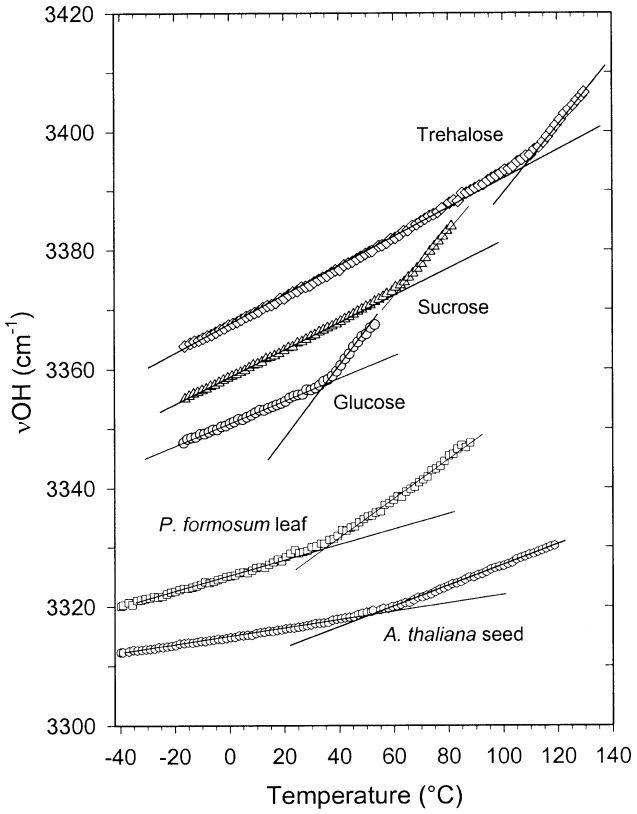

FIG. 2. Temperature plots of the wavenumber position of *ν*OH (FT-IR) for dried glucose, sucrose and trehalose glasses and for a leaf of the moss *Polytrichum formosum* and a seed of *Arabidopsis thaliana* (Columbia). The data points are from second heating scans. Procedures were as described in Figure 1 according to Wolkers *et al.* (1998*a*). Circular CaF_2 and diamonds windows were used for the sugar glasses and the anhydrobiotic specimens, respectively. The breakpoints in the curves represent *T*g.

an important role in the maintenance of the native structure of membranes and biopolymers (Crowe *et al.*, 1992, 1998). In addition, they form glasses at room temperature when the water content drops below approximately 10% of the dry weight (Crowe *et al.*, 1998; Sacandé *et al.*, 2000). Sugar glasses produced by drying have been extensively studied by Fourier transform infrared spectroscopy (Wolkers *et al.*, 1998*a*, *b*, *c*, 2001, 2004). This method can reveal glassy properties, also in dried anhydrobiotes, which might give insight into the molecular basis of the differences in longevity.

Sugar glasses

An IR-absorption spectrum of a dried sucrose glass is shown in Figure 1, in which the OH-stretching vibration band (*ν*OH) dominates. When the wavenumber position of *ν*OH is followed with temperature (Fig. 2), a break in the curve can be observed at approximately 60°C for sucrose, which has been found to represent *T*g (Wolkers *et al.*, 1998*b*). Also, the behaviors with temperature of trehalose and glucose glasses are shown in Figure 2, with breaks at 107°C and 34°C, respectively. The lines indicate the change in wavenumber position of *ν*OH with temperature. Above *T*g, these lines are steeper than below *T*g. The angle of the lines, also called Wavenumber-Temperature-Coefficient (*WTC* [Wolkers *et al.*, 1998*b*]) is characteristic for the type of sugar that formed the glass. Thus, the *WTC* values for sucrose were 0.23 and 0.54 cm^{-1}/°C, below and above *T*g, respectively. For trehalose, these values were 0.24 and 0.55 cm^{-1}/°C, and for glucose, 0.19 and 0.55 cm^{-1}/°C. Sucrose and trehalose had relatively high *WTC* and *T*g values in the glassy state as compared with glucose, and the wavenumber position of their *ν*OH, also, was higher (Fig. 2). A general trend had thus been established that with increasing molecular weight of a sugar, *T*g, *WTC*, and wavenumber position of *ν*OH become higher (Wolkers *et al.*, 2004). Above *T*g, sugars appear to have similar *WTC* values of between 0.5 and 0.6 cm^{-1}/°C (also in Fig. 2). The wavenumber position of *ν*OH at a given temperature characterizes the length of the intermolecular hydrogen bonding—a relatively high wavenumber position reflecting a greater length (Wolkers *et al.*, 2004). A low *WTC* value in the glassy state indicates that the OH vibrational energy changes little with temperature increase and is interpreted to represent a relatively strong intermolecular hydrogen bonding (Wolkers *et al.*, 2004). This is typical for low molecular weight sugars. Glasses of high molecular weight sugars are characterized by relatively low hydrogen bonding strength. As expected, a greater hydrogen bonding strength (lower *WTC*) goes together with a reduced length of hydrogen bonding (lower *ν*OH wavenumber position).

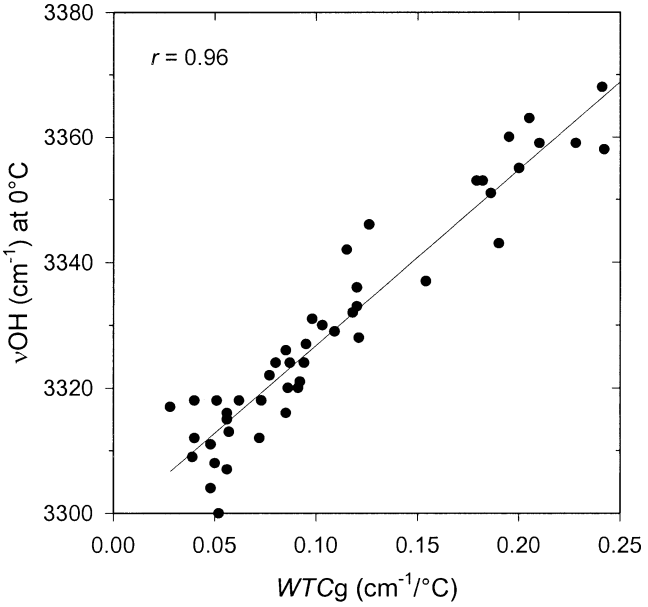

FIG. 3. Plot of the intermolecular hydrogen bonding strength (*WTC*) *versus* the wavenumber position of νOH at 0°C for different sugar glasses with and without added protein (mass ratio = 1:1). The sugars used here are fructose, glucose, surose and trehalose, and the proteins are poly-L-lysine and Lea proteins from soybean (3) and *Medicago truncatula* (2). All data (also from repeated scanning) are presented. FT-IR spectra were recorded as described earlier (Wolker *et al.*, 1998*a*) using circular CaF_2 windows. The data points are from second heating scans.

Protein/sugar glasses

The addition of a peptide (poly-L-lysine, Wolkers *et al.*, 1998*c*) or a protein (Lea from pollen, Wolkers *et al.*, 2001) to sucrose has been shown to lead to high *T*g glasses having reduced *WTC* and wavenumber position of νOH. In the case of poly-L-lysine there hardly is a spectral contribution of the peptide to the OH-stretching region of sucrose. Therefore, the increased hydrogen bonding strength and lower νOH can be entirely attributed to the interaction of sucrose with the peptide. Other proteins may confound the νOH region, also because of their greater N-H vibrations contribution in the 3200–3300 cm^{-1} region, which could disturb the linear relationship between the wavenumber position of νOH and *WTC*. However, the relationship between the two parameters was linear when testing the sugars, fructose, glucose, sucrose and trehalose alone or in the presence of 5 different Lea proteins (1:1, mass ratio) (Fig. 3, $r = 0.96$). Also above *T*g, a linear relationship was found between wavenumber position of νOH at 0°C and *WTC* (Figure not shown, $r = 0.86$), indicating that the proteins also increase hydrogen bonding strength of the mixture above *T*g. Thus, in model protein/sugar glasses, the wavenumber position of νOH as well as *WTC* report equally well on the quality of hydrogen bonding. For all the sugars tested, the Lea proteins increased *T*g, but to varying extent. The analogous effects of proteins and sugar molecular weight on *T*g, on the one hand, and the opposite effects on *WTC*, on the other hand, exclude that *WTC* and *T*g are correlated. Apart from proteins, some metabolites, particularly tricarboxylic acid salts, have been found to reduce *WTC* and increase *T*g (Kets *et al.*, 2004).

Cytoplasmic glasses

Also, FT-IR spectra from dried anhydrobiotes are characterized by a dominant νOH band, as exemplified in Figure 1 for a dried leaf of the moss *Polytrichum formosum*. From temperature scans information about the *in vivo* properties of the cytoplasmic glass can be extracted, in a similar way as for the model systems. Thus, it has been shown that the *T*g of dried leaves of the resurrection plant, *Craterostigma plantagineum*, is higher than expected on the basis of the sucrose that constitutes most of the carbohydrate reserves (Wolkers *et al.*, 1998*b*). This is proof that compounds other than sucrose also contribute to the glassy cytoplasm. Temperature plots of the wavenumber position of νOH of a dried *Arabidopsis thaliana* seed and a dried leaf of *Polytrichum formosum* are shown in Figure 2. The *WTC* and νOH values were much lower than those of the sugars. This is an indication of extensive interaction of carbohydrates with other molecules, most likely, proteins.

Using various *A. thaliana* mutant seeds that are developmentally deficient to various extents, a range of *WTC* values in the glassy state has been obtained, with the wild type having the lowest value (Wolkers *et al.*, 1998*a*). A negative correlation has thus been established between *WTC* and longevity, which suggested that there may be a causal relationship between life span and hydrogen bonding strength in the cytoplasmic glass. In addition, proteins in the most developmentally deficient mutant seeds denature at a much lower temperature than those in wild type seeds, which has been ascribed to the weak hydrogen bonding interactions in the mutant seeds. Strong hydrogen bonding, rendering an organism relatively insensitive to temperature fluctuations, thus extending longevity, would make an attractive hypothesis. However, from some of these mutant seeds it is known that they accumulate less Lea proteins than the wild type does (de Bruijn *et al.*, 1997; Bies-Etheve *et al.*, 1999). The negative correlation could thus reflect compositional differences associated with deficient seed development rather than a direct involvement of intermolecular hydrogen bonding strength in long-term stability.

In an attempt to unravel this issue, a comparative study was undertaken. FT-IR spectra were recorded from a wide variety of dried anhydrobiotic plant propagules, including pollens, seeds, the spores of ferns, mosses, horsetails and clubmosses, and leaves of angiosperm resurrection plants and mosses. From the temperature scans, the glass parameters wavenumber position of νOH at 0°C, *WTC* in the glassy state, and *T*g were calculated and are presented in Table 2. Also, the protein content and the approximate life span of the various dried propagules under laboratory conditions (approximately 20–25°C and 40–50% relative

TABLE 2. *Parameters of the glassy cytoplasm (wavenumber-temperature coefficient in the glass [*WTC*g], wavenumber position of the OH stretching vibration band at 0°C [*ν*OH] and T*g*) for various dried, anhydrobiotic plant propagules from different phyla.**

Species	*WTC*g ($cm^{-1}/°C$)	*ν*OH (cm^{-1})	*T*g (°C)	Protein (%)	Longevity (d)	Source
P. formosum spores	0.116	3,340	52.0	0.8	3,500	F.A.H., unpublished data
H. vulgare seeds	0.119	3,320	53.2	18.1	2,600	Priestley (1986)
A. thaliana seeds	0.051	3,310	42.7	20.1	2,100	Hay *et al.* (2003)
C. pumilum rootstock	0.244	3,347	41.8	2.2	700	F.A.H., unpublished data
C. purpureus leaf	0.217	3,356	40.3	4.2	700	F.A.H., unpublished data
A. filix femina spores	0.051	3,321	37.9	2.0	600	Lindsay *et al.* (1992)
C. pumilum leaf	0.221	3,350	40.1	1.9	350	F.A.H., unpublished data
P. sylvestris pollen	0.131	3,327	57.0	9.9	279	Pfundt (1910)
M. flabellifolius leaf	0.199	3,354	50.0	3.1	250	Farrant and Kruger (2001)
B. spicant spores	0.028	3,310	24.3	5.1	240	Lindsay *et al.* (1992)
Q. ilex pollen	0.105	3,326	54.6	5.2	150	F.A.H., unpublished data
T. latifolia pollen	0.159	3,329	49.4	7.6	150	Hoekstra (1986)
N. tabacum pollen	0.107	3,307	56.7	20.0	100	F.A.H., unpublished data
N. poeticus pollen	0.068	3,303	72.2	20.8	83	Hoekstra (1986)
P. formosum leaf	0.151	3,329	41.3	4.0	70	F.A.H., unpublished data
P. rhoeas pollen	0.072	3,308	51.1	23.5	36	Hoekstra (1986)
E. arvense spores	0.071	3,308	45.2	21.4	14	Lebkuecher (1997)
I. glandulifera pollen	0.068	3,313	25.5	5.9	10	Hoekstra (1992)
S. cereale pollen	0.169	3,335	65.0	9.4	0.25	Lichte (1957)
L. clavatum spores	0.058	3,336	67.7	5.2	?	
L. annotinum spores	0.092	3,348	71.6	5.3	?	

* Protein content of the propagules and longevity in the dried state are also indicated, including the source from which longevity data were extracted. Longevity is expressed as the number of days until half or less of the original viability is left. FT-IR spectra were recorded as described earlier (Wolkers *et al.*, 1998*a*) using diamond windows. The data obtained by FT-IR spectroscopy are from second heating scans. Protein content was determined as in Hoekstra and Bruinsma (1975). The specimens used were the mosses, *Polytrichum formosum* and *Ceratodon purpureus*, the clubmosses *Lycopodium annotinum* and *Lycopodium clavatum*, the ferns, *Athyrium filix-femina* and *Blechnum spicant*, the horsetail *Equisetum arvense*, and the angiosperms *Arabidopsis thaliana*, *Craterostigma pumilum*, *Hordeum vulgare*, *Impatiens glandulifera*, *Myrothamnus flabellifolius*, *Narcissus poeticus*, *Nicotiana tabacum*, *Papaver rhoeas*, *Pinus sylvestris*, *Quercus ilex*, *Secale cereale* and *Typha latifolia*.

humidity) are indicated, including the source from which the information on longevity was extracted. Unfortunately, longevity data of spores are scarce. For example, I could not find longevity data for *Lycopodium* spores.

From Table 2 it can be learned that, in contrast with what would be expected, there was no correlation between intermolecular hydrogen bonding strength in the glassy cytoplasm and life span (r of linear fit = −0.05). Short-lived pollen and horsetail spores had particularly low *WTC* values in the glassy state (*WTC*g), *i.e.*, strong hydrogen bonding. Also, there was no correlation between the wavenumber position of *ν*OH and life span (r of linear fit = 0.14), but there was a positive linear correlation between the parameters *WTC* and *ν*OH (r = 0.76; graph not shown) as in the model glass/protein systems presented in Figure 3. Also, the *WTC* values above *T*g correlated positively with *ν*OH (r of linear fit = 0.73). This means that when *WTC* values were low below *T*g, they were also comparatively low above *T*g. This is typical for proteins in model sugar-protein glasses. In the dried specimens from Table 2 characterized by relatively low hydrogen bonding strength, proteins were never found to denature up to a temperature of 120°C, in contrast with what Wolkers *et al.* (1998*a*) reported for the *A. thaliana* developmentally deficient mutant seeds. It can be concluded that strong hydrogen bonding interactions in the glassy state are not linked with survival, at least on the basis of comparisons among anhydrobiotic plant propagules of different phyla.

An attempt to link low *WTC*g and *ν*OH values with elevated protein contents indicated that there was indeed such a trend (Fig. 4; r of the linear fits were −0.42 and −0.74, respectively). Some spores and pollen had high protein contents of over 20% of their dry weight. These propagules always had low *WTC* values. Some others had less protein, but nevertheless low *WTC* values. This may be explained by the presence of abundant oil in some of the propagules (clubmoss and fern spores) or the presence of some metabolites. Oil does contribute to the dry weight, but excludes the protein from its environment. This may mean that locally the glass could nevertheless have high protein content. Low oil and protein specimens, such as *C. pumilum* leaves and rootstock, and leaves from other anhydrobiotic plants, typically had high *WTC* and *ν*OH values. The forgoing reveals the general drawback of content measurements without information about the exact cellular location of the compound under investigation. In conclusion, it might be possible that *WTC*g and *ν*OH are controlled by the protein content in the glassy cytoplasm.

In model systems, proteins tend to increase *T*g of sugar glasses (Wolkers *et al.*, 1998*b*). Because Figure 4 suggested a trend for low *WTC*g and *ν*OH values in the high protein specimens, *T*g could be high in these specimens. The *T*g values measured for the propagules

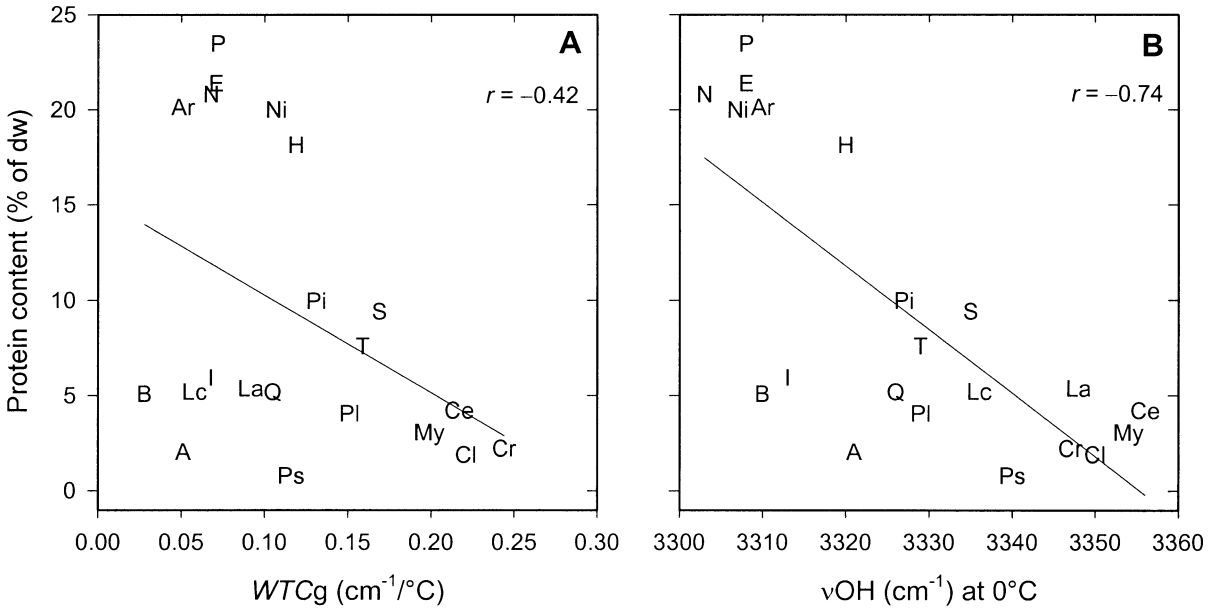

FIG. 4. Plot of the protein content of various dried plant propagules *versus* hydrogen bonding strength in the glass (*WTC*g) (A) and wavenumber position of *ν*OH at 0°C (B). The abbreviations in the graphs are as follows: **A,** *Athyrium filix-femina* spores; **B,** *Blechnum spicant* spores; **Ce,** *Ceratodon purpureus* leaf, **CI** and **Cr,** *Craterostigma pumilum* leaf and root stock, respectively; **E,** *Equisetum arvense* spores; **I,** *Impatiens glandulifera* pollen; **La,** *Lycopodium annotinum* spores; **Lc,** *Lycopodium clavatum* spores; **N,** *Narcissus poeticus* pollen; **Ni,** *Nicotiana tabacum* pollen; **P,** *Papaver rhoeas* pollen; **PI** and **Ps,** *Polytrichum formosum* leaf and spores, respectively; **Q,** *Quercus ilex* pollen; **S,** *Secale cereale* pollen; **T,** *Typha latifolia* pollen. IR-spectra were recorded as described earlier (Wolkers *et al.*, 1998*a*) using diamong windows. The FT-IR data are from second heating scans. Protein contents were determined as in Hoekstra and Bruinsma (1975).

in Table 2 were plotted against the two parameters (figure not shown). However, there was no correlation between *T*g and both parameters. This could be the result of the fact that *T*g is much influenced by traces of residual water in the specimens, but since the *T*g measurements are from second heating scans it could be expected that differences in residual water be minimized. There was also no correlation between longevity and *T*g (figure not shown; $r = 0.03$).

POLAR LIPID ACYL CHAIN UNSATURATION

It is reasonable to assume that the aging rate depends on the decay rate of the most sensitive component in a dried anhydrobiote. The aging rate increases as a result of a rise in temperature and/or water content, which has been shown to correlate with an increased molecular mobility in the (glassy) cytoplasm (Buitink *et al.*, 2000). Although it seems attractive to think that these features are causally linked, it has to be kept in mind that water and temperature influence mobility of membrane components in a similar way that of the glassy cytoplasm (Golovina and Hoekstra, 2002, 2003; E.A. Golovina and F.A.H., unpublished data). In addition, the glassy cytoplasm is far more immobile than the acyl chain area of membranes in dried anhydrobiotes. These acyl chains are thought to retain a sort of mobile state at room temperature as a result of the interaction of sugars with the polar headgroups (Crowe *et al.*, 1992). Therefore, membranes might be more easily accessible to free radicals than the glassy cytoplasm. Moreover, the acyl chains of the polar lipids may contain (poly)unsaturated bonds, which are known to be particularly sensitive to free radical attack.

Some twenty years ago, I found that pollens that germinate within a few minutes after hydration have membrane lipids that consist of up to 70% of the polyunsaturated fatty acid, linolenic acid (18:3). These pollens appeared to be extremely short-lived, although they were generally desiccation tolerant (Hoekstra, 1986). In contrast, the low linolenic acid conifer pollens have relatively long lag periods, exhibit slow tube growth, and are relatively long-lived. It has been argued that pollen tube competition has led to an evolutionary trend towards short lag times and great speed of tube growth through the style (Mulcahy, 1974; Hoekstra, 1986). Insect pollination, which allows vast numbers of pollen to be deposited on the stigma once the flower is visited, is thought to promote selection in the haploid phase of the angiosperm life cycle. Poorly performing pollen grains are thus eliminated and metabolically vigorous ones are advanced. This mechanism may have contributed to the dominance of the angiosperms (Mulcahy, 1979). The reason why wind-pollinated coniferous pollens have relatively long lag periods and slow tube growth might be the result of the fact that pollination is more erratic in wind pollinated flowers, which discourages pollen competition.

The link between high levels of linolenic acid in the membranes and fast tube growth might be explained as follows. Pollen tube growth occurs at the tip, where

cell wall precursors are transported over the plasma membrane to the outside via exocytosis. The greater membrane fluidity that results from the high degree of poly-unsaturation is supposed to expedite this process. A reduced life span may be the price that has to be paid, but for pollens lacking dormancy, this might not be an evolutionary disadvantage. For seeds, having a dormancy system, a reduced life span would pose problems. Thus, the level of linolenic acid in the polar lipids is expected to be considerably less in seeds than in pollens.

To test a possible general validity of a high degree of unsaturation of the polar lipids acyl chains limiting life span, a comparative study was undertaken. Fatty acids compositions were determined from a wide variety of dried anhydrobiotic plant propagules, mainly from the organisms listed in Table 2 and some additional specimens from the literature. For simplicity only the double bond index (DBI), representing the average number of double bonds per polar lipid molecule is given. Figure 5 shows the correlation between longevity and DBI. There was a trend that long-lived specimens contain relatively low numbers of double bonds in their polar lipids acyl chains, the sacred lotus seeds (*N. nucifera*) having the lowest. The short-lived specimens are characterized by a considerable poly-unsaturation of their acyl chains. Apart from pollen, also the chloroplast-containing horsetail spores are extremely short-lived. Furthermore, the leaves of resurrection plants contained a relatively large number of double bonds in their polar lipids, which is a general characteristic of chloroplasts (Somerville *et al.*, 2000). The life span of these specimens is generally limited to a few years maximally (Fig. 5). The rootstock of *C. pumilum* thus had a longer life span than its leaves. The negative correlation between longevity and DBI suggests that for a long lifespan the number of double bonds should be minimized.

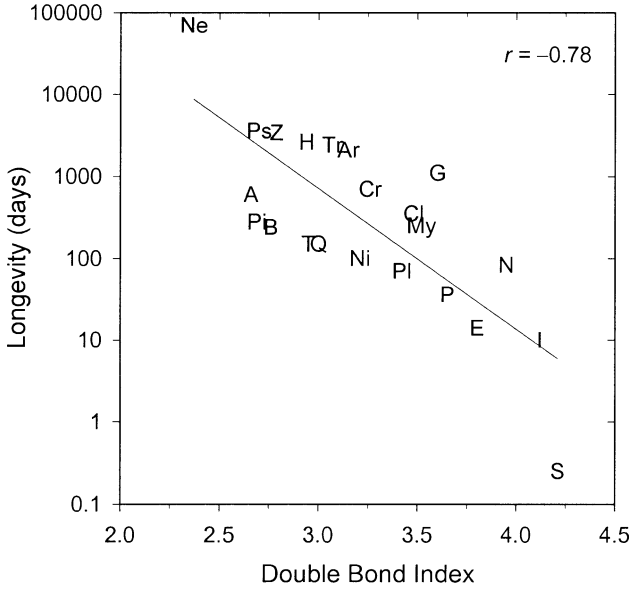

FIG. 5. Correlation between longevity of various dried plant propagules and average number of double bonds per polar lipid molecule (Double bond Index). Longevity is expressed as the number of days until half or less of the original viability is left (storage conditions approx. 25°C and 40–50% relative humidity). The abbreviatinos in the graphs, followed by a reference for longevity, are as follows: **A,** *Athyrium filix-femina* spores (Lindsay *et al.*, 1992); **Ar,** *Arabidopsis thaliana* (Columbia) seeds (Hays *et al.*, 2003); **B,** *Blechnum spicant* spores (Lindsay *et al.*, 1992); **CI** and **Cr,** *Craterostigma pumilum* leaf and root stock, respectively (F.A.H, unpublished data); **E,** *Equisetum arvense* spores (Lloyd and Klekowski, 1970; Lebkuecher, 1997); **G,** *Glycine max* axis (Priestley, 1986); **H,** *Hordeum vulgare* axis (Priestley, 1986); **I,** *Impatiens glandulifera* pollen (Hoekstra, 1992); **M,** *Myrothamnus flabellifolius* leaf, (Farrant and Kruger, 2001; Kranner *et al.*, 2002); **N,** *Narcissus poeticus* pollen (Hoekstra, 1992); **Ne,** *Nelumbo nucifera* seeds (Priestley, 1986); **Ni,** *Nicotiana tabacum* pollen (F.A.H., unpublished data); **Pi,** *Pinus sylvestris* pollen (Pfundt, 1910); **P,** *Papaver rhoeas* pollen (Hoekstra, 1992); **PI** and **Ps,** FA Hoekstra *Polytrichum formosum* leaf and spores, respectively (F.A.H., unpublished data); **Q,** *Quercus ilex* pollen (F.A.H., unpublished data); **S,** *Secale cereale* pollen (Lichte 1957); **T,** *Typha latifolia* pollen (Hoekstra, 1992); **Tr,** *Triticum aestivum* axis (Priestley, 1986); **Z,** *Zea mays* axis (Priestley, 1986). Determinations of the polar lipid acyl chain composition were carried out as in Hoekstra *et al.* (1992). Literature data on acyl chain composition of *Arabidopsis thalinana* (Columbia) seeds, *Glycine max* axes, *Nelumbo nucifera* seeds and *Zea mays* axes are from Lemieux *et al.* (1990), Wang *et al.* (1989), Priestley and Posthumus (1982) and Chen and Burris (1991), respectively.

CONCLUDING COMMENTS

Although hydrogen bonding strength in the cytoplasmic glass of dried *A. thaliana* (Ler) mutant seeds has been shown to correlate with level of desiccation tolerance and longevity (Wolkers *et al.*, 1998*a*), this trend could not be established when anhydrobiotic specimens of widely different groups in the plant kingdom were compared. From Figure 4, it appears that, at least in part, the protein content could be responsible for the strong hydrogen bonding. Variation in protein content might thus explain the observed relationship in the developmentally deficient *A. thaliana* mutant seeds, from which it is known that they are precociously blocked in their protein accumulation (de Bruijn *et al.*, 1997; Bies-Etheve *et al.*, 1999). Among the accumulating proteins are those that are involved in the acquisition of desiccation tolerance. Also, the accumulation of compounds other than proteins, such as membrane-soluble antioxidants could have been terminated precociously in the mutant seeds. The lack of correlation between longevity and glassy parameters as shown in Table 2 may indicate a lack of a general strategy of increasing life span via improvement of the hydrogen bonding strength. This does not exclude that molecular stability might be superior in strong hydrogen bonding glasses. Alternatively, the lack of correlation might indicate that the glassy cytoplasm is not the system that is the most sensitive to aging.

The most rapid aging is likely to occur in the phase that is most mobile. Lipid bodies and membranes make up such a phase. Particularly the integrity of membranes is important for an anhydrobiote to properly function in the rehydrated state, because even a slight loss of integrity has a major impact on its biochemical functioning, for example, the failure to gen-

erate chemical energy. It is clear that for a long life span, large amounts of poly-unsaturated fatty acids in membranes should be avoided because of the extreme sensitivity to oxidation reactions. The short-lived pollens and *Equisetum* spores have comparatively high DBI values (Fig. 5), mainly because of the contribution of linolenic acid of up to 70% of the total. It is striking that chloroplast-containing fern spores that lack dormancy also have short life spans (Lloyd and Klekowski, 1970). These authors have argued that the chloroplasts are responsible for the short life span, but that the faster germination of these green spores outweigh the disadvantage of a short life span. It can be expected that such spores contain large amounts of poly-unsaturated fatty acids, because this is a characteristic of chloroplasts (up to 70% of linolenic acid [Sommerville *et al.*, 2000]). The question remains whether these spores have short life spans because of a high number of double bonds, or because the presence of chloroplasts implicates the risk of generation of free radicals. In this respect it is interesting to consider whether poikilochlorophylly—when specimens reversibly lose their chlorophyll and dismantle their chloroplasts—is aimed at lowering poly-unsaturation levels or avoiding the generation of free radicals associated with illumination of dried chloroplasts, or both. In the case of the PUFA-rich, short-lived pollens, the chloroplast issue does not play a role. On the other hand, both short lived pollen and green spores are characterized by high metabolic potential. From the foregoing it emerges that membrane associated processes could play an important role in aging, but that the quality of glasses is less important in that respect.

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