26 Evolutionary Consequences of Transition to a Lichen Symbiotic State and Physiological Adaptation to Oxidative Damage Associated with Poikilohydry

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I.	Introduction	592
П.	 Origin and Evolution of Lichen Symbioses A. Transitions to a lichen symbiotic state are rare in fungi B. Consequences of a transition to a lichenized state C. Stresses associated with a transition to a lichen symbiosis D. Fungal predispositions for a transition to a mutualistic state E. Correlation between mutualism and increased oxidative stress 	592 592 594 597 598 599
ш.	 Physiological Adaptation to Oxidative Damage Associated with Poikilohydry A. Toxic effects of oxygen on plant and fungal tissues B. Dehydration leads to the formation of free radicals C. DNA damage caused by desiccation D. Physiological adaptation to desiccation requires a complex interplay of several adaptational mechanisms including protection from oxidative damage E. Free-radical-scavenging pathways F. Physiological adaptation to desiccation-induced oxidative damage G. Glutathione plays a major role for desiccation-tolerant plants and lichens 	600 601 602 603 606 608 612
IV.	Summary	618
	References	620

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Kranner and Lutzoni

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I. INTRODUCTION

Lichenization is one of the most successful ways whereby fungi fulfill their requirement for carbohydrates, with about one-fifth of all known species of fungi being lichenized (Hawksworth 1988a), i.e., forming an obligatory mutualistic association with a photoautotrophic green alga and/or a cyanobacterium. Although lichen-forming fungi account for a major part of fungal diversity, these obligatory symbiotic species are not randomly distributed within the fungal kingdom. Lichenization is almost restricted to the Ascomycota (ascolichens), which claims more than 98% of all lichen-forming species (Hawksworth 1988b). Also, lichenized species are concentrated in specific lineages within the Ascomycota. Although 42% of all known Ascomycota species are lichenized, only 15 orders out of 46 include lichen-forming taxa (Hawksworth et al. 1995). The largest portion of lichen diversity is classified within a single order, the Lecanorales, suggesting that a large part of lichen-forming ascomycetes share a common ancestor. The remaining lichen-forming fungi are mostly members of the Basidiomycota (basidiolichens), representing less than 2% of lichen diversity. Only 6 of the 1428 genera classified in the Basidiomycota include lichen-forming species (Hawksworth et al. 1995; Oberwinkler 1984).

A large portion of lichen-forming fungi are heliophilous, and most are xerophilous compared with their nonlichenized relatives. Moreover, the fungal component of lichens is in constant contact with an oxygen-producing alga and/or cyanobacterium during its entire life cycle, except for the sexually generated spores. Consequently, fungal lineages that were involved in a transition to a lichenized state are likely to have been exposed to higher levels of solar radiation, desiccation, and ambient oxygen generated by the photobiont. These stresses are associated with increased formation of free radicals. Given the poikilohydric and heliophilic nature of most lichens, it is very likely that a fungal lineage with adequate protection mechanisms against solar radiations, desiccation, and oxidative stresses is well-suited to forming and maintaining a mutualistic association with an oxygenproducing, heliophilic photobiont. Within a phylogenetic framework, Section II of this chapter explores the predispositions and consequences on fungal lineages that have survived various stresses associated with a transition to a lichenized state. To overcome extreme drought (some lichens can survive in a desiccated state for many years), lichens are equipped with extraordinary mechanisms protecting them from oxidative stress. Among these, a powerful antioxidant system is found. Therefore, Section III of this chapter is focused on the phenomenon of desiccation tolerance. A review of mechanisms that protect desiccation-tolerant organisms from the deleterious effects of drought is provided. Given the paucity of papers published on desiccation-induced oxidative stress and on antioxidant protection in lichens, we will discuss these rare reports along with the more abundant papers published on other desiccation-tolerant organisms, such as resurrection plants and dormant seeds.

II. ORIGIN AND EVOLUTION OF LICHEN SYMBIOSES

A. Transitions to a Lichen Symbiotic State Are Rare in Fungi

Phylogenetic studies on lichenized and nonlichenized fungal species aimed at studying the origin and evolution of lichenization can provide important clues to determine the nature and intensity of the initial stress on the fungal and photobiotic (green alga and/or cyanobacterium) partners in lichens. If successful transitions to a lichenized state occurred frequently during the evolution of ascomycetes and basidiomycetes this would indicate that

this type of transition is simple with low stress on the symbionts. On the other hand, if most of the lichens' diversity was derived from one or very few independent origins of lichenization, this would suggest that this transition to a symbiotic state is complex and initially involves high stresses on at least one of the symbionts.

The acceptance by the mycological and lichenological community, more than 12 years ago, that some basidiomycetes associated with green algae or cyanobacteria are lichens, simultaneously established, for the first time, that lichens (including both ascoand basidiolichens) originated more than once (Lutzoni et al. in press). At that time, it was also known that more than one independent transition to a lichenized state took place within the Basidiomycota. This was because of the paucity of basidiolichen species and their affiliation with drastically divergent groups within this phylum (e.g., coral fungi versus mushrooms). Because more than 98% of the diversity of lichens is within the Ascomycota, one crucial question needs to be answered: How many independent origins of lichens took place during the evolution of Ascomycota?

Based on a cladistic analysis of nucleotide sequences from the small subunit nrRNA gene for 7 lichenized ascomycetes, representing 3 of the 15 orders of ascomycetes that include lichenized taxa, and 34 nonlichenized ascomycete species, representing 14 orders of ascomycetes out of 31 orders that do not include lichenized taxa (Hawksworth et al. 1995), Gargas et al. (1995) concluded that there were multiple origins of lichen symbiosis within the Ascomycetes. They detected two independent origins for ascolichens, but predicted that at least four more would be revealed as more orders containing lichen-forming species would be sampled.

Spatafora and Lutzoni (Spatafora et al. 1994; Lutzoni et al. 1996, and in press), using nucleotide sequences from the small subunit nrRNA gene, selected 74 taxa, representing 19 orders of the phylum Ascomycota. Eleven of these orders include lichen-forming ascomycetes. Depending on the evolutionary model used for the cost of gains and losses of lichenization, Lutzoni et al. (in press) indicated that the number could range from one to four independent origin(s) for ascolichens. It also revealed, for the first time, that lichenization was also lost during the evolution of ascolichens. Contrary to the conclusion and prediction of multiple origins for ascolichens by Gargas et al. (1995), Spatafora and Lutzoni's studies demonstrated that the small subunit nrDNA alone was insufficient to reject the hypothesis of a single origin for ascolichens. Balanced sampling of lichenized and nonlichenized taxa, the use of multiple models for the evolution of lichenization, and wellsupported topologies provided by multiple data sets are essential to conduct a conclusive study on the origin of ascolichens.

In an attempt to gather a second data set from a different coding region, Lutzoni et al. (1997) sequenced a 1.4-kb fragment at the 5' end of the large subunit nrRNA gene and gathered a set of 44 sequences representing 22 orders of ascomycetes, 9 of which include lichen-forming ascomycetes. The preliminary results from this ongoing study provide evidence for a single origin for ascolichens, with at least two losses of lichenization. The two data sets (large and small subunit nrRNA genes) had 28 taxa in common. Because the two 28-taxon data sets revealed different phylogenetic affinities, a test was implemented to determine if these differences could be explained by sampling error (Lutzoni and Vilgalys 1995a; Lutzoni 1997; Rodrigo et al. 1993) and, therefore, could be combined into one data set. The combinability test showed that the discrepancies between the topologies derived from the two data sets when analyzed separately were not significant. The phylogenetic analysis of the combined data set generated one most parsimonious tree. With a model in which gains and losses of lichenization have the same cost, two equivocal solutions were found: one with one gain and two losses of lichenization, the other with three independent gains. If it is assumed that gains have a slightly higher cost than losses, only one unequivocal solution was found, with a single origin of ascolichens and two losses. If losses have a slightly higher cost than gains, a maximum of three independent lichenization events was revealed. Even if the exact number of independent origin(s) of ascolichens and their phylogenetic relationships have not been solved, this series of studies by Spatafora and Lutzoni clearly demonstrated that successful transitions to lichenization were potentially rare during the evolution of ascomycetes, especially, given the tremendous adaptive radiation (> one-fifth of all fungi) that followed.

B. Consequences of a Transition to a Lichenized State

Successful transitions to a lichen symbiotic state are relatively rare, given the tremendous diversity of extant lichen species. If the current lichen diversity is derived from a few lichenization events, this strongly suggests that the initiation and successful maintenance of a lichen symbiotic interaction are difficult and involve stresses on at least one of the symbionts during their early coevolutionary history. To study the predispositions that might confer a higher potential to a fungal lineage to survive a transition to a mutualistic association with a photobiont, and the consequences of this transition, Lutzoni (Lutzoni and Vilgalys 1995b; Lutzoni 1997) proposed the Omphalina (basidiomycete)-Coccomyxa (green alga) lichen model system. Phenotypic and genotypic differences between the closely related species O. velutipes, O. epichysium, O. sphagnicola (nonlichenized species), and O. ericetorum (lichen-forming species) are small (Fig. 1). These four taxa are most similar to the fungal lineages that flanked the transition to mutualism. It is believed that the origin of this symbiotic association is relatively recent compared with those that gave rise to most ascolichens (Hawksworth and Hill 1984). A model system that includes a relatively recent transition to a lichen state is essential to minimize the number of incidental differences between the lichenized and nonlichenized taxa. With such a model system the observed differences are more likely to be directly associated with the transition to a lichenized state. This can point to potential genes necessary for the transition and maintenance of the lichen symbiosis that can be targeted by future molecular genetic studies. It is yet to be determined to what extent each conclusion derived from this basidiomycete model system can be extended to evolutionary mechanisms associated with the origin of ascolichens. The available evidence provided by ongoing molecular systematic studies suggests an old origin(s) for ascolichens (i.e., soon after the origin of filamentous ascomycetes; Spatafora et al. 1994; Lutzoni et al. 1996, and in press). Consequently, too much divergence and extinction might have taken place between extant lichenized and nonlichenized ascomycetes to allow the reconstruction of ancestral evolutionary mechanisms associated with the origin(s) of most ascolichens. If this were true, it might not be possible to determine how relevant these evolutionary mechanisms associated with the origin of lichenized Omphalina are to the origin(s) of lichenized ascomycetes other than by comparison with other mycobiont-photobiont symbioses.

The discovery of phenotypic changes resulting from a transition to a lichen symbiosis can reveal the nature of the stresses associated with lichenization and potential requirements that a given lineage would need to be more likely to establish and maintain a successful mutualistic interaction with a photosynthetic organism. With the *Omphalina–Coccomyxa* model system along with a comparative phylogenetic approach, Lutzoni and co-workers (Lutzoni and Vilgalys 1995b; Lutzoni 1997; Lutzoni and Pagel 1997) have shown that several drastic changes took place in the *Omphalina* lineage subsequent to the single successful transition to mutualism that occurred in this genus (see Fig. 1). Contrary



internode at which the most parsimonious transition to a lichen state (lichenization) took place. Potential requirements and consequences flanking this transition to lichen mutualism are listed. The number on the right of each internode is the percentage of 350 bootstrap replicates supporting the same binary partition as shown in this figure. Bootstrap values ≤50% are not shown. (From Lutzoni and Vilgalys 1995b; Lutzoni 1997; Lutzoni and Pagel ()1997.)

Lichen Symbiosis and Adaptation to Desiccation

595

Kranner and Lutzoni

to the nonlichenized sister groups, the five lichen-forming species (O. ericetorum, O. grisella, O. hudsoniana, O. luteovitellina, and O. velutina) are extremely difficult to grow in axenic culture. The only published successful attempt to grow the Omphalina mycobiont in axenic culture was by Langenstein (1994), with the species O. ericetorum. However, these cultures could be maintained alive for only 6 months. Basidiospores of O. hudsoniana would germinate, but died shortly after. The difficulty in growing these mycobionts in axenic culture and their slow growth suggest a strong dependency on the phycobiont for their survival in nature.

The second major consequence detected in lichenized *Omphalina* species was a trend towards the loss of the dikaryotic state. Based on a karyological study of more than 600 collections of lichenized *Omphalina* species, Lamoure (1968, 1969, 1993) reported that many of these individuals were uninucleate. This state was not observed for species belonging to the nonlicheninzed sister group (Lamoure 1989). This trend was confirmed by phylogenetic studies on the genus- *Omphalina* and related species (Lutzoni and Vilgalys 1995b; Lutzoni 1997). Lamoure (1968, 1969) proposed that these single nuclei were haploid and that the formation of the basidiocarp was parthenocarpic. A preliminary study by Lutzoni (unpublished) using flow cytometry to quantify the DNA from nuclei in the basidiocarps and from nuclei in the spores generated by these basidiocarps supports the hypothesis that meiosis was taking place and that these single nuclei in the basidiocarp is not likely to be parthenocarpic. The uninucleate state more likely results from exobasidial karyogamy, probably early after mating.

Lichenized species of *Omphalina* can reproduce asexually. The resulting vegetative propagules contain both the algal and fungal partners. This consequence could represent an advantage over sexual reproduction for which the fungal meiotic spores (basidiospores) need to germinate to form an haplont that needs to mate to form a dikaryon. This dikaryotic mycelium needs to find a specific alga (*Coccomyxa*, in this case) to form the lichen thallus. The asexual reproduction mode by thallus fragmentation is always functional, whereas the sexual mode needs the appropriate environmental conditions to produce the basidiocarp and basidiospores. If the asexual mode of reproduction provides a larger portion of the progeny than the sexual mode in the lichenized species of *Omphalina*, and exobasidial karyogamy occurs frequently soon after mating, a trend toward the loss of the dikaryotic state is expected. Because genetic isolation is intrinsic to clonal reproduction, it is very likely that some of the species in the lichen-forming clade originated from a single asexual propagule with the uninucleate state. This could well be so for *O. hudsoniana* in which 98% of the individuals examined by Lamoure (1993) were uninucleate.

The third consequence of this transition to mutualism is meiotic anomalies observed in the lichenized lineage (Lamoure 1969). As for mushrooms in general, basidia of nonlichenized *Omphalina* species produce four spores. The number of spores per basidium can be extremely variable (a mixture of one to four spores per basidium on a single lamella) for the lichenized species.

Finally, a remarkable increase in rates of nucleotide substitution for the nuclear ribosomal DNA was recorded for the lichenized *Omphalina* species. By using phylogenetic comparative methods, Lutzoni and Pagel (1997) demonstrated that the transition to the lichen state preceded the rate acceleration in these lineages. Their study suggests that the increased rate of evolution was generalized across the genome of these mutualist fungi. In addition to the propagation of uninucleate individuals by asexual reproduction, selection for the uninucleate state, described in the foregoing, could result from this exceptional increase in rates of nucleotide substitution. The uninucleate state might enhance or enable

additional "recombination-repair" mechanisms at almost every stage of the life cycle by providing a template, from the homologous chromatid, that could be necessary for some specific types of DNA retrieval systems. These specific repair systems are not likely to be operational during the dikaryotic state. In basidiomycetes and ascomycetes, karyogamy occurs only in the hyphal compartments in which meiosis will occur (basidium and ascus, respectively). Therefore, it is possible that these fungi might not have a mechanism to prevent recombination after karyogamy has taken place, and this would explain why the two parental nuclei are kept separate until meiosis. However, these additional or enhanced "recombination-repair" systems might also be necessary to survive a higher mutational stress (see following section), resulting from a transition to a lichen symbiotic condition.

C. Stresses Associated with a Transition to a Lichen Symbiosis

Given these potential consequences resulting from a transition to a lichen state, three types of stresses were identified that could explain the observed changes in the lichenized taxa: increased solar radiation, desiccation levels, and ambient oxygen. A saprophytic lifestyle usually involves mycelial growth in the substrate, protecting it from damaging wavelengths, such as UV-B and from excessive desiccation. To enable an interaction with a photobiont that must carry out photosynthesis, the mycelium of the mycobiont has to be at the substrate surface, exposed to solar radiation, desiccation, and oxygen produced by the photobiont. Because the mycelium of nonlichenized fungi is not exposed to light, it probably does not have the capability to produce pigments and other secondary compounds to provide adequate protection against damaging radiation. For the same reason, newly lichenized individuals probably did not have the appropriate biochemical mechanisms to protect against desiccation. In lichen-forming Omphalina species, each photobiotic cell is surrounded by hyphae. The hyphal sheet surrounding the photobiont is, therefore, exposed to the oxygen produced by the photobiont. In addition, increased levels of ambient oxygen for the mycobiont may have resulted from the nonoptimal symbiotic interactions between the symbionts associated with the origin of lichenization, in which the mycobiont probably maximizes its contact with the photobiotic cells (Galun 1988; Langenstein 1994) and fails (for example) to translocate efficiently the carbon provided by the photobiont. Under such conditions, the photobiotic moiety of early lichen symbioses would account for a large part of the lichen biomass, as is true for the ancestral crustose-globulose thallus of lichenized Omphalina (Lutzoni and Vilgalys 1995b; Oberwinkler 1984). Under this scenario it is expected that the stresses would be higher on the mycobiont than on the photobiont.

Increased levels of oxygen, desiccation, ionizing radiation, or UV-A and UV-B radiations between 320 and 380 nm can cause increased levels of free oxygen radicals (Halliwell 1987; Halliwell and Gutteridge 1989; Mattimore and Battista 1996; Friedberg et al. 1995). As explained later, these free radical species can damage membranes, proteins, and DNA (e.g., double strand breaks). UV radiation can also have a direct effect on DNA by causing the formation of cyclobutane-pyrimidine dimers (Li and Graur 1991; Singer and Ames 1970).

The acceleration of nucleotide substitutions recorded in the mutualist fungi studied by Lutzoni and Pagel (1997) also could have resulted from deficient DNA repair systems, shorter generation time, and reduced sexual reproduction (Law and Lewis 1983), coupled with smaller population size (Moran 1996). Lutzoni and Pagel concluded that the observed acceleration of nucleotide substitutions in the lichenized *Omphalina* is mostly due to selection of mutations that disrupt the potential formation of thymine dimers. However, this factor could not explain the accelerated rate of nucleotide substitutions observed in two other independent transitions to mutualism included in their study. For these two other cases, they proposed that an increase in the level of mutagenic free radicals could be responsible for the acceleration.

D. Fungal Predispositions for a Transition to a Mutualistic State

Do all fungal lineages have the same potential for a successful transition to a lichen symbiotic state? The identification of sources of stresses, described in the foregoing, associated with a transition to a lichen symbiosis can be used to deduce the advantageous traits a fungal lineage would need to be particularly well suited for a successful transition to a mutualistic state. Three major predispositions are outlined here (see Fig. 1): (1) high phenotypic plasticity/broad ecological amplitude; (2) low fungal virulence/high photobiont infection resistance; (3) desiccation and sun irradiation tolerant/efficient DNA repair mechanisms (this third predisposition is discussed in section III.C).

A broad ecological amplitude and high phenotypic plasticity are attributes that provide opportunities to colonize different substrates and adapt readily to new environments. For fungi, new environments can include growing on or in photoautotrophic organisms, such as living plants, algae, and cyanobacteria. If molecular rates of evolution are any indication of the overall rate of species evolution (Omland 1997), *O. ericetorum*, with the slowest evolutionary rate among the lichen-forming *Omphalina* clade, is likely to be the most similar extant species to the lichenized ancestor of this clade (see Fig. 1). *Omphalina velutipes*, *O. spagnicola*, and *O. epichysium*, three basal species with the slowest rates of nucleotide substitutions in the nonlichenized sister group to this lichen clade, are likely to be most similar to the ancestral lineage in which the transition to a lichen state occurred.

Interestingly, O. ericetorum is the most plastic species of the lichenized Omphalina group, the most common, the most broadly distributed, and with the broadest range of substrates. Omphalina ericetorum is also the species in this group of lichenized fungi with the most variable degree of lichenization. Some individuals are almost without a thallus (where the fungal-algal interaction occurs), whereas the thallus of other individuals densely covers the substrate. It, therefore, is quite possible that individuals from the ancestral lineage where lichenization took place were phenotypically plastic, with broad ecological amplitudes, and these attributes were one of the reasons a transition to mutualism was possible. Redhead and Kuyper (1987) came to the same conclusion. They reported that O. epichysium, a lignicolous species, can also be found associated with bryophytes and algae. They further suggested that these facultative fungus-alga and fungusbryophyte associations are precursors to the development of lichenization. These conclusions are in conflict with Gargas et al. (1995), who concluded that basidiolichens (such as lichenized Omphalina) arose from predominantly mycorrhizal fungi. This discrepancy can be partly explained by the sampling of Gargas et al., in which the closest relative to the lichenized Omphalina was the distantly related genus Pleurotus. Lutzoni (1997) included all known lichenized Omphalina species, and 25 species selected from nonlichenized Omphalina and closely related genera such as Arrhenia, Chrysomphalina, and Clitocybe. None of these taxa are mycorrhizal. Moreover, it is not the most parsimonious solution for the mycorrhizal state to be ancestral to basidiolichens (see Fig. 1 of Gargas et al. 1995).

The ability of algae or cyanobacteria to provide good sources of carbon for their fungal partners is a major factor in determining which alga or cyanobacterium will associate with which fungus. The next necessary condition for a successful transition to mutualism is the ability of the fungus not to kill the alga (low pathogenicity) that it is colonizing and/or the ability of the alga to defend itself from lethal colonization by the

fungus. Origins of lichen symbioses involving *Coccomyxa*, such as for the basidiolichen genera *Omphalina* and *Multiclavula*, favor the latter explanation. *Coccomyxa* cell walls contain sporopollenin that seems to confer resistance to fungal penetration (Honegger and Brunner 1981; Galun 1988). This case scenario has the advantage of broadening the range of fungal species with which the photobiont can form a successful mutualistic interaction, by including some pathogenic fungal species that would otherwise be lethal to the alga or cyanobacterium.

It is not known which photobiont was associated with ascolichen origin(s), but based on Spatafora and Lutzoni's phylogenetic studies (Spatafora et al. 1994; Lutzoni et al. 1996, and in press) it is likely to have involved a photobiont without sporopollenin. In such a case, a fungus with low pathogenicity, or that had lost its pathogenicity, is more likely to have been responsible for the initial stage of a transition to mutualism. Galun (1988) reports that structurally simple crustose lichen species feature intracellular invasion in which the haustorium pierces and grows through the algal cell wall at all phases of its normal life cycle. In more differentiated crustose species, fungal invasion is limited to invaginations that appear only in mature and senescent algal cells. Finally, in lichens with a derived foliose or fruticose growth form, haustoria were almost restricted to preexisting decayed or entirely distorted algal cells. This trend toward the loss of haustoria from plesiomorphic to apomorphic thalli growth forms suggests that the haustorium may be a vestigial remnant of an initial parasitic fungus–alga relation (Ahmadjian and Jacobs 1981; Galun 1979, 1988).

E. Correlation between Mutualism and Increased Oxidative Stress

In general, oxygen is a two-edged sword for aerobic organisms: on the one hand, it enables efficient energy production by enzymatic combustion of organic compounds; on the other hand, it leads to damage of cells by the formation of reactive oxygen species, such as singlet oxygen, the hydroxyl radical, the superoxide anion radical, and hydrogen peroxide. Reactive oxygen species interact with all classes of biomolecules and can thus damage almost all cellular compartments. Moreover, various environmental stresses, such as irradiance, soil contamination, air pollution, chilling, nutritional disorders, flooding, or drought (see Smirnoff 1993; Elstner and Osswald 1994; Bartosz 1997) cause formation of free radicals in plants.

For fungi, the transition to a mutualistic association with a photoautotrophic organism is very likely correlated with increased oxidative stress for at least two reasons: first, they are associated with oxygen-producing photobionts and, second, the exposure to desiccation and sun irradiation causes the formation of free radicals. Given the poikilohydric and heliophylic nature of most lichens, it is very likely that a fungal lineage tolerant to oxidative stress caused by desiccation and sun irradiation, and/or with efficient DNA repair mechanisms would be more suited to forming a successful mutualistic association with terrestrial cyanobacteria or green algae. Because desiccation tolerance plays such a major role in the ecological success and diversification of lichens, in the remaining part of this chapter we will concentrate on mechanisms of free radical production, on desiccationinduced oxidative stress, and finally on protection mechanisms against free radical- and desiccation-induced damages. When possible, we incorporate results obtained from physiological studies on lichens; however, lichen stress physiology is a rather neglected field of study. Therefore, we will discuss the rare reports on desiccation and oxidative stress in lichens in addition to the more numerous publications using poikilohydric plants, as well as desiccation-tolerant propagules, such as seeds and spores, from various organisms.

(3)

III. PHYSIOLOGICAL ADAPTATION TO OXIDATIVE DAMAGE ASSOCIATED WITH POIKILOHYDRY

A. Toxic Effects of Oxygen on Plant and Fungal Tissues

Generally, the energetic benefit of aerobic metabolism is associated with the generation of reactive oxygen species capable of damaging biologically relevant molecules, such as DNA, proteins, carbohydrates, and lipids. Free oxygen radicals and other active oxygen species naturally produced during plant metabolism are particularly found in chloroplasts and mitochondria (see Halliwell 1987). These highly reactive oxygen species such as hydrogen peroxide (H₂O₂), superoxide (O_2^{--}), singlet oxygen ($^{1}O_2$), or the hydroxyl radical (OH'), have the potential to inactivate enzymes and damage important cellular components. They are thus associated with several physiological disorders in plants. Moreover, oxygen has a toxic effect because thiol (SH)-groups-containing enzymes are inactivated by oxygen. In the following we give a short overview of some major pathways for generation of reactive oxygen species. For broader reviews refer to Elstner (1982), Halliwell (1987), and Halliwell and Gutteridge (1989).

Most oxygen taken up by aerobic cells is reduced to water by the addition of four electrons to each molecule. This reaction [Eq. (1)] is catalyzed by the cytochrome oxidase complex of the inner mitochondrial membrane.

$${}^{3}O_{2} + 4e^{-} + 4H^{+} \rightarrow 2H_{2}O$$
 (1)

Other oxidases, such as glycollate oxidase, urate oxidase, and amino acid oxidases, transfer two electrons onto each oxygen molecule to form hydrogen peroxide (see citations in Halliwell 1987; Halliwell and Gutteridge 1989), following Eq. (2).

$${}^{3}O_{2} + 2e^{-} + 2H^{+} \rightarrow H_{2}O_{2}$$
 (2)

The high electronic excitation state, resulting from the illumination of the chlorophyll molecule, can transfer energy onto the oxygen molecule and raise it from its ground state (triplet oxygen, ${}^{3}O_{2}$) to a more reactive state known as singlet oxygen ${}^{1}O_{2}$, Eqs. (3) and (4). Singlet oxygen is not subjected to spin rule and reacts rapidly with most organic molecules, especially at double bonds, to produce hydroperoxides (ROOH). Because the trapping of light by photosystems (PS) I and II causes the formation of excited states of the chlorophyll molecules, excess excitation can cause production of ${}^{1}O_{2}$ in chloroplasts, which can react directly with polyunsaturated fatty acid side chains to form lipid peroxides (Halliwell 1987).

$$P + light \rightarrow P^*$$
 (excited pigment) (3)

$$P^* + {}^3O_2 \rightarrow {}^1O_2 + P \tag{4}$$

Some cellular systems, such as nitropropane dioxygenase, galactose oxidase, and xanthine oxidase, catalyze oxidation reactions in which a single electron is transferred from the substrate onto oxygen to produce superoxide O_2^{-} [Eq. (5)]. Autoxidation of some reduced compounds [e.g., flavins, pteridines, diphenols, and ferredoxin; Eq. (6)] can also transfer a single electron to oxygen to produce O_2^{-} (citations in Halliwell 1987 and Halliwell and Gutteridge 1989).

$${}^{3}O_{2} + e^{-} \rightarrow O_{2}^{-} \tag{5}$$

 $Ferredoxin_{reduced} + {}^{3}O_{2} \rightarrow ferredoxin_{oxidized} + O_{2}^{-}$ (6)

Compared with other oxygen radicals, O_2^{-} is rather unreactive. For example, it can-

not react directly with membrane lipids to cause peroxidation. However, most of O_2^{-1} formed in biochemical systems reacts with itself (nonenzymatic) to form H_2O_2 [Eq. (7)]. Hydrogen peroxide and O_2^{-} can react together to form the hydroxyl radical 'OH [Eq. (8)] in an iron-catalyzed reaction, known as the Haber-Weiss reaction. The hydroxyl radical is the most reactive species known to chemistry. It is capable of attacking and damaging almost every molecule found in living cells. It can hydroxylate purine and pyrimidine bases in DNA, leading to an increase in mutation rates (Halliwell 1987). It can also abstract hydrogen radicals from membranous lipids to initiate peroxidation. Once initiated, peroxidation is autocatalytic. For example, the perhydroxy radical (HOO') generated by the protonation of O_2^{-} and HO' can extract the bis-allylic hydrogen atom from an unsaturated fatty acid (LH) to form a lipid alkyl radical (L') that is further oxidized by molecular oxygen to generate a lipid peroxy radical (LOO'). LOO' reacts with LH yielding LOOH and L'. Thus, a radical chain reaction is propagated. For these reasons, the superoxide radical and its derivatives induce membrane rigidity as well as peroxidation of membrane lipids, and can also attack sulfhydryl-containing proteins. Consequently, the inactivation of membrane-bound enzymes and changes in lipid structure may contribute to a loss of membrane integrity and selective permeability.

$$O_2^{-} + O_2^{-} + 2H^+ \rightarrow H_2O_2 + {}^{3}O_2$$
 (7)

$$H_2O_2 + O_2^{-} \rightarrow {}^3O_2 + {}^{\circ}OH + OH^{-}$$
(8)

Lipid peroxidation involves the reaction of oxygen with conjugated dienes to produce lipid peroxides. Lipid peroxides decompose to give aldehydes, such as 4-hydroxynonenal (HNE) and malondialdehyde and other products (including volatile hydrocarbons such as ethane and pentane). These aldehydes are considered as second toxic messengers which disseminate initial free radical events. This way, lipid peroxides and their degradation products cause extensive damage. The chemistry and biochemistry of such aldehydes resulting from lipid peroxidation is well reviewed by Esterbauer et al. (1991).

In lichens, oxygen free radicals produced in the photobiont are not likely to pass through the chloroplast membranes and plasmalemma of the alga to subsequently damage the mycobiont's cellular components. However, the triplet oxygen itself can react with several molecules in the mycobiont, thereby leading to generation of oxygen free radicals. To the best of our knowledge, production of oxygen free radicals in lichenized fungi has not yet been investigated, but it was reported that oxidative stress can cause DNA damage in yeast (Frankenberg et al. 1993; Brennan et al. 1994; Woodford et al. 1995). Moradas-Ferreira et al. (1996) reviewed molecular defenses against reactive oxygen species in yeast.

B. Dehydration Leads to the Formation of Free Radicals

Reports on the formation of free radicals in response to many plant stresses, including drought, are being published with increased frequency (for reviews, see Smirnoff 1993; Elstner and Osswald 1994; Bartosz 1997). Here, we will consider mainly the generation of desiccation-induced free radicals. Because of the economic significance of storing seeds, most investigations have been carried out on seeds and seedlings of vascular plants, such as *Glycine max* (Buchvarov and Gantcheff 1984; Senaratna et al. 1985a,b; Priestley et al. 1985; Simontacchi and Puntarulo 1992), *Helianthus annuus* (Quartacci and Navari-Izzo 1992), *Zea mays* (Priestley et al. 1985; Leprince et al. 1990, 1994a,b, 1995), *Phaseolus vulgaris* (Leprince et al. 1995), *Quercus robur* (Hendry et al. 1992), *Castanea sativa* (Finch-Savage et al. 1994), and on other grasses and cereals (Price and Hendry 1991).

Kranner and Lutzoni

Navari-Izzo et al. (1994) reported superoxide radical production in illuminated thylakoids during rapid and slow desiccation and rehydration of the resurrection plant *Boea hygroscopica*. Superoxide radical production was higher in control and rehydrated plants than in slowly and rapidly dehydrated plants. O_2^- production was higher in rapidly than in slowly dehydrated leaves. In thylakoids of rapidly dried leaves, SH groups of proteins were maintained in the reduced state at low levels (6.6%). They amounted to more than 50% in slowly dehydrated and to 70% in rehydrated leaves.

There are few reports on the formation of free radicals in cryptogamic plants. Generation and accumulation of desiccation-induced free radicals have not been reported from lichenized fungi. Seel et al. (1991) used electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) to demonstrate that, besides short-lived oxygen radicals, relatively stable, carbon-centered radicals accumulate during desiccation in mosses. Such stable free radicals also accumulated in germinating maize (Leprince et al. 1990, 1994a,b, 1995), in bean (Leprince et al. 1995), and in oak (Hendry et al. 1992). The most common free radical produced may be a semiquinone, resulting from the final trapping out of radicals involved in a sequence of desiccation-promoted free radical reactions (Hendry et al. 1992). In orthodox seeds, the formation of such desiccation-induced stable free radicals was reported to be a function of oxygen and temperature. In the desiccation-intolerant stage the concentration of stable radicals was lower if tissues were dried below 15°C and higher when tissues were dried above 30°C, and high oxygen concentrations dramatically increased the buildup of free radicals (Leprince et al. 1995). Furthermore, senescence in plants was correlated with the generation of free radicals derived from a quinone (Atherton et al. 1993).

Secondary free radicals, other than carbon- and oxygen-centered radicals formed by the interaction of oxygen or carbon radicals with organic molecules, may originate during desiccation. One such main radical species is the thiyl radical S'. However, the formation and reactions of thiyl radicals under drought conditions have not been studied in plants or in lichens. It might be possible to apply to plants and lichens a new analytical method developed to investigate stable thiyl radicals in yeast (Sievers et al. 1996). In animal and human tissues, this topic has been investigated more extensively (see Cadenas 1995; Kundu and Willson 1995; van der Vliet et al. 1995; Wardmann and von Sonntag 1995). For these organisms, the formation of the S'. radicals is discussed relative to numerous metabolic functions of thiols, such as the repair of carbon-centered radicals and freeradical-scavenging activities.

C. DNA Damage Caused by Desiccation

Most studies on DNA damage resulting from desiccation stress have been carried out on bacteria. DNA exposure (in vivo and in vitro) to dry environments leads to the accumulation of double-strand breaks (DSBs) and to DNA becoming cross-linked with other cellular constituents, such as proteins (Dose and Gill 1995). Unique tandem-base change mutations leading to mutagenic and lethal consequences have also been reported for bacterial spores exposed to extreme dryness (Munakata et al. 1997).

Mattimore and Battista (1996) reported that adaptations needed to survive prolonged desiccation in *Deinococcus radiodurans* are also necessary to survive ionizing radiation. Because the high levels of radiation necessary to cause detectable lethality are absent in nature, the authors suggested that resistance to ionizing radiation in *D. radiodurans* wildtype is a consequence of its adaptation to dehydration. Given this premise, it would be expected that desiccation-resistant organisms, such as most lichen-forming fungi and their

photobionts, would also be more resistant to ionizing radiation than desiccation-sensitive species. In *D. radiodurans* wild-type, dehydration-induced DSBs accumulate as a function of time (Mattimore and Battista 1996). In this species, it took a minimum of 8 days before DNA DSBs accumulated to detectable amounts by agarose gel electrophoresis. *Escherichia coli*, an ionizing radiation-sensitive bacterium, accumulates DSBs at the same rate. This suggests that the resistance of *D. radiodurans* to desiccation is not a function of an enhanced mechanism to protect DNA from damage caused by desiccation, but rather, of its ability to efficiently repair DNA damage on rehydration. However, this does not seem to apply to all bacteria.

Setlow and co-workers (Setlow 1995; Setlow and Setlow 1995; Fairhead et al. 1994) reported a desiccation-phase mechanism that protects DNA from damage through its saturation with a group of DNA-binding proteins, termed α/β -type small, acid-soluble spore proteins (SASP). The saturation of spore DNA with α/β -type SASP provides protection against DNA single-strand breakage caused by desiccation and hydrogen peroxide. Setlow and Setlow (1996) report that, although this mechanism also provides significant protection against spore DNA damage caused by dry heat, the killing of wild-type spores by this specific treatment is, in large part, due to DNA damage. Because DNA damage contributes significantly to the killing of wild-type spores subjected to dry heat and to the killing of $\alpha^{-}\beta^{-}$ spores by all treatments, Setlow and Setlow (1996) proposed that DNA repair is an important rehydration-phase mechanism of spore resistance to dry heat. DNA repair-related genes (*dinR*, *recA*, and *uvrC*) were induced during outgrowth of wild-type spores treated with dry heat or UV.

Because high levels of dehydration-induced lethality were detected after only 1 day for *E. coli*, and no DNA repair was detected during the desiccation phase for *D. radiodurans* (Mattimore and Battista 1996), it seems that resistance to desiccation can involve two distinct series of mechanisms, as described by Oliver and Bewley (1997): (1) a series of desiccation-phase mechanisms that confers protection against damage to membranous and proteinaceous components as well as DNA during the drought period; and (2) a series of rehydration-phase mechanisms that provides efficient DNA repair once the cells starts to rehydrate. Any given desiccation-resistant organism could have one or the other series of mechanisms, or a combination of both. Mattimore and Battista (1996) also noted that weekly exposure to ambient humidity was harmful to dried cells during a long-term desiccation experiment. Because the survival of drought-tolerant lichens is probably dependent on short dry-wet cycles (see Kershaw 1985), the resistance of these lichens to desiccation is likely to involve additional mechanisms.

The work of Esterbauer and co-workers suggests that mutagens can be derived from lipids and lipid precursors (for review see Esterbauer et al. 1991). More specifically, free radical damage leading to mutations might result from aldehydic lipid peroxidation products, such as malondialdehyde, 4-hydroxynonenal (HNE), and others. These aldehydes can react with DNA bases and induce mutations (see Vaca et al. 1988 for review). HNE can induce the incidence of micronuclei, sister chromatid exchange, and chromosomal aberrations (citations in Esterbauer et al. 1991). Presumably, these compounds may also inhibit certain DNA repair systems.

D. Physiological Adaptation to Desiccation Requires a Complex Interplay of Several Adaptational Mechanisms Including Protection from Oxidative Damage

Cellular desiccation imposes multifaceted stresses that are lethal for most organisms. Along with increased oxidative stress (Smirnoff 1993; Elstner and Osswald 1994), water removal

Kranner and Lutzoni

affects ionic strength and pH, and it can lead to the crystallization of solutes and denaturation of proteins. Conformational changes in protein structure are probably primary sites of desiccation injury, particularly for irreversible formation of intramolecular disulfides (Levitt 1980). Moreover, water deficit leads to increased lipid peroxidation. A consequence of lipid peroxidation and protein denaturation is membrane disruption, leading to leakage of solutes and loss of compartmentalization. Finally, severe water removal can cause cellular collapse.

By contrast, desiccation-tolerant (DT) plants and lichens are able to revive and show normal physiological characteristics on rehydration after having been desiccated and remaining in the air-dried state for long or short periods. Desiccation tolerance occurs in organisms ranging from the most simple levels of cellular organization, such as bacteria. to much more complex and multicellular nature, such as arthropods. In the plant kingdom, desiccation tolerance occurs in species belonging to almost all major classes (for review, see Oliver and Bewley 1997). However, it is a rare phenomenon in the vascular plants. Most desiccation-tolerant plants are found in nonvascular cryptogamic plants, especially among algae and bryophytes. In the fungal kingdom desiccation-tolerant species are found in all major phyla. However, almost all of these species are lichen-forming ascomycetes, representing approximately one-fifth of all known fungi (Hawksworth 1988a). Many bryophytes, lichen mycobionts, and photobionts (including green algae and cyanobacteria), as well as some specialized vascular plants, withstand drying of their photosynthetic cells to water contents of 10% of their dry weight, or even less. In this condition they appear completely dry, but they recover normal function rapidly during rehydration. Such desiccation-tolerant photoautotrophic organisms are divided into two groups: (1) Those that retain their chlorophyll content during desiccation are termed homoiochlorophyllous (HDT), and (2) those that lose most of their chlorophylls are poikilochlorophyllous (PDT) (Oliver and Bewley 1997). In PDT plants the photosynthetic apparatus with its pigments and thylakoids disintegrates and disappears during desiccation (Tuba et al. 1993b, 1996b) and has to be resynthesized de novo on rehydration (Tuba et al. 1993a, 1994; Sherwin and Farrant 1996). In contrast, HDT plants preserve their photosynthetic apparatus during desiccation in a state from which it can recover quickly and fully at rehydration (Sherwin and Farrant 1996; Tuba 1996a).

For vascular plants, 60-70 species of pteridophytes, and at least 60 species of angiosperms exhibit desiccation-tolerant vegetative tissues. The only major class of vascular plants without representative desiccation-tolerant species is the gymnosperms (for reviews, see Bewley and Krochko 1982; Oliver 1996; Oliver and Bewley 1997). However, many angiosperms and gymnosperms do produce desiccation-tolerant propagules in the form of seeds and pollen. Thus, desiccation tolerance is one of the most fundamental principles for resting stages of plant development, including dormancy of seeds, spores, and pollen. as well as the survival of resurrection plants and lichens under drought. In spite of the drastic variation in anatomy and physiology of desiccation-tolerant organisms, such as algae, bacteria, mosses, lichen-forming fungi, or vascular plants, these organisms could have developed similar molecular mechanisms that protect them from the deleterious effects of cellular desiccation. For comprehensive reviews of desiccation tolerance in plants. including physiological responses to desiccation and rehydration, refer to Bewley (1979), Gaff (1989), and Bewley and Krochko (1982). Oliver and Bewley (1997) concentrated on the idea that desiccation tolerance is a balance between two fundamental processes: cellular protection from desiccation- and rehydration-induced damage, and the repair of the damage, that does occur. Most DT plants probably employ aspects of both (Oliver 1996).

Several strategies for coping with damages resulting from cellular desiccation during seed maturation have been recently reviewed (Leprince et al. 1993). For a review on deteriorative changes associated with the loss of viability of DT and desiccation-sensitive seeds, refer to Smith and Berjak (1995). Leprince et al. (1993) discuss the presence of high amounts of nonreducing sugars, the expression of desiccation and/or abscisic-acid (ABA)-regulated genes, and the capability of free-radical-scavenging systems as important mechanisms to overcome desiccation. Nonreducing sugars, such as trehalose and sucrose, may substitute for water by forming hydrogen bonds, thereby maintaining hydrophilic structures in their hydrated orientation (Crowe et al. 1988, 1992). This way, they may help stabilize proteins and membranes under dry conditions. Nonreducing sugars are also thought to promote the formation of a "glass-phase" in the cytoplasm, a process that is known as vitrification. In the glassy state, the cytoplasm is characterized as a liquid solution, with the viscous properties of a solid. Benefits resulting from vitrification for the quiescent seed are numerous. Owing to the high viscosity of the cytoplasm, chemical reactions are strongly slowed down. Consequently, degenerative processes, such as alterations in ionic strength and pH, and crystallization of solutes, are prevented. The glass phase also fills space, thus preventing cellular collapse following desiccation.

Popp and Smirnoff (1995) review the role of polyols in plants during water stress. Polyols (polyhydric alcohols), such as sorbitol and mannitol, also accumulate in plants during water deficit. Cyclic polyols are termed *cyclitols* (e.g., inositol). Such polyols may have the property of scavenging hydroxyl radicals. Moreover, cyclitols may play a role as cryoprotectants, similar to that suggested for sugars (Crowe et al. 1984), through interaction between cyclitols and membranes. Whether sugars and polyols play a role in desiccation tolerance in lichens is unknown, but certainly they contain high concentrations of these compounds (Roser et al. 1992).

Water deficit or any treatments affecting the cellular water potential, such as ABA, lead to expression of the so-called late embryogenesis abundant (LEA) proteins. The latter are rich in hydrophilic amino acids and have only few hydrophobic residues. Therefore, they are largely water-soluble and have high hydration levels that may contribute to seed adaptation to desiccation (citations in Leprince et al. 1993). Such water stress proteins (dehydrins) are believed to accommodate water loss (see Oliver and Bewley 1997). Moreover, analysis of protein profiles on rehydration of desiccation-tolerant tissues has enabled the identification of rehydration-phase-specific proteins, the so-called rehydrins (Oliver 1996), Bauemlein et al. (1995) suggested that the present-day seed globulins of spermatophytes have evolved from a group of ancient proteins functioning in cellular desiccationhydration processes. These authors reported that the amino acid sequences of legumin and vicilin domains share significant similarities to the germination-specific germins of wheat, as well as to the spherulation-specific spherulins of myxomycetes that are thought to be involved in tissue desiccation or hydration. Dehydrin-like proteins are also inducible by the desiccation-rehydration process in cyanobacteria, most extensively investigated in Nostoc commune (citations in Oliver and Bewley 1997). It was also suggested that desiccation tolerance of the lichen Peltigera horizontalis may depend on the accumulation of dehydrin-like proteins (e.g., Schulz 1995).

Abscisic acid appears to be involved in the acquisition of desiccation tolerance (Oliver and Bewley 1997, for review), as described, for example, for alfalfa seeds, (Xu and Bewley 1995) and in the enhancement of the ability to withstand drying conditions of the desiccation-tolerant fern *Polypodium virginianum* (Reynolds and Bewley 1993). ABA was also reported to be involved in desiccation tolerance of liverworth gametophytes (Hellwege et al. 1994, 1996) and moss protonema (Werner et al. 1991), possibly through the induction of dehydrin-like proteins synthesis. The presence of dehydrins was also correlated with high ABA contents in various recalcitrant seeds by Farrant et al. (1996). However, the significance of ABA to overcome desiccation in lichens has not yet been studied.

Beckett (1995) found that desiccation tolerance in lichens was directly related to the proportion of potassium contributing to the osmotic potential, suggesting that low concentrations of cytoplasmatic potassium are important for desiccation tolerance.

Desiccation tolerance of dormant seeds is also based on their capability to scavenge desiccation-induced free radicals (Leprince et al. 1993). Indeed, desiccation-induced free radicals were found in numerous tissues, and desiccation tolerance has been correlated with the maintenance or synthesis of antioxidants, such as glutathione (γ -glutamyl-cysteinyl-glycine; GSH), ascorbic acid (AA), or tocopherols, and/or of enzymes scavenging cytotoxic oxygen species, such as superoxide dismutase, catalase, or peroxidases (PO) (citations in Kranner and Grill 1996, 1997c).

E. Free-Radical-Scavenging Pathways

Much evidence exists that the function of antioxidants in plant and animal tissue is to scavenge free radicals. The main free radical scavengers found in biological systems are reduced glutathione, ascorbic acid, α -tocopherol, and carotenes (for review of oxidative stress-induced reactions of antioxidants see Smirnoff 1993; Elstner and Oßwald 1994). The xanthophyll cycle pigments serve to dissipate excess excitation energy, thus preventing formation of singlet oxygen. Antioxidant functions are associated with lowered DNA damage and diminished lipid peroxidation. By investigating the GSH content of algae and fungi, isolated from the lichen Usnea filipendula, Kranner et al. (1992) demonstrated that the major part of GSH is present in the mycobiont. This may mean that the mycobiont has a substantial requirement for antioxidants.

GSH reacts rapidly with 'OH, 'O₂ and, together with AA is involved in removal of H_2O_2 . AA also reacts rapidly with O_2^- , 'OH, and 'O₂ (Halliwell 1987). α -Tocopherol is the major radical scavenger in biological lipid phases, such as membranes. It is a powerful scavenger of 'O₂ and of lipid peroxides, Eq. (9). The resulting tocopheryl radical can be reduced back to tocopherol by ascorbic acid. In plants, α -tocopherol appears to be concentrated in photosystem II; however, it was not found in ascomycetes (Esterbauer, personal communication). GSH and AA are present in aqueous compartments, their reducing power being used in radical (such as the reduction of the tocopheryl radical) and nonradical redox reactions.

$$\text{Lipid-O}_{-}^{-} + \alpha \text{TH} \rightarrow \text{lipid-OOH} + \alpha \text{T}$$
(9)

Carotenoids, such as β -carotene and the xanthophyll-cycle pigments, act as antioxidants in lipid phases by quenching ${}^{1}O_{2}$ and electronically excited molecules that are produced by photoexcitation or chemiexcitation reactions. They further react with peroxyl and alkoxyl radicals.

The xanthophyll cycle is thought to protect plants from excess excitation energy that would lead to formation of singlet oxygen [see Eqs. (3) and (4)]. Here, the zeaxanthin formed from a precursor (violaxanthin, by the intermediate antheraxanthin) is thought to act as a protective pigment. In the xanthophyll cycle, stepwise removal (deepoxidation) of two oxygen functions (the epoxy groups) in violaxanthin results in a lengthening of the conjugated system of double bonds from 9 in violaxanthin, to 10 in antheraxanthin, to 11 in zeaxanthin (e.g., Siefermann-Harms 1977; Demmig-Adams and Adams 1996a,b). Epoxidation and deepoxidation are enzyme-catalyzed events. The deepoxidase reaction requires

light, reducing conditions, and probably ascorbate [Eqs. (10) and (11)]. The epoxydase reaction requires molecular oxygen and NADPH [Eqs. (12) and (13)]. Demmig-Adams and co-workers have investigated the xanthophyll cycle in lichens in detail.

Violaxanthin + ascorbate \rightarrow anthe	$raxanthin + H_2O$	+ dehydroascorbate	(10)
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- Antheraxanthin + ascorbate \rightarrow zeaxanthin + H₂O + dehydroascorbate (11)
- Zeaxanthin + NADPH + H⁺ + O₂ \rightarrow antheraxanthin + NADP⁺ + H₂O (12)

Antheraxanthin + NADPH + H^+ + $O_2 \rightarrow violaxanthin$ + NADP⁺ + H_2O (13)

Other compounds, such as flavonoids, sugars, polyols, proline, and polyamines are believed to have antioxidant properties (citations in Smirnoff 1993). Phenolic substances in lichens are also likely to be involved in antioxidant processes. There are numerous papers demonstrating the antioxidant activities of phenolic compounds in higher plants (for review, see Rice-Evans et al. 1996). By exposing conifers to oxidative stress (ozone), Sandermann et al. (1989) reported the stress-induced production of phenolic components in Pinus silvestris. When we consider the extraordinary high amounts of various phenolic compounds in lichens (Elix et al. 1984), studies on the antioxidant properties of phenolic compounds and on formation of phenoxy radicals may greatly improve our understanding of the lichen symbiosis. For a comprehensive review on the chemistry of lichen products and of secondary biochemistry of lichens refer to Elix et al. (1984), Fahselt (1994), and to the work of Culberson (such as Culberson 1969, 1970; Culberson et al. 1977; Culberson and Elix 1989). In lichens, the formation and reactions of phenoxyl radicals have not yet been investigated. Phenoxyl radicals are intermediates of one-electron oxidation of phenolic compounds by various peroxidases. Reactions of phenoxyl radicals with NADPHcytochrome P-450 oxidoreductase and NADPH have been studied in human tissues (Goldman et al. 1997). This study provides evidence that the phenoxyl radical can be reduced enzymatically by transfer of electrons from NADPH by the FAD/FMN of the NADPHcytochrome P-450 oxidoreductase pathway. Antioxidant activity of lichen depsides and depsidones was assessed by studying their effects as inhibitors of rat brain homogenate and β-carotene oxidation (Hidalgo et al. 1994). Armaleo (1995) suggested that, in the isolated lichen mycobiont Cladonia grayi, the grayanic acid pathway might function in scavenging oxygen radicals. However, he did not find a specific response of the mycobiont to oxidants, indicating that this pathway may not act as a general redox sink for free radicals.

Finally, cytotoxic oxygen species are scavenged by enzymatic pathways that include reactions of superoxide dismutase (SOD), ascorbate peroxidase (AP), and other peroxidases, as well as mono- and dehydroascorbate reductases, glutathione reductase (GR), and catalase (for an overview, see Elstner and Oßwald 1994).

All aerobic organisms contain superoxide dismutases that are metalloproteins catalyzing the dismutation of superoxide to hydrogen. Thus, O_2^{-} is removed rapidly, further conversion into 'OH is prevented, and ground-state oxygen, rather than singlet oxygen, is formed, Eq. (14).

$$O_{2}^{*-} + O_{2}^{*-} + 2H^{+} \rightarrow H_{2}O_{2} + {}^{3}O_{2}$$
 (14)

Peroxidases catalyze hydrogen peroxide-dependent oxidation of substrates (S) following Eq. (15), here described for AP, Eq. (16), and glutathione peroxidase [GP; Eq. (17)].

Kranner and Lutzoni

$$SH_2 + H_2O_2 \rightarrow S + 2H_2O \tag{15}$$

 $2H^+ + ascorbate + H_2O_2 \rightarrow dehydroascorbate + 2H_2O$ (16)

$$2 \text{ GSH} + \text{H}_{2}\text{O}_{2} \rightarrow \text{GSSG} + 2\text{H}_{2}\text{O} \tag{17}$$

Plants probably do not possess a selenium-containing GP, as frequently described for animal tissues. Plants contain a wide range of peroxidases, with a broad specificity for substrate, detected by using artificial substrates (guaiacol or o-dianisdine). The identity of the natural substrate is often unknown. Yeast contains a membrane-bound glutathione peroxidase that is inducible by lipid hydroperoxide (LOOH) or by reactive oxygen species (Miki et al. 1996).

Catalases break down high concentrations of H_2O_2 [Eq. (18)] very rapidly, but are much less effective than peroxidases at removing H_2O_2 present in low concentrations because of their low affinity (High K_m) for this substrate.

$$2H_2O_2 \rightarrow H_2O + O_2 \tag{18}$$

A well-balanced interplay of antioxidants and enzymes in scavenging cytotoxic oxygen species was first suggested by Foyer and Halliwell (1976). They postulated an ascorbate-glutathione cycle (Fig. 2) for the scavenging of the H_2O_2 derived from superoxide, catalyzed by SOD. This cycle is best known for eliminating the risk of oxidation of enzymes by H_2O_2 in chloroplasts. It involves reactions of GSH, AA, GR, AP and monoand dehydroascorbate reductases. Moreover, this cycle may also be linked to the lipidsoluble antioxidant α -tocopherol that protects membrane proteins from free radical reactions (Finckh and Kunert 1985).

F. Physiological Adaptation to Desiccation-Induced Oxidative Damage

Seel et al. (1991) showed that the formation and accumulation of free radicals were the same in desiccation-sensitive (Dicranella palustris) and desiccation-tolerant (Tortula ruraliformis) mosses. However, Seel et al. (1992) showed that, unlike the sensitive species, the desiccation-tolerant moss seemed to have the capacity for activating and maintaining its antioxidant system on dehydration. Desiccation caused a significant increase in SOD activity, but did not affect the activities of peroxidase and AP in T. ruraliformis. In contrast, desiccation, in combination with irradiance, led to a decrease in peroxidase activity in D. palustris, but had little effect on the activities of other oxygen-processing enzymes. The extrachloroplastic enzyme catalase was sevenfold more active in hydrated T. ruraliformisthan in D. palustris, but desiccation resulted in significantly decreased enzyme activity in both species. During desiccation, a depletion of AA occurred in both species. When T. ruraliformis was desiccated in light, the authors reported synthesis of y-tocopherol and maintenance of α -tocopherol and glutathione. Activities of the antioxidant-recycling enzymes, dehydroascorbate reductase and GR, were not significantly increased by desiccation in either moss. These authors did not find that desiccation caused any cellular damage in the desiccation-tolerant moss, but water removal in the desiccation-sensitive species led to destruction of chlorophyll, loss of carotenoids, and increased lipid peroxidation. In the desiccation-sensitive species, this cellular damage was correlated with the depletion of the antioxidant system (Seel et al. 1992). These results indicate that in T. ruraliformis a series of desiccation- and rehydration-phase mechanisms, providing protection from desiccationinduced damage, may be more important than rehydration-phase mechanisms that provide repair during rehydration. These results agree with reports from higher plants. Hendry et



Figure 2 The ascorbate-glutathione cycle, as described in Kunert and Foyer (1993). This interplay of GSH, AA, GR, mono- and dehydroascorbate reductases in scavenging H_2O_2 was first suggested by Foyer and Halliwell (1976). Monodehydroascorbate is unstable and disproportionate to ascorbate and dehydroascorbate. Additionally, this cycle may also be linked to α -tocopherol. (From Kranner and Grill 1996.)

609

al. (1992) associated desiccation in seeds of the recalcitrant (i.e., desiccation-sensitive) species *Quercus robur* with loss of viability, a rise in lipid peroxidation, and a buildup of free radicals. These authors suggest that the decay of the defense mechanisms occurring during the storage and, thus desiccation of the seeds, was directly linked with lipid peroxidation and free radical formation. These events could contribute to a loss of viability for recalcitrant seeds.

Desiccation of pea nodules subjected to water stress (Gogorcena et al. 1995) caused a decrease in the activities of catalase, AP, dehydroascorbate peroxidase, and SOD, and in the contents of AA, GSH, GSSG, NAD(P)⁺, and NAD(P)H⁺. The authors suggested that the decline in antioxidant capacity of the drought-stressed nodules might result from restricted supply of NADPH required for the ascorbate–glutathione pathway, and from the Fe-catalyzed Fenton reaction of AA and GSH with activated oxygen. Drought tolerance of desiccation-tolerant varieties of *Sorghum bicolor* was also correlated with diverse responses in the antioxidant system, such as increased levels of SOD and catalase (Jagtap and Bhargava 1995). Drought tolerance was also correlated with the enzymatic defense against lipid peroxidation and high proline content in sugar beet cultivars (Stajner et al. 1995). For maize, it was demonstrated that the ability to reduce damaging effects of chilling-induced free radicals was associated with increased enzymatic antioxidant systems, such as catalase, SOD, or peroxidases (Zhang et al. 1995).

Other important studies on antioxidants and activated oxygen-processing enzymes in desiccated and subsequently rehydrated mosses include those of Dhindsa (1987, 1991), who examined glutathione and its effects on protein synthesis in T. ruralis. Dhindsa demonstrated that the speed of dehydration affects the oxidation-reduction processes of glutathione during rehydration. Oxidation of glutathione and activation of the enzymes GR, GP, and glutathione-S-transferase (GST) were induced only by desiccating the moss slowly. Rapid dehydration did not lead to either oxidation of glutathione or activation of these enzymes. However, glutathione was oxidized and these enzymes were activated on rehydration, following the rapid dehydration (Dhindsa 1987, 1991). This leads to the conclusion that the moss had received a drought stress-induced signal for oxidizing glutathione and activating GR, GP, and GST, but the response to this signal was not possible when the moss was desiccated too rapidly. The plant could respond to the signal only during the subsequent rehydration. Dhindsa also found a positive correlation between levels of oxidized glutathione (glutathione disulfide, GSSG) during rehydration following rapid dehydration and levels of lipid peroxidation and solute leakage, but a negative correlation with the rate of protein synthesis. The inhibition of protein synthesis by high GSSG concentrations was also reported by several other authors (Fahey et al. 1980; Jackson et al. 1983). Ernst et al. (1978, 1979) described such a mechanism for animal tissues. They demonstrated that GSSG activates a protein kinase that phosphorylates and thereby, inactivates the α -subunit of initiation factor 2.

Tuba et al. (1996a) reported photosynthetic responses of the DT moss *T. ruralis* and two DT lichens, *Cladonia convoluta* and *C. furcata*, to short periods of desiccation. They reached the conclusion that desiccation tolerance in lichens and bryophytes must be largely achieved through cellular protection implemented during the dehydration process. They mentioned that the ability of desiccated cells to retain most of their normal configuration and structural integrity is an important feature of DT plants. Indeed, during desiccation, neither the DT moss nor lichen photobiont showed significant changes in chlorophyll and carotenoid contents nor in the chlorophyll/carotenoid ratio. The authors remarked that, in spite of the importance of repair processes, a mechanism necessitating major repair after each desiccation period would not be viable in a rapidly changing environment in which

conditions allowing photosynthesis are restricted to short periods of the day. Mosses with unistratose leaves, and lichens with water-permeable thalli lacking a cuticule, can absorb and lose water very rapidly and are thus highly adapted to a rapidly changing environment.

Anatomical studies on lichens aimed at visualizing structural alterations induced by drought at the mycobiont-photobiont interface were completed for two cyanobacterial and three green algal macrolichens (Honegger et al. 1996). In the hydrated state, free water was confined to the symplast and the apoplast; no intercellular water was found in the gas-filled thallus interior of all species. Drought stress caused dramatic shrinkage and deformation in all cell types. Although the cytoplasm of both partners was strongly condensed in desiccated lichens, the membrane systems were very well preserved. This indicates a well-functioning protection from desiccation-induced lipid peroxidation.

Ascaso et al. (1988) reported that, in phycobiont cells of *Parmelia laevigata*, desiccation causes collapse of tubules in pyrenoids. On rehydration the lumen of these tubules reappeared. Brown et al. (1987) reported desiccation-induced ultrastructural changes in the pyrenoid structure of *Parmelia sulcata*. As rehydration restored pyrenoids to their original dimensions, they noted that pyrenoid proteins probably become dispersed, rather than degraded by desiccation, thereby indicating the importance of a desiccation-rehydrationphase protection mechanism.

It was clearly demonstrated by Lange and co-workers that lichens with green-algal photobionts can absorb enough moisture from atmospheric water vapor to photosynthesize. In contrast, lichens with cyanobacterial photobionts, in general, require liquid water for recovering the ability to photosynthesize after dehydration (e.g., Lange et al. 1989). However, desiccated cyanobacteria in axenic cultures (isolated from lichens) show water vapor activation of net photosynthesis (Lange et al. 1994a). Generally, poikilohydric DT plants made use of liquid water from dew, mist droplets, or rain, to recover from desiccation. For most lichens, desiccation and rehydration cycles are physiologically important factors. The effect of water content in lichen thalli on photosynthesis (for review on photosynthesis of poikilohydric plants, see Green and Lange 1994) and respiration (for review on water relations of lichens, refer to Rundel 1988) have been abundantly documented (e.g., Lange et al. 1990, 1993, 1994b, 1996; Nash et al. 1990; Bilger et al. 1989; Büdel and Lange 1991; Green et al. 1994; Scheidegger et al. 1995). However, studies on antioxidant defense mechanisms for protection against desiccation-induced oxidative damage in lichens are rare. Only recently were antioxidants, especially glutathione, shown to play an important role in lichens to overcome desiccation periods (Kranner and Grill 1994, 1997a,b; for review, see Kranner and Grill 1996, 1997c).

Demmig-Adams and co-workers revealed the importance of the xanthophyll cycle for scavenging activated oxygen in lichens. Zeaxanthin (but not the epoxides of the xanthophyll cycle) was frequently found in lichens with cyanobacterial photobionts that also contained remarkable amounts of ketocarotenoids (Adams et'al. 1993). By investigating the effect of high light levels on the two symbiotic partners of a *Pseudocyphellaria* phycosymbiodeme (*P. rufovirescens* with a green algal photobiont and *P. murrayi* with a cyanobacterial photobiont), Demmig-Adams et al. (1990b) reported that thallus lobes with green algae exhibited strong nonphotochemical fluorescence quenching, indicative of the radiation-free dissipation of excess excitation energy, whereas lobes with blue-green algae did not possess this capacity. In contrast, a group of cyanobacterial lichens that had formed zeaxanthin slowly through reactions other than the xanthophyll cycle, showed a very similar response to that of leaves and green algal lichens (Demmig-Adams et al. 1990a). These authors correlated the pronounced difference in the capacity for photoprotective energy dissipation in the antenna chlorophyll between (zeaxanthin-containing) green algal lichens and (zeaxanthin-free) cyanobacterial lichens with the presence or absence of zeaxanthin. They proposed that this difference can explain the greater susceptibility to high light stress in lichens with cyanobacterial photobionts.

For investigating adaptation mechanisms that allow air pollution-resistant lichens surviving in polluted areas, Silberstein et al. (1996) evaluated possible air pollution-protection mechanisms in *Xanthoria parietina*, a lichen persisting in fairly heavily polluted areas, and in *Ramalina duriaei* that is disappearing readily in air-polluted regions. *Xanthoria parietina* has multiple protective mechanisms, among which there are increased detoxification of active oxygen species, increased GSH content, and the occurrence of anthraquinones, mainly parietin, that might have antioxidant properties, according to Lawrey (1984).

G. Glutathione Plays a Major Role for Desiccation-Tolerant Plants and Lichens

Glutathione, the most abundant low molecular weight thiol in plants, fulfills significant biological functions. Besides its function in protecting cells from oxidative damage, it regulates enzyme activities and synthesis of proteins and DNA (see Meister and Anderson 1983; Meister 1995). In higher plants, glutathione is the transport form of reduced sulfur (Rennenberg 1982). It has also been postulated that this tripeptide plays a role in the detoxification of heavy metals (Rauser 1993) and xenobiotics (Lamoureux and Russness 1993). One of the functions ascribed to glutathione is the maintenance of protein thiol groups in the reduced state. In general, glutathione is the main redox buffer of cells (Gilbert 1995). Mutants of the yeast Saccharomyces cerevisiae deficient in glutathione synthesis, were hypersensitive to H_2O_2 , O_2^{-} (Stephen and Jamieson 1996) and t-butyl hydroperoxide (Grant et al. 1996). Despite this, these mutants were still able to induce adaptive stress responses to oxidants (Stephen and Jamieson 1996). These results indicate, first, the importance of glutathione and, second, the involvement of various other antioxidants and enzymes in cellular protection from oxidative stress. The requirement for GSH in protection against oxidative stress is analogous with that in other eukaryotes, but unlike the situation in bacteria, for which it is dispensable for growth during both normal and oxidative stress conditions (Grant et al. 1996).

For DT plants, glutathione probably acts, first, as antioxidant (Kranner and Grill 1995), and secondly, as a protective for protein thiol groups (PSH) by forming proteinbound glutathione (PSSG). This way, SH-compounds are protected from irreversible formation of intramolecular disulfide bonds (Kranner and Grill 1996, 1997c). Support for this hypothesis was given by Butt and Ohlrogge (1991), who demonstrated that glutathione protects the acyl carrier protein (ACP) from oxidative damage in dry spinach seeds. They showed that half of the ACP in mature seeds is conjugated to glutathione as ACP-S-S-G, which accumulates during the final stages of seed development when the seed is in the process of dehydration. Imbibition caused a dramatic decrease in ACP-S-S-G and appearance of acylated and reduced ACP. Navari-Izzo et al. (1997) also discussed the glutathionedependent protection of SH groups of proteins during desiccation. They reported a loss in total glutathione content (total glutathione = sum of GSH + GSSG) content during a 25day desiccation period in Boea hygroscopica. On day 12 of dehydration, the total glutathione content was reduced to 24% of the control level and was present mainly in the oxidized form. Simultaneously, oxidation of SH groups of soluble proteins occurred. After 22 days of desiccation, glutathione started to accumulate, and protection from further oxidation of the SH groups of soluble proteins was established. However, during the whole

desiccation period, SH groups of thylakoid proteins were maintained in the reduced form, and the authors suggested a primary role for glutathione in protecting SH groups of thylakoid proteins from desiccation-induced oxidation in *B. hygroscopica*.

Poikilohydric plants and lichens increase their pools of GSSG (Table 1) during desiccation, and they reduce these disulfides at recovery (Dhindsa 1987, 1991; Kranner and Grill 1994, 1997a,b; Sgherri et al. 1994a,b; Navari-Izzo et al. 1997). GSSG and PSSG levels also increase in seeds and conidia of fungi during dormancy and the first stages of germination (Fahey et al. 1975, 1978, 1980; Butt and Ohlrogge 1991; Kranner and Grill 1993; deVos et al. 1994).

The reduction of GSSG to GSH during the recovery of desiccated plants and lichens that are tolerant to desiccation needs the activities of two enzymes: glutathione reductase and glucose-6-phosphate dehydrogenase. The latter is the key enzyme of the oxidative pentose shunt that provides the NADPH required for the action of GR. Presumably this pathway has special importance for resurrection plants that are not able to provide NADPH from photosynthesis in the first stages of recovery following desiccation (Fig. 3).

Glucose-6-phosphate dehydrogenase was also reported to be induced by oxidative stress in yeast (Tran et al. 1995; Miki et al. 1996), which suggests a role for G6PDH in recycling the GSH pool in yeast exposed to oxidative stress (Fig. 4). These authors discussed the role of the oxidative pentose phosphate pathway in providing NADPH required for the action of GR for the oxidative stress response in *Hansenula mrakii* exposed to lipid peroxide.

Recently, changes in the redox status of glutathione and in the activities of GR and G6PDH have been investigated during desiccation and rehydration of three lichens differing in their desiccation tolerance (Kranner and Grill 1997a,b). In general, all three species are desiccation-tolerant; however, within these species there are different grades of desiccation tolerance, with *Pseudevernia furfuracea* being the most desiccation-tolerant and *Peltigera polydactylon* the least desiccation-tolerant of these three species. *Lobaria pulmonaria* has an intermediate position.

Desiccation of these lichens for 2 months resulted in the loss of glutathione (15– 30% of the control content; Table 2), which can be explained by GSH-consuming processes, such as scavenging of desiccation-induced free radicals or the formation of proteinbound glutathione. Simultaneously, the GSH present in lichens in their natural habitat was oxidized in all three lichens. Thalli desiccated for 2 months contained up to 93% of the total glutathione in the oxidized form. This is the highest amount of GSSG, expressed as a percentage of total glutathione, that was ever reported for any living organism. Several authors have reported oxidation of GSH during desiccation of lichens and cryptogamic plants, and during the maturation of seeds of angiosperms (see Table 1). It seems to be a feature of drought-tolerant plants and lichens to oxidize GSH during dehydration. However, such desiccation-tolerant plants have a strong demand to reduce the accumulated glutathione disulfide on recovery for at least two reasons: first, to prevent the GSSGmediated inhibition of protein synthesis and, second, to recycle and then maintain their GSH pool.

Wetting thalli that were in a desiccated state for 2 months resulted in a rapid reduction of GSSG in the most desiccation-tolerant lichens, *P. furfuracea* and *L. pulmonaria*. A pronounced delay was recorded for the most desiccation-sensitive lichen, *P. polydactylon* (Kranner and Grill 1997b). The authors suggested that lichens obtained the NADPH required for the action of GR from the oxidative pentose shunt. The activity of G6PDH was decreased dramatically by dehydrating *P. polydactylon* and *L. pulmonaria* for 2 months, whereas its activity was not affected in *P. furfuracea*. On rehydration, the G6PDH activity

Table 1Redox Reactions of GlutathiSeeds and Spores During Dormancy and	one During Dehydration-Rehydration P d the First Stages of Germination	rocesses in Desiccation-Tolerant Plants a	nd Lichens as Well as in
Species; organ	Thiol-disulfide exchange during dehydration	Thiol-disulfide exchange during rehydration	Ref.
Neurospora crassa; conidia	GSSG and PSSG increasing under aging or drying conditions	Reduction of both PSSG and GSSG during	Fahey et al. 1975
Triticum aestivum and Hordeum sativum; embryos	High GSSG and PSSG levels in dormant seeds	Reduction of both PSSG and GSSG during	Fahey et al. 1980
Tortula ruralis; leaves	Oxidation of GSH to GSSG during slow dehydration	Reduction of GSSG to GSH	Dhindsa 1987, 1991
Spinacia oleracea; seeds	ACP-SSG accumulation during seed maturation	Reduction of ACP-SSG to reduced and acylated	Butt and Ohlrogge 1991
Pisum sativum; seeds	High GSSG levels in dormant seeds	Reduction of GSSG to GSH during imbibition	Kranner and Grill 1993
Pseudevernia furfuracea, Lobaria pulmonaria, Peltiyera polvdactylon: thalli	Oxidation and loss of GSH during desiccation	Reduction of GSSG to GSH during rehydration	Kranner and Grill 1994, 1997a,b
Lycopersicum esculentum; seeds	High GSSG levels in dormant seeds, increasing during aging	Reduction of GSSG during imbibition	DeVos et al. 1994
Boea hygroscopica; leaves Boea hygroscopica; leaves	Oxidation of GSH Oxidation and loss of GSH during desiccation, oxidation of SH groups in soluble proteins	Further oxidation of GSH	Sgherri et al. 1994a Sgherri et al. 1994b; Navari-Izzo et al. 1997
Source: Kranner and Grill (1996, 1997c).			

614

Kranner and Lutzoni





The latter is the key enzyme of the oxidative pentose phosphate pathway that provides NADPH + H⁺ under nonphotosynthetic conditions. The oxidative pentose phosphate pathway has special importance for polkilohydric plants that are, during desiccation, accumulating GSSG following an adaptive strategy for overcoming desiccation (see Fig. 5). (From Kranner 1998.)

Lichen Symbiosis and Adaptation to Desiccation

615





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Species	Desiccation	Rehydration
Pseudevernia furfuracea	Oxidation of GSH (up to 89% GSSG), loss of total glutathione (30% of the content of nondesiccated thalli); GR activated, G6PDH not affected	Reduction of GSSG to the control level (18%) within 5 min; GR not affected, G6PDH slightly increased
Lobaria pulmonaria	Oxidation of GSH (up to 93% GSSG), loss of total glutathione (23% of the content of nondesiccated thalli); GR not affected, G6PDH dramatically decreased	Reduction of GSSG to the control level (18%) within 5 min; GR not affected, G6PDH dramatically increased
Peltigera polydactylon	Oxidation of GSH (up to 71% GSSG), loss of total glutathione (15% of the content of nondesiccated thalli); GR slightly, G6PDH dramatically decreased	Reduction of GSSG only to 30% of total glutathione within 60 min (not to the control level); GR not affected, G6PDH not significantly increased

Table 2 Oxidation of GSH During a Desiccation Period of 2 Months and Reduction of GSSG^aDuring Their Rehydration as Described for Three Lichen Species

"GSSG is given as a percentage of total glutathione. The three investigated lichen species show different grades of desiccation tolerance. Of these three species, *P. furfuracea* is the most tolerant to desiccation, *P. polydactylon* the least tolerant, and *L. pulmonaria* is intermediate.

Source: Kranner and Grill (1997a,b,c).

significantly increased in *P. furfuracea* and in *L. pulmonaria*, but not in *P. polydactylon*. From these results, the authors concluded that the reduction of GSSG during rehydration of *P. polydactylon* might be limited by the availability of NADPH. Furthermore, they suggested a correlation between desiccation tolerance and the capacity to reduce GSSG on rehydration, and that this reduction of GSSG depends on the ability to activate or maintain the activities of the enzymes GR and G6PDH. Moreover, in these three lichen species the capability to activate G6PDH reflects the lichens different grades of desiccation tolerance.

The glutathione metabolism of desiccated-rehydrated lichens appears similar to that occurring in dormant or germinating pea seeds (Kranner and Grill 1993). Dormant pea seeds contained rather high amounts of GSSG (24% of total glutathione) that were reduced to 3% of total glutathione within the first 14 h of imbibition; GR and G6PDH were active during this time. As for the recovery of desiccation-tolerant plants on rehydration, the authors concluded that during the initial stages of germination, pea seeds obtain NADPH required, as cosubstratum of GR, from the pentose phosphate pathway. This indicates that the biochemical pathways that allow recovery from desiccation may depend on similar principles both for seeds during dormancy and the first stages of germination, and for desiccation-tolerant plants and lichenized fungi during desiccation and rehydration.

In most tissues, glutathione is maintained in the reduced state by the action of GR. If we consider the antioxidant properties of GSH, the accumulation of GSSG is correlated with increased oxidative stress. However, from several observations reported in the literature, it was recently proposed that GSSG plays a central role in overcoming the stresses associated with the resting stages of plant development, including dormancy of seeds and

Kranner and Lutzoni

desiccation of resurrection plants (Kranner and Grill 1996). This hypothesis assumes that protein thiol groups are protected from irreversible autooxidation by the reaction of GSSG with protein thiol groups (PSH), thus forming protein-bound glutathione (PSSG) and GSH during desiccation. With extreme desiccation, the GSH formed by the reaction of GSSG with PSH is oxidized further leading to high concentrations of PSSG and GSSG in the desiccated tissue. In this state, PSH and GSH are protected from desiccation-induced oxidative injury, such as irreversible formation of intramolecular cross links in proteins or uncontrolled oxidation of SH groups (e.g., to sulfonic acids).

Although the mechanism and significance of the formation and the accumulation of GSSG in dehydrated tissues are not clearly understood, GSSG can be formed from GSH by several pathways. First, the oxidation of GSH by oxygen may occur spontaneously as a metal-catalyzed reaction. Moreover, a high concentration of thiyl radicals will lead to dimerization of these radicals, leading to autooxidation of GSH or PSH. Another possibility for the oxidation of GSH is an enzyme-catalyzed reaction that leads to the formation of GSSG, As outlined for horseradish peroxidase by Pichorner et al. (1992) and Burner and Obinger (1997), plant peroxidases have a thiol-oxidase function in vitro. In the course of oxidation of thiols by peroxidases, thiyl radicals are formed that undergo several free radical conjugative reactions. In aqueous solutions thiyl radicals show complex behavior. Several reactions of thiyl radicals are outlined by Burner and Obinger (1997), among them dimerization of thiyl radicals to the corresponding disulfides. An increase in the activity of glutathione peroxidase has been reported by Dhindsa (1991) for mosses under drought stress. Such enzyme-catalyzed oxidation of GSH to GSSG during drought could be interpreted as an adaptation mechanism that provides the required high levels of GSSG for the reaction of GSSG and PSH (DH 2; Fig. 5). After reoxidation of the GSH derived from the last reaction, PSSG and GSSG represent stable states that allow a protection from further oxidation of both.

The results of Kranner and Grill (1997a,b) suggests that, in lichens, cellular protection from desiccation- and rehydration-induced damage may be at least as important as repair of the damage during rehydration. Moreover, these results indicate that lichens are extremely well adapted to the dehydration—rehydration process. Considering that the major part of GSH seems to be present in the mycobiont (Kranner et al. 1992), this may lead to the conclusion that, during transition to a lichenized state, adaptation to desiccation (including adaptation to desiccation-induced oxidative damage) has evolved from increased oxidative stress on the mycobiont.

IV. SUMMARY

Studying the origin and evolution of lichen symbioses can provide important clues for determining the nature and intensity of the initial stress on the fungal and photobiotic (green alga and/or cyanobacterium) partners. Recent molecular phylogenetic studies based on the large and small subunit nrRNA genes provide evidence that successful transitions to a lichenized state were very rare during the evolution of ascomycetes, in which more than 98% of the lichen diversity is found. These results imply that evolutionary transitions toward the establishment of stable lichen symbioses are complex and initially involve high stresses on at least one of the symbionts. To identify the nature of these stresses and the symbiont affected by them, the *Omphalina–Coccomyxa* lichen model system was used. Four major evolutionary consequences were found in the fungal species derived from this specific transition to a lichenized state: (1) a slow growth in axenic culture of the myco-



Figure 5 Glutathione disulfide-dependent protection of protein thiol groups during desiccation of plants and lichens, as described by Kranner and Grill (1996). This cycle is organized in dehydration (DH) and rehydration (RH) steps. The cycle starts in the fully hydrated state (left part of the cycle) during which glutathione is mainly present as GSH. Dehydration step 1 starts with the oxidation of GSH to GSSG. The authors propose an enzymatic reaction, maybe catalyzed by peroxidases that use GSH as substrate. In dehydration step 2 the formation of mixed disulfides yields PSSG and GSH. The participation of enzymes remains unclear. In dehydration step 3 the GSH formed in dehydration step 2 is oxidized to GSSG, possibly by peroxidases, as described for dehydration step 1. At this point, the tissue is in the dehydrated state. On rehydration step 1 GSSG is reduced to GSH by the action of GR. Rehydration step 2 implicates the reaction of GSH and PSSG, yielding reduced protein thiol groups and GSSG. After rehydration step 3 the fully hydrated state is reached, leading to reduction of GSSG to GSH by GR. If no further dehydration follows, the normal metabolic processes are started. During desiccation, more than 90% of total glutathione present in lichens can occur as GSSG. At rehydration they are able to reduce this high GSSG content within 2-5 min to the control content of 10-20%. Glutathione reductase and glucose-6-phosphate dehydrogenase have high activities in desiccation-tolerant lichens during the desiccation-rehydration process (Kranner and Grill 1997a,b). During a prolonged rehydration process, lichens may be able to switch between the pentose phosphate pathway (fungi and algae) and photosynthesis (algae). Such interactions between algae and fungi have been neglected so far. (From Kranner and Grill 1997c; adapted from Kranner and Grill 1996.)

biont, suggesting a strong dependency on the algal partner (this dependency was not reciprocal); (2) a trend toward the loss of the dikaryotic state, which could provide a mechanism for a more efficient repair of DNA damage; (3) a variable number of spores per basidium, which could reflect a meiotic defect; and (4) a remarkable increase in rates of nucleotide substitution. From these potential consequences resulting from a transition to a lichen state, three sources of stress were identified: higher solar radiation, higher desiccation levels, and higher ambient oxygen levels. Therefore, a transition to a lichenized state is very likely associated with increased oxidative stress on the mycobiont. First, the fungus is associated with oxygen-producing photobionts; second, they are exposed to increased environmental stresses, such as sun irradiation and desiccation, leading to formation of free radicals. Elevated oxygen levels lead to formation of reactive oxygen spe-

619

cies, such as hydrogen peroxide, superoxide, singlet oxygen, and the hydroxyl radical. These radicals are capable of damaging almost all biologically relevant molecules, such as proteins, carbohydrates, lipids, and DNA. Plants and lichenized fungi possess several mechanisms that help reduce the deleterious effects of oxidative stress, including desiccation-induced oxidative stress. Although these include the well-known free-radical-scavenging pathways, recent studies on lichens suggest that glutathione may play a more important role than previously suspected. In addition to overcoming free radical attack, glutathione may be involved in a thiol-disulfide cycle that protects protein thiol groups from desiccation-induced autooxidation. The significance of other antioxidants from overcoming desiccation have not yet been elucidated in lichens.

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REFERENCES

- Adams WW III, Demmig-Adams B, Lange OL (1993). Carotenoid composition and metabolism in green and blue-green algal lichens in the field. Oecologia 94:576-584.
- Ahmadjian V, Jacobs JB (1981). Relationship between fungus and alga in the lichen *Cladonia* cristatella Tuck. Nature 289:169-172.
- Armaleo D (1995). Factors affecting depside and depsidone biosynthesis in a cultured lichen fungus. Crypt Bot 5:14-21.
- Ascaso C, Brown DH, Rapsch S (1988). The effect of desiccation on pyrenoid structure in the oceanic lichen *Parmelia laevigata*. Lichenologist 20:31-39.
- Atherton NM, Hendry GAF, Möbius K, Rohrer M, Törring JT (1993). A free radical ubiquitously associated with senescence in plants: evidence for a quinone. Free Radic Res Commun 19: 297-301.
- Bauemlein H, Braun H, Kakhovskaya IA, Shutov AD (1995). Seed storage proteins of spermatophytes share a common ancestor with desiccation proteins of fungi. J Mol Evol 41:1070-1075.
- Bartosz G (1997). Oxidative stress in plants. Acta Physiol Plant 19:47-64.
- Beckett RP (1995). Some aspects of water relations of lichens from habitats of contrasting water status studied using thermocouple psychrometry. Ann Bot 76:211-217.
- Bewley DJ (1979). Physiological aspects of desiccation tolerance. Annu Rev Plant Physiol 30: 195-238.
- Bewley JD, Krochko JE (1982). Desiccation tolerance. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. Encyclopedia of Plant Physiology, Vol 12. Physiological Ecology II. Berlin: Springer, pp 325-378.
- Bilger W, Rimke S, Schreiber U, Lange OL (1989). Inhibition of energy transfer to photosystem II in lichens by dehydration: different properties of reversibility with green and blue-green phycobionts. J Plant Physiol 134:261-268.
- Brennan RJ, Swoboda BEP, Schiestl RH (1994). Oxidative mutagens induce intrachromosomal recombination in yeast. Mutat Res 308: 159-167.

- Brown DH, Ascaso C, Rapsch S (1987). Ultrastructural changes in the pyrenoid of the lichen *Parmelia sulcata* stored under controlled conditions. Protoplasma 136:136-144.
- Buchvarov P, Gantcheff T (1984). Influence of accelerated and natural aging on free radical levels in soybean seeds. Physiol Plant 60:53-56.
- Büdel B, Lange OL (1991). Water status of green and blue-green phycobionts in lichen thalli after hydration by water vapor uptake: do they become turgid? Bot Acta 104: 361-366.
- Burner U, Obinger C (1997). Transient-state and steady-state kinetics of the oxidation of aliphatic and aromatic thiols by horseradish peroxidase. FEBS Lett 411:269-274.
- Butt AD, Ohlrogge JB (1991). Acyl carrier protein is conjugated to glutathione in spinach seed. Plant Physiol 96:937-942.
- Cadenas E (1995). Thiyl radical formation during thiol oxidation by ferrylmyoglobin. Methods Enzymol 251:106-116.
- Crowe JH, Crowe LM, Carpenter JF, Rudolf AS, Wistrom CA, Spargo BJ, Anchordoguy TJ (1988). Interactions of sugars with membranes. Biochim Biophys Acta 947:367-384.

Crowe JH, Hoekstra FA, Crowe LM (1992). Anhydrobiosis. Annu Rev Physiol 54:579-599.

- Crowe LM, Mouradian R, Crowe JH, Jackson SA, Womersley C (1984). Effects of carbohydrates on membrane stability at low water activities. Biochim Biophys Acta 769:141-150.
- Culberson CF (1969). Chemical and Botanical Guide to Lichen Products. Chapel Hill: University of North Carolina Press.
- Culberson CF (1970). Supplement to "Chemical and Botanical Guide to Lichen Products." Bryologist 73:177-377.
- Culberson CF, Culberson WL, Johnson A (1977). Second Supplement to "Chemical and Botanical Guide to Lichen Products." St. Louis: Missouri Botanical Garden, American Bryological and Lichenological Society.
- Culberson CF, Elix JD (1989), Lichen substances. In: Harborne JB, ed. Methods in Plant Biochemistry. Vol 1. New York: Academic Press, pp 509-535.
- DeVos CHR, Kraak HL, Bino RJ (1994). Ageing of tomato seeds involves glutathione oxidation. Physiol Plant 92:131-139.
- Demmig-Adams B, Adams WW III (1996a). Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. Planta 198:460-470.
- Demmig-Adams B, Adams WW III (1996b). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci 21-27.
- Demmig-Adams B, Adams WW III, Czygan FC, Schreiber U, Lange OL (1990a). Differences in the capacity for radiationless energy dissipation in the photochemical apparatus of green and blue-green algal lichens associated with differences in carotenoid composition. Planta 180: 582-589.
- Demmig-Adams B, Adams WW III, Green TGA, Czygan FC, Lange OL (1990b). Differences in the susceptibility to light stress in two lichens forming a phycosymbiodeme, one partner possessing and one lacking the xanthophyll cycle. Oecologia 84:451-456.
- Dhindsa RS (1987). Glutathione status and protein synthesis during drought and subsequent rehydration in *Tortula ruralis*. Plant Physiol 83:816-819.
- Dhindsa RS (1991). Drought stress, enzymes of glutathione metabolism, oxidation injury, and protein synthesis in *Tortula ruralis*. Plant Physiol 95:648-651.
- Dose K, Gill M (1995). DNA stability and survival of *Bacillus subtilis* spores in extreme dryness. Origins Life Evol Biosph 25:277-293.
- Elix JA, Whitton AA, Sargent MV (1984). Recent progress in the chemistry of lichen substances. Prog Chem Organ Nat Prod 45:103-234.
- Elstner EF (1982). Oxygen activation and oxygen toxicity. Annu Rev Plant Physiol 33:76-96.
- Elstner EF, Oßwald W (1994). Mechanisms of oxygen activation during plant stress. Proc R Soc Edinb B102:131-154.
- Ernst V, Levin DH, London IM (1978). Inhibition of protein synthesis initiation by oxidized glutathione: activation of a protein kinase that phosphorylates the α -subunit of eucaryotic initiation factor 2. Proc Natl Acad Sci USA 75:4110-4114.

- Ernst V, Levin DH, London IM (1979). In situ phosphorylation of the α-subunit of eucaryotic initiation factor 2 in reticulocyte lysates inhibited by heme deficiency, double-stranded RNA, oxidized glutathione, or the heme-regulated protein kinase. Proc Natl Acad Sci USA 76:2118-2122.
- Esterbauer H, Schaur RJ, Zollner H (1991). Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radic Biol Med 11:81-128.
- Fahey RC, Brody S, Mikolajczyk SD (1975). Changes in the glutathione thiol-disulfide status of Neurospora crassa conidia during germination and aging. J Bacteriol 121:144-151.
- Fahey RC, Mikolajczyk SD, Brody S (1978). Correlation of enzymatic activity and thermal resistance with hydration state in ungerminated *Neurospora conidia*. J Bacteriol 135:868-875.
- Fahey RC, Di Stefano DL, Meier GP, Bryan RN (1980). Role of hydration state and thiol-disulfide status in the control of thermal stability and protein synthesis in wheat embryo. Plant Physiol 65:1062-1066.
- Fahselt D (1994). Secondary biochemistry of lichens. Symbiosis 16:117-165.
- Fairhead H, Setlow B, Waites WM, Setlow P (1994). Small, acid-soluble proteins bound to DNA protect *Bacillus subtilis* spores from being killed by freeze-drying. Appl Environ Microbiol 60:2647-2649.
- Farrant JM, Pammenter NW, Berjak P, Farnsworth EJ, Vertucci CW (1996). Presence of dehydrinlike proteins and levels of abscisic acid in recalcitrant (desiccation sensitive) seeds may be related to habitat. Seed Sci Res 6:175-182.
- Finch-Savage WE, Hendry GAF, Atherton NM (1994). Free radical activity and loss of viability during drying of desiccation-sensitive tree seeds. Proc R Soc Edinb B102:257-260.
- Finckh BF, Kunert KJ (1985). Vitamin C and E: an antioxidative system against herbicide-induced lipid peroxidation in higher plants. J Agric Food Chem 33:574-577.
- Foyer CH, Halliwell B (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133:21-25.
- Frankenberg D, Frankenberg-Schwagner M, Harbich R (1993). Mechanisms of oxygen radiosensitization in irradiated yeast. I. DNA double-strand breakage. Int J Radiat Biol 64:511-521.
- Friedberg EC, Walker GC, Siede W (1995). DNA Repair and Mutagenesis. Washington, DC: American Society for Microbiology Press.
- Gaff DF (1989). Responses of desiccation tolerant "resurrection" plants to water stress. In: Kreeb KH, Richter H, Hinckley TM, eds. Structural and Functional Responses to Environmental Stresses: Water Shortages. The Hague: SPB Academic Publishing, pp 264-311.
- Galun M (1979). Symbiotic relationships in plants. In: Zuckermann A, ed. Dynamic Aspects of Host-Parasite Relationship. Vol 3. Jerusalem: Israel University Press, pp 204-228.
- Galun M (1988). The fungus-alga relation. In: Galun M, ed. Handbook of Lichenology. Vol 1. Boca Raton: CRC Press, pp 147-158.
- Gargas A, DePriest PT, Grube M, Tehler A (1995). Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. Science 268:1492-1495.
- Gilbert HF (1995). Thiol-disulfide exchange equilibria and disulfide bond stability. Methods Enzymol 251:8-28.
- Gogorcena Y, Itrube-Ormaetxe I, Escuredo PR, Becana M (1995). Antioxidant defenses against activated oxygen in pea nodules subjected to water stress. Plant Physiol 108:753-759.
- Goldman R, Tsyrlov IB, Grogan J, Kagan VE (1997). Reactions of phenoxyl radicals with NADPH cytochrome P-450 oxidoreductase and NADPH—reduction of the radicals and inhibition of the enzyme. Biochemistry 36:3186-3192.
- Grant CM, Maciver FH, Dawes IW (1996). Glutathione is an essential metabolite required for resistance to oxidative stress in the yeast Saccharomyces cervisiae. Curr Genet 29:511-515.
- Green TGA, Lange OL (1994). Photosynthesis in poikilohydric plants: a comparison of lichens and bryophytes. In: Schulze ED, Caldwelll MC, eds. Ecophysiology of Photosynthesis. Ecological Studies 100. Berlin: Springer Verlag, pp 319-341.
- Green TGA, Lange OL, Cowan IR (1994). Ecophysiology of lichens photosynthesis: the role of water status and thallus diffusion resistances. Crypt Bot 4:166-178.

- Halliwell B (1987). Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. Chem Phys Lipids 44:327-340.
- Halliwell B, Gutteridge JMC (1989). Free Radicals in Biology and Medicine. Oxford: Clarendon Press.
- Hawksworth DL (1988a). The variety of fungal-algal symbioses, their evolutionary significance, and the nature of lichens. Bot J Linn Soc 96:3-20.
- Hawksworth DL (1988b). The fungal partner. In: Galun M, ed. Handbook of Lichenology. Vol 1. Boca Raton: CRC Press, pp 35-38.
- Hawksworth DL, Hill DJ (1984). The Lichen-Forming Fungi. New York: Chapman & Hall.
- Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995). Ainsworth and Bisby's Dictionary of the Fungi. Kew: CMI.
- Hellwege EM, Dietz KJ, Volk OH, Hartung W (1994). Abscisic acid and the induction of desiccation tolerance in the extremely xerophilic liverwort *Exormotheca holstii*. Planta 194:525-531.
- Hellwege EM, Dietz KJ, Hartung W (1996). Abscisic acid causes changes in gene expression involved in the induction of the landform of the liverwort *Riccia fluitans* L. Planta 198:423– 432.
- Hendry G, Finch-Savage WE, Thorpe PC, Atherton NM, Buckland SM, Nilsson KA, Seel WE (1992). Free radical processes and loss of seed viability during desiccation in the recalcitrant species *Quercus robur* L. New Phytol 122:273-279.
- Hidalgo ME, Fernandez E, Quilhot W, Lissi E (1994). Antioxidant activity of depsides and depsidones. Phytochemistry 37:1585-1587.
- Honneger R, Brunner U (1981). Sporopollenin in the cell walls of *Coccomyxa* and *Myrmecia* phycobionts of various lichens: an ultrastructural and chemical investigation. Can J Bot 59:2713– 2734.
- Honegger R, Peter M, Scherrer S (1996). Drought-induced structural alterations at the mycobiontphotobiont interface in a range of foliose marcolichens. Protoplasma 190:221-232.
- Jackson RJ, Campbell EA, Herbert P, Hunt T (1983). The roles of sugar phosphates and thiol reducing systems in the control of reticulocyte protein synthesis. Eur J Biochem 131:313-324.
- Jagtap V, Bhargava S (1995). Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of *Sorghum bicolor* (L.) Moench. exposed to high light, low water and high temperature stress. J Plant Physiol 145:195-197.
- Kershaw KA (1985). Physiological Ecology of Lichens. Cambridge Studies in Ecology. Cambridge: Cambridge University Press.
- Kranner I (1998). Determination of glutathione, glutathione disulfide, and two related enzymes, glutathione reductase, and glucose-6-phosphate dehydrogenase, in fungal and plant cells. In: Varma A, ed. Mycorrhiza Manual. Heidelberg: Springer Verlag, pp 227-240.
- Kranner I, Grill D (1993). Content of low-molecular-weight thiols during the imbibition of pea seeds. Physiol Plant 88:557-562.
- Kranner I, Grill D (1994). Rapid changes of the glutathione status and the enzymes involved in the reduction of glutathione disulfide during the initial stage of wetting of lichens. Crypt Bot 4: 203-206.
- Kranner I, Grill D (1995). The role of glutathione and related enzymes in seeds and poikilohydric plants during desiccation and rehydration. Acta Pharm 2:157--163.
- Kranner I, Grill D (1996). Significance of thiol-disulfide exchange in resting stages of plant development. Bot Acta 109:8-14.
- Kranner I, Grill D (1997a). Desiccation of lichens: changes in the glutathione status. In: Brunold CH, Cram J, DeKok LJ, Rennenberg H, Stulen I, eds. Sulfur Metabolism in Higher Plants. Fundamental Molecular, Ecophysiological and Agricultural Aspects. Leiden: Backhuys Publishers, pp 249-252.
- Kranner I, Grill D (1997b). The glutathione status during recovery of desiccated lichens: is desiccation tolerance correlated with a high reducing capacity for glutathione disulphide? In: Brunold CH, Cram J, DeKok LJ, Rennenberg H, Stulen I, eds. Sulfur Metabolism in Higher

Plants. Fundamental Molecular, Ecophysiological and Agricultural Aspects. Leiden: Backhuys Publishers, pp 253-255.

- Kranner I, Grill D (1997c). Desiccation and the subsequent recovery of cryptogamics that are resistant to drought. Phyton Special issue: Oxygen, Free Radicals and Environmental Stress in Plants 37:139-150.
- Kranner I, Guttenberger H, Grill D, Delefant M, Türk R (1992). Investigations of thiols in lichens. Special Issue: Sulfur metabolism. Phyton 32:69-73.
- Kundu SC, Wilson RL (1995). Thiyl (sulfhydryl/thiol) free radical reactions, vitamins, β-carotene, and superoxide dismutase in oxidative stress: design and interpretation of enzymatic studies. Methods Enzymol 251:69-81.
- Kunert KJ, Foyer C (1993). Thiol/disulfide exchange in plants. In: DeKok LJ, Stulen I, Rennenberg H, Brunold CH, Rauser WE, eds. Sulfur Nutrition and Assimilation in Higher Plants. The Hague: SPB Academic Publishing, pp 139-151.
- Lamoure D (1968). Preuve caryologique que le basidiomycète Omphalina ericetorum (Pers. ex Fr.) M. Lange peut être le mycobionte du lichen Botrydina vulgaris Bréb. C R Acad Sci Paris 266:2339-2340.
- Lamoure D (1969). Évolution nucléaire dans la baside de formes parthénogénétiques tétrasporiques de trois espèces d'*Omphalina* (Argaricales). Bull Soc Mycol Fr 80:247-249.
- Lamoure D (1989). Répertoire des données utiles pour effectuer les tests d'intercompatibilité chez les basidiomycètes V. Agaricales sensu lato. Crypt Mycol 10:41-80.
- Lamoure D (1993). Nuclear behavior in some omphaloid fructifications of the basidiolichens Botrydina and Coriscium. In: Petrini O, Laursen GA, eds. Arctic and Alpine Mycology 3. Bibl Mycol 150. Berlin: J Cramer, pp 155-160.
- Lamoureux GL, Rusness DG (1993). Glutathione in the metabolism and detoxification of xenobiotics in plants. In: DeKok LJ, Stulen I, Rennenberg H, Brunold CH, Rauser WE, eds. Sulfur Nutrition and Assimilation in Higher Plants. The Hague: SPB Academic Publishing, pp 221– 237.
- Lange OL, Bilger W, Rimke S, Schreiber U (1989). Chlorophyll fluorescence of lichens containing green and blue-green algae during hydration by water vapour uptake and by addition of liquid water. Bot Acta 102:306-313.
- Lange OL, Pfanz H, Kilian E, and Meyer A (1990). Effect of low water potential on photosynthesis in intact lichens and their liberated algal components. Planta 182:467-472.
- Lange OL, Büdel B, Heber U, Meyer A, Zellner H, Green TGA (1993). Temperate rainforest lichens in New Zealand: high thallus water content can severely limit photosynthetic CO₂ exchange. Oecologia 95:303-313.
- Lange OL, Meyer A, Büdel B (1994a). Net photosynthesis activation of a desiccated cyanobacterium without liquid water in high air humidity alone. Experiments with *Microcoleus sociatus* isolated from a desert soil crust. Funct Ecol 8:52-57.
- Lange OL, Meyer A, Zellner H, Heber U (1994b). Photosynthesis and water relations of lichen soil crusts: field measurements in the coastal fog zone of the Namib Desert. Funct Ecol 8:253–264.
- Lange OL, Green TGA, Reichenberger H, Meyer A (1996). Photosynthetic depression at high thallus water content in lichens: concurrent use of gas exchange and fluorescence techniques with a cyanobacterial and a green algal *Peltigera* species. Bot Acta 109:43-50.
- Langenstein B (1994). Experimentell-ontogenetische Studien zur Lichenisation am Beispiel von *Omphalina* (Basidiomycota) und *Coccomyxa* (Chlorophyta). Diplomarbeit der Fakultät für Biologie der Universität Tübingen.
- Law R, Lewis DH (1983). Biotic environments and the maintenance of sex—some evidence from mutualistic symbioses. Bot J Linn Soc 20:249-276.

Lawrey JD (1984). Biology of Lichenized Fungi. New York: Praeger.

Leprince O, Deltour R, Thorpe PC, Atherton NM, Hendry GAF (1990). The role of free radicals and radical processing systems in loss of desiccation tolerance in germinating maize (Zea mays L.). New Phytol 116:573-580.

- Leprince O, Hendry GAF, McKersie BD (1993). The mechanisms of desiccation tolerance in developing seeds. Seed Sci Res 3:231-246.
- Leprince O, Atherton NM, Deltour R, Hendry GAF (1994a). The involvement of respiration in free radical processes during loss of desiccation tolerance in germinating Zea mays L. An electron paramagnetic resonance study. Plant Physiol 104:1333–1339.
- Leprince O, Hendry GAF, Atherton NM (1994b). Free radical process induced by desiccation in germinating maize. The relationship with respiration and loss of desiccation tolerance. Proc R Soc Edin 102B:211-218.
- Leprince O, Vertucci CW, Hendry GAF, Atherton NM (1995). The expression of desiccation-induced damage in orthodox seeds is a function of oxygen and temperature. Physiol Plant 94:233-240.
- Levitt J, ed. (1980). Responses of Plants to Environmental Stress 1: Chilling, Freezing and High Temperature Stress. New York: Academic Press.
- Li W-H, Graur D (1991). Fundamentals of Molecular Evolution. Sunderland, MA: Sinauer Associates.
- Lutzoni F (1997). Phylogeny of lichen- and non lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Syst Biol 46:373-406.
- Lutzoni F, Vilgalys R (1995a). Integration of morphological and molecular data sets in estimating fungal phylogenies. Can J Bot 73(suppl 1):S649-S659.
- Lutzoni FM, Vilgalys R (1995b). *Omphalina* (Basidiomycota, Agaricales) as a model system for the study of coevolution in lichenized fungi. Crypt Bot 5:82-97.
- Lutzoni F, Pagel M (1997). Accelerated evolution as a consequence of transitions to mutualism. Proc Natl Acad Sci USA 94:11422-11427.
- Lutzoni F, Crodian J, Reeb V (1997). Phylogenetic relationships of ascomycetes as revealed by the small and large subunit nuclear ribosomal DNA with emphasis on lichen-forming ascomycetes and their origin. Am J Bot (Abstr)84:10–11.
- Lutzoni F, Spatafora JW, Armaleo D, Søchting U, Johnson J, Mahoney B, Lagreca S, Culberson WL, (1996). Relationships among lichenized and nonlichenized ascomycota based on phylogenetic analyses of ribosomal DNA. Third International Lichenological Congress (Salzburg, Austria): Abstr p 6.
- Lutzoni F, Spatafora JW, Armaleo D, Søchting U, Johnson J, Mahoney B, Lagreca S, Culberson WL (in press). Relationships among lichenized and nonlichenized ascomycota based on phylogenetic analyses of ribosomal DNA.
- Mattimore V, Battista JR (1996). Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J Bacteriol 178:633-637.
- Meister A (1995). Glutathione metabolism. Methods Enzymol 251:3-7.
- Meister A, Anderson ME (1983). Glutathione. Annu Rev Biochem 52:711-760.
- Miki T, Tsujimoto Y, Miyabe S, Sugiyama KI, Izawa S, Inoue Y, Kimura A (1996). Oxidative stress response in yeast: purification and some properties of oxidative stress-inducible glucose-6-phosphate dehydrogenase from *Hansenula mrakii*. Biosci Biotechnol Biochem 60:966-970.
- Moradas-Ferreira P, Costa V, Piper P, Mager W (1996). The molecular defences against reactive oxygen species in yeast. Mol Microbiol 19:651-658.
- Moran N (1996). Accelerated evolution and Muller's rachet in endosymbiotic bacteria. Proc Natl Acad Sci USA 93:2873-2878.
- Munakata N, Saitou M, Takahashi N, Hieda K, Morohoshi F (1997). Induction of unique tandembase change mutations in bacterial spores exposed to extreme dryness. Mutat Res 390:189– 195.
- Nash TH III, Reiner A, Demmig-Adams B, Kilian E, Kaiser WM, Lange OL (1990). The effect of atmospheric desiccation and osmotic water stress on photosynthesis and dark respiration of lichens. New Phytol 116:269-276.
- Navari-Izzo F, Pinzino C, Quartacci MF, Sgherri CLM (1994). Intracellular membranes: kinetics of superoxide production and changes in thylakoids of resurrection plants upon dehydration and rehydration. Proc R Soc Edin 102B:187-191.

Navari-Izzo F, Meneguzzo S, Loggini B, Vazzana C, Sgherri CLM (1997). The role of the glutathione system during dehydration of *Boea hygroscopica*. Physiol Plant 99 (1):23-30.

Oberwinkler F (1984). Fungus-alga interactions in basidiolichens. Beih Nova Hedwigia 79:739-774.

- Oliver MJ (1996). Desiccation tolerance in vegetative plant cells. Physiol Plant 97:779-787.
- Oliver MJ, Bewley JD (1997). Desiccation-tolerance of plant tissues: a mechanistic overview. Hort Rev 18:171-213.
- Omland KE (1997). Correlated rates of molecular and morphological evolution. Evolution 51:1381-1393.
- Pichorner H, Couperus A, Korodi SAA, Ebermann R (1992). Plant peroxidase has a thiol oxidase function. Phytochemistry 31:3371-3376.
- Popp M, Smirnoff N (1995). Polyol accumulation and metabolism during water deficit. In: Smirnoff N, ed. Environment and Plant Metabolism. Flexibility and Acclimation. Oxford: Bios Scientific, pp 199-215.
- Price AH, Hendry GAF (1991). Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. Plant Cell Environ 14:477-484.
- Priestley DA, Werner BG, Leopold AC, McBride MB (1985). Organic free radical levels in seeds and pollen: the effects of hydration and aging. Physiol Plant 64:88-94.
- Quartacci MF, Navari-Izzo F (1992). Water stress and free radical mediated changes in sunflower seedlings. J Plant Physiol 139:621-625.
- Rauser W (1993). Metal-binding peptides in plants. In: DeKok LJ, Stulen I, Rennenberg H, Brunold C, Rauser WE, eds. Sulfur Nutrition and Assimilation in Higher Plants. The Hague: SPB Academic Publishing, pp 239–251.
- Redhead SA, Kuyper TW (1987). Lichenized agarics: taxonomic and nomenclatural riddles. In: Laursen GA, Ammirati JF, Redhead SA, eds. Arctic and Alpine Mycology II. New York: Plenum Press, pp 319-348.
- Rennenberg H (1982). Glutathione metabolism and possible biological roles in higher plants. Phytochemistry 21:2771-2781.
- Reynolds TL, Bewley JD (1993). Abscisic acid enhances the ability of the desiccation-tolerant fern *Polypodium virginianum* to withstand drying. J Exp Bot 44:1771-1779.
- Rice-Evans CA, Miller NJ, Paganga G (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radic Biol Med 20:933-956.
- Rodrigo AG, Kelly-Borges M, Bergquist PR, Bergquist PL (1993). A randomisation test of the null hypothesis that two cladograms are sample estimates of a parametric phylogenetic tree. NZ J Bot 31:257-268.
- Roser DJ, Melick DR, Ling HU, Seppelt RD (1992). Polyol and sugar content of terrestrial plants from continental Antarctica. Antarctic Sci 4:413-420.
- Rundel PW (1988). Water relations. In: Galun M, ed. CRC Handbook of Lichenology. Boca Raton: CRC Press, pp 17-36.
- Sandermann H Jr, Schmitt R, Heller W, Rosemann D, Langebartels C (1989). Ozone-induced early biochemical reactions in conifers. In: Longhurst JWS, ed. Acid Deposition. Sources, Effects, and Controls. Oxford: Information Press, pp 243–253.
- Scheidegger C, Schroeter B, Frey B (1995). Structural and functional processes during water vapour uptake and desiccation in selected lichens with green algal photobionts. Planta 197:399-409.
- Schultz M (1995). Protein and ubiquitin conjugate patterns of *Peltigera horizontalis* (Huds.) Baumg. during desiccation and rehydration. In: Daniels FJA, Schultz M, Peine J, eds. Flechten Follmann. Contributions to Lichenology in Honour of Gerhard Follmann. Geobotanical and Phytotaxonomical Study Group. University of Cologne: Botanical Institute, pp 87–96.
- Seel W, Hendry G, Atherton N, Lee J (1991). Radical formation and accumulation in vivo, in desiccation-tolerant and intolerant moss. Free Radic Res Commun 15:133-141.
- Seel WE, Hendry GAF, Lee JA (1992). Effects of desiccation on some activated oxygen processing enzymes and anti-oxidants in mosses. J Exp Bot 43:1031-1037.
- Seneratna T, McKersie BD, Stinson RH (1985a). Simulation of dehydration injury to membranes from soybean axes by free radicals. Plant Physiol 77:472-474.

- Seneratna T, McKersie BD, Stinson RH (1985b). Antioxidant levels in germinating soybean seed axes in relation to free radical and dehydration tolerance. Plant Physiol 78:168-171.
- Setlow P (1995). Mechanisms for the prevention of damage to DNA in spores of *Bacillus* species. Annu Rev Microbiol 49:29-54.
- Setlow B, Setlow P (1995). Small, acid-soluble proteins bound to DNA protect *Bacillus subtilis* spores from killing by dry heat. Appl Environ Microbiol 61:2787-2790.
- Setlow B, Setlow P (1996). Role of DNA repair in *Bacillus subtilis* spore resistance. J Bacteriol 178:3486-3495.
- Sgherri CLM, Loggini B, Bochicchio A, Navari-Izzo F (1994a). Antioxidant system in *Boea hy*groscopica: changes in response to desiccation and rehydration. Phytochemistry 37:377-381.
- Sgherri CLM, Quartacci MF, Bochicchio A, Navari-Izzo F (1994b). Defence mechanisms against production of free radicals in cells of "resurrection" plants. Proc R Soc Edinb B102:291-294.
- Sherwin HW, Farrant JM (1996). Differences in rehydration of three desiccation-tolerant angiosperm species. Ann Bot 78:703-710.
- Siefermann-Harms D (1977). The xanthophyll cycle in higher plants. In: Tevini M, Lichtenthaler HK, eds. Lipids and Lipid Polymers in Higher Plants. Berlin: Springer Verlag, pp 219-230.
- Sievers C, Deters D, Hartmann HJ, Weser U (1996). Stable thiyl radicals in dried yeast Cu(I)-6thionein. J Inorg Biochem 62:199-205.
- Silberstein L, Siegel BZ, Siegel SM, Mukhtar A, Galun M (1996). Comparative studies on Xanthoria parientina, a pollution-resistant lichen, and Ramlina duriaei, a sensitive species. II. Evaluation of possible air pollution-protection mechanisms. Lichenology 28:367-383.
- Simontacchi M, Puntarulo S (1992). Oxygen radical generation by isolated microsomes from soybean seedlings. Plant Physiol 100:1263-1268.
- Singer CE, Ames BN (1970). Sunlight ultraviolet and bacterial DNA base ratios. Science 170: 822-826.
- Smirnoff N (1993). The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27-58.
- Smith MT, Berjak P (1995). Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation-sensitive seeds. In: Kigel J, Galili G, eds. Seed Development and Germination, New York: Marcel Dekker, pp 701-746.
- Spatafora JW, Lutzoni F, Armaleo D, Mahoney D, LaGreca S, Johnson J, Clerc P, Culberson WL (1994). The integration of lichenized and nonlichenized ascomycetes in molecular systematics. Fifth International Mycological Congress: Abstr, p 130.
- Stajner D, Mimica-Dukic N, Gasic O (1995). Adaptability to drought in sugar beet cultivars. Biol Plant 37:107-112.
- Stephen DWS, Jamieson DJ (1996). Glutathione is an important antioxidant molecule in the yeast Saccharomyces cervisiae. FEMS Microbiol Lett 141:207-212.
- Tran LT, Miki T, Kamakura M, Izawa S, Tsujimoto Y, Miyabe S, Inoue Y, and Kimura A (1995). Oxidative stress response in yeast: induction of glucose-6-phosphate dehydrogenase by lipid hydroperoxide in *Hansenula mrakii*. J Ferment Bioeng 80:606-609.
- Tuba Z, Lichtenthaler HK, Csintalan Z, Pocs T (1993a). Regreening of desiccated leaves of the poikilochlorophyllous *Xerophyta scabrida* upon rehydration. J Plant Physiol 142:103-108.
- Tuba Z, Lichtenthaler HK, Maroti I, Csintalan Z (1993b). Resynthesis of thylakoids and chloroplast ultrastructure in the desiccated leaves of the poikilochlorophyllous *Xerophyta scabrida* upon rehydration. J Plant Physiol 142:742-748.
- Tuba Z, Lichtenthaler HK, Csintalan ZS, Nagy Z, Szente K (1994). Reconstitution of chlorophylls and photosynthetic CO₂ assimilation in the desiccated poikilochlorophyllous plant *Xerophyta scabrida* upon rehydration. Planta 192:414-420.
- Tuba Z, Csintalan Z, Proctor MCF (1996a). Photosynthetic responses of a moss, *Tortula ruralis*, spp. *ruralis*, and the lichens *Cladonia convoluta* and *C. furcata* to water deficit and short periods of desiccation, and their ecophysiological significance: a baseline study at presentday CO₂ concentration. New Phytol 133:353-361.

- Tuba Z, Lichtenthaler HK, Csintalan ZS, Nagy Z, Szente K (1996b). Loss of chlorophylls, cessation of photosynthesis, CO₂ assimilation, and respiration in the poikilochlorophyllous plant Xerophyyta scabrida during desiccation. Physiol Plant 96:383-388.
- Vaca CE, Wilhelm J, Harms-Ringdahl M (1988). Interaction of lipid peroxidation products with DNA. A review. Mutat Res 195:137-149.
- Van der Vliet A, Cross CE, Halliwell B, O'Neil CA (1995). Plasma protein sulfhydryl oxidation: effect of low molecular weight thiols. Methods Enzymol 251:448-455.
- Wardmann P, Von Sonntag C (1995). Kinetic factors that control the fate of thiyl radicals in cells. Methods Enzymol 251:31-35.
- Werner O, Ros Espin RM, Bopp M, Atzorn R (1991). Abscisic-acid-induced drought tolerance in *Funaria hygrometrica* Hedw. Planta 186:99-103.
- Woodford DV, Parish JH, Moradas-Ferreira P (1995). Hydrogen peroxide induces DNA damage in Saccharomyces cervisiae. Yeast 11: p 149.
- Xu N, Bewley JD (1995). The role of abscisic acid in germination, storage protein synthesis and desiccation tolerance in alfalfa (*Medicago sativa* L.) seeds, as shown by inhibition of its synthesis by fluoridone during development. J Exp Bot 46:687-694.
- Zhang J, Cui S, Li J, Wei J, Kirkham MB (1995). Protoplasmic factors, antioxidant responses, and chilling resistance in maize. Plant Physiol Biochem 33:567-575.

628