*Symbiosis*, 31 (2001) 113–121
Balaban, Philadelphia/Rehovot


# The Effect of Desiccation on the Activities of Antioxidant Enzymes in Lichens from Habitats of Contrasting Water Status

NOSISA MAYABA and RICHARD P. BECKETT*
*School of Botany and Zoology, University of Natal, Private Bag X01, Pietermaritzburg, Scottsville 3209, Republic of South Africa, Tel. +27-33-2605141, Fax. +27-33-2605897, E-mails. beckett@nu.ac.za or rick_beckett@yahoo.com*

Received October 29, 2000; Accepted February 11, 2001

**Abstract**

Changes in the activities of the antioxidant enzymes ascorbate peroxidase, catalase and superoxide dismutase were measured during wetting and drying cycles in the lichens *Peltigera polydactyla*, *Ramalina celastri* and *Teloschistes capensis*. These species normally grow in moist, xeric, and extremely xeric microhabitats respectively. Enzyme activity was measured shortly after collection, after rehydration for 48 h, after desiccation for 14 d and 28 d, and during the first 30 min of subsequent rehydration with liquid water. In all species, enzyme activities tended to rise or stay the same following rehydration. After desiccation for 14 d, enzyme activities decreased, then decreased further to very low values after desiccation for 28 d. In all species, including the *T. capensis* from an extremely xeric habitat, the activities of all enzymes remained at very low values during the 30 min following rehydration, and were therefore unavailable to remove any reactive oxygen species accumulating in lichen tissues as a result of desiccation stress. We suggest that the enzymatic antioxidants are more likely to be involved in removing reactive oxygen species produced during moderate stress or the normal metabolic processes of lichens.

Keywords: Lichens, desiccation, ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), antioxidants

*Presented at the Fourth International Association of Lichenology Symposium, September 3–8, 2000, Barcelona, Spain*

*The author to whom correspondence should be sent.

0334-5114/2001/$05.50 ©2001 Balaban

## 1. Introduction

Most lichens are "poikilohydric" organisms, and can survive in the air-dried state for long or short periods even at relative water contents below 10%, a state that would be lethal to most vascular plants (Kranner and Lutzoni, 1999; Gaff, 1997). In the desiccated state lichens appear completely dry, but can revive and show normal physiological characteristics rapidly during rehydration. Desiccation is harmful to plants for many reasons (Oliver et al., 1998). These reasons include damage to the cytoskeleton as a result of the enormous changes in cell volume that accompany desiccation. Water removal can lead to damage to membranes, and increases ionic strength and changes pH, causing the crystallization of solutes and protein denaturation. Water deficit induces the formation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), the hydroxyl radical (·OH), the superoxide anion radical ($O_2{\cdot}^-$) and hydrogen peroxide ($H_2O_2$) (Smirnoff, 1993). Although the stress-induced formation of intracellular ROS in lichen tissues has never been directly demonstrated, Minibayeva (personal communication) have recently shown that in some species desiccation induces a burst of extracellular superoxide production. ROS can react with many classes of biomolecules and thus damage almost all cellular compartments. Although reactive oxygen species are produced during normal metabolism, especially in chloroplasts and mitochondria (Halliwell, 1987), desiccation greatly enhances their production.

Lichens probably survive desiccation by using a complex interplay of many mechanisms, rather than one simple adaptive feature. In other desiccation tolerant organisms e.g. resurrection plants, bryophytes, and in the so-called orthodox seeds of angiosperms, these mechanisms have been shown to include the production of non-reducing sugars to promote vitrification of the cytoplasm and protect membranes (Scott, 2000), and the synthesis of dehydrin proteins that may act as surfactants inhibiting the coagulation of a range of macromolecules (Close, 1997). In addition, desiccation tolerant plants appear to use a variety of free-radical scavenging systems. Antioxidants include molecules such as the tri-peptide glutathione (γ-glutamyl-cysteinyl-glycine, GSH), ascorbic acid or tocopherols, and also a variety of enzymes (Gaff, 1997). Although very few studies have been carried out on lichens, recent evidence reviewed by Kranner and Lutzoni (1999) suggests that GSH probably plays a pivotal role in conferring desiccation tolerance in these organisms.

The role of enzymic antioxidants in removing ROS from lichens during desiccation has received very little attention. In higher plants, ROS are scavenged by enzymatic pathways that include reactions of superoxide dismutase (SOD), ascorbate peroxidase (APX) and other peroxidases, as well as mono- and dehydroascorbate reductases, glutathione reductase (GR) and catalase (CAT) (for an overview see Elstner and Osswald, 1994). All aerobic

organisms contain superoxide dismutases, metalloproteins catalysing the dismutation of superoxide ($O_2^{\cdot -}$) to hydrogen peroxide and $^3O_2$. Thus, $O_2^{\cdot -}$ is removed rapidly, preventing further possible conversion into ·OH. Peroxidases catalyse hydrogen peroxide-dependent oxidation of substrates. Plants contain a wide range of peroxidases with a broad specificity for substrate, but the identity of the natural substrate is often unknown. Catalases break down high concentrations of $H_2O_2$ very rapidly, but are much less effective than peroxidases at removing $H_2O_2$ present in low concentrations because of their low affinity for this substrate. Foyer and Halliwell (1976) were the first to suggest that in healthy plants a well-balanced interplay of antioxidants and enzymes scavenges cytotoxic oxygen species. ROS are especially likely to be produced in the chloroplast, and here an ascorbate-glutathione cycle eliminates the risk of oxidation of enzymes by $H_2O_2$ in chloroplasts. This cycle involves reactions of glutathione, ascorbic acid, glutathione reductase, APX, and mono- and dehydroascorbate reductases (Kunert and Foyer, 1993). The aim of this study was to measure changes in the activities of the antioxidant enzymes APX, CAT and SOD during desiccation and rehydration in three lichen species from contrasting habitats. The species chosen were *Peltigera polydactyla,* a desiccation sensitive species that grows in moist microhabitats, *Ramalina celastri,* an intermediate species from tree trunks and *Teloschistes capensis,* a lichen from the Namib Desert. We hypothesised that if these enzymes are involved in conferring desiccation tolerance, during water stress or during recovery following water stress their activities should be higher in species from xeric microhabitats.

## 2. Materials and Methods

### *Lichen material*

Lichens were collected from contrasting habitats: *Peltigera polydactyla* (Necker) Hoffm. from moist covered boulders under a tree canopy, *Ramalina celastri* (Sprengel) Krog and Swinscow, from exposed tree branches and *Teloschistes capensis* (L.f.) Vain. from an extremely xeric habitat in the Namib desert. Lichens were gradually rehydrated using air at a relative humidity of 100% for 24 h (over distilled water) at 20°C and a light intensity of 75 $\mu mol\ m^{-2}\ s^{-1}$, followed by contact with wet filter paper for a further 24 h. Enzyme activity was assayed shortly after collection, and again after rehydration. Lichens were then desiccated over silica gel for 28 d. Samples were taken after desiccation for 14 d, and after 28 d material remaining was rapidly rehydrated by incubation in deionized distilled water. Samples were taken at intervals for 30 minutes during rehydration.

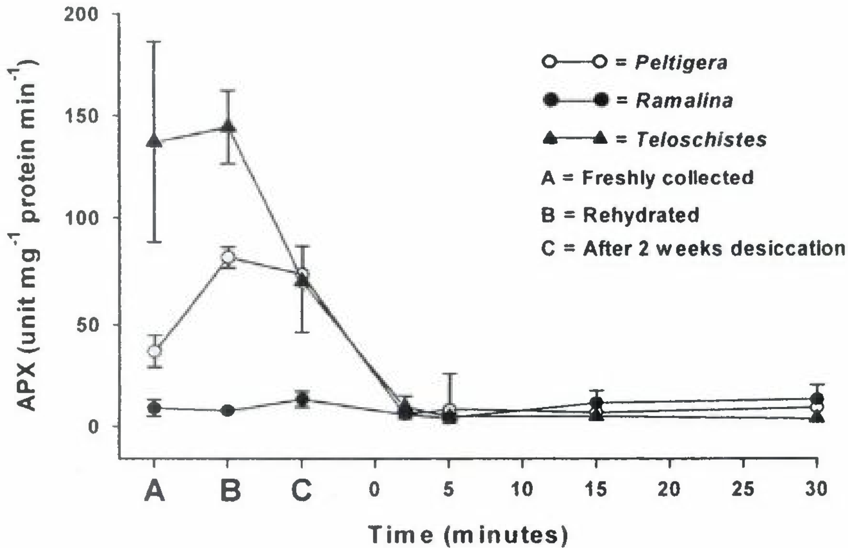


Figure 1. The effect of desiccation and rehydration on the activities of ascorbate peroxidase in *P. polydactyla*, *R. celastri* and *T. capensis*. In this and subsequent figures, points represent fitted values with 95% confidence limits calculated using the "Spline" program of Hunt and Parsons (1974). Overlapping error bars have been removed. A = enzyme activities in freshly collected material; B = enzyme activities in material rehydrated for 48 h; C = enzyme activity in material desiccated for 14 d.

### *Enzyme extraction*

All samples were ground in chilled 0.2 M Tris/HCl buffer (pH 7.8) containing 1 mM DTT, 2 mM NaEDTA and 2% PVP in a ratio material:buffer = 1:3. Homogenates were filtered through 4 layers of muslin cloth and centrifuged at 12000 G for 5 min. The supernatant containing soluble proteins was then collected in 2 ml Eppendorf tubes and stored at –20°C for further analysis. Protein estimation was carried out spectrophotometrically following Bradford (1976) using 1% bovine serum albumin as a protein standard.

### *Enzyme activities*

The activities of APX (EC 1.11.1.11), CAT (EC 1.11.1.6) and SOD (EC 1.15.1.1) were measured spectrophotometrically. APX activity was determined by monitoring the decrease in absorbance of ascorbic acid at 270 nm as described by Nakano and Asada (1981). One unit of APX was defined as the amount of

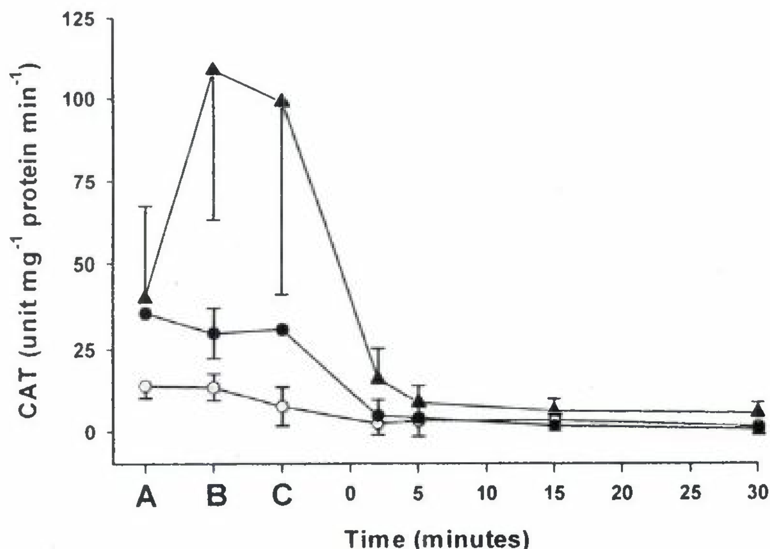


Figure 2. The effect of desiccation and rehydration on the activities of catalase in *P. polydactyla*, *R. celastri* and *T. capensis*. Legend as for Fig. 1.

enzyme that hydrolyses 1 μmol of ascorbic acid $min^{-1}$ at room temperature, and activity expressed as units $mg^{-1}$ protein $min^{-1}$. CAT activity was determined following the consumption of $H_2O_2$ at 240 nm according to Chance and Meahly (1955). One unit of CAT was defined as the amount of enzyme that hydrolyses 1 μmol of $H_2O_2$ $min^{-1}$ at room temperature and activity expressed as units $mg^{-1}$ protein $min^{-1}$. SOD activity was detected by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium at 560 nm (Beauchamp and Fridovich, 1971). One unit SOD was defined as the amount of enzyme causing a 50% inhibition of formazin formation, and activity expressed as units $mg^{-1}$ protein. This assay determines all the isozymes of SOD.

## 3. Results

Freshly collected material of *T. capensis* from the Namib desert consistently displayed the highest activities of APX, CAT and SOD (Figs. 1–3). *R. celastri* from a moderately xeric habitat had lower activities of APX than *P. polydactyla* from a moist habitat, higher activities of CAT and similar activities of SOD. In all species, enzyme activities tended to rise or stay the same following rehydration for 48 h. After desiccation for 14 d, enzyme

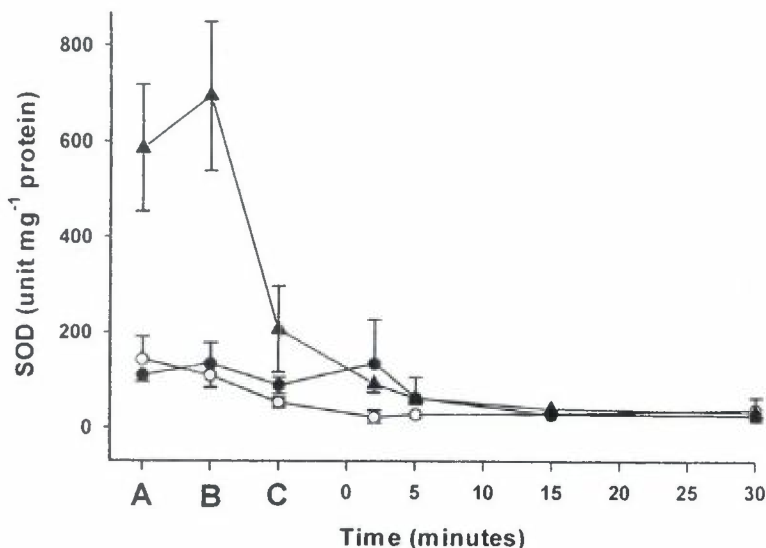


Figure 3. The effect of desiccation and rehydration on the superoxide dismutase activities of *P. polydactyla*, *R. celastri* and *T. capensis*. Legend as for Fig. 1.

activities decreased, and decreased further to low values after 28 d desiccation. In all cases, activities declined even further during the 30 min following rehydration.

## 4. Discussion

Results presented here clearly show that the activities of the enzymes APX, CAT and SOD are very low in lichens during rehydration following desiccation for 28 d, even in species from xeric habitats. During rehydration, enzymic antioxidants will be unavailable to remove ROS accumulating in lichen tissues as a result of desiccation. Taken with the limited data available in the literature, our results suggest that when desiccation is severe, non-enzymic antioxidants are more important than enzymic antioxidants in protecting lichens from desiccation-induced ROS during rehydration. Reviewing the available literature, Kranner and Lutzoni (1999) concluded that good evidence exists that GSH in particular has an important role in desiccation tolerance in lichens. Two enzymes involved in GSH metabolism, glutathione reductase and glucose-6-phosphate dehydrogenase, become active within minutes of rehydration in lichens desiccated for even as long as 60 d. Calatayud et al. (1997) and Chakir and Jensen (1999) provided evidence that the xanthophyll

cycle may reduce ROS formation during desiccation in lichens. High levels of non-photochemical quenching (NPQ) occurred in desiccation-stressed lichens, and in the study of Calatayud et al. (1997), increased NPQ was accompanied by the conversion of the xanthophyll cycle compounds violaxanthin to zeaxanthin. Operation of the xanthophyll cycle can prevent excess excitation energy from being passed to oxygen from photo-excited chlorophyll pigments, forming singlet oxygen, and also the production of superoxide and hydrogen peroxide at photosystem II (McKersie and Lesham, 1994). In bryophytes, Seel et al. (1992) found that the main difference between desiccation tolerant and sensitive mosses was more the ability of tolerant species to maintain levels of α-tocopherol and GSH rather than maintain the activities of antioxidant enzymes. Interestingly, in the resurrection angiosperm *Myrothamnus flabellifolia* Sherwin and Farrant (1998) only found limited evidence that APX and SOD are involved in desiccation tolerance, while Kranner (personal communication) has shown that non-enzymic antioxidants play an important role in tolerance in this species. Results presented here indicate that, at least when the stress is severe, the enzymes APX, CAT and SOD play only a minor role in the removal of desiccation-induced ROS in lichens.

Possibly the enzymes APX, CAT and SOD are more involved in removing ROS produced during the normal metabolic functioning of poikilohydric plants or during moderate stress. In plant and animal tissues, the production of ROS occurs naturally as a result of the side reactions of metabolism. In a healthy, normally functioning organism a multilevel antioxidant system, including APX, CAT and SOD, keeps the levels of ROS at safe levels (Fridovich, 1984). However, it is worth noting that unstressed material of the desert lichen *Teloschistes capensis* had higher activities of all three enzymes than the other species. Similarly, Seel et al. (1992) found that unstressed material of the desiccation tolerant moss *Tortula ruralis* had much higher activities of SOD and CAT, but not APX, than the sensitive moss *Dicranella palustris*. Further work is needed, but a general correlation seems to exist between the stressfulness of the environment a cryptogam grows in and the normal levels of antioxidant enzymes. Our most important finding is that the activities of these enzymes during rehydration following desiccation for 28 d are very low, even in the species from the xeric microhabitats. In lichens, rather than detoxify ROS produced during severe desiccation, APX, CAT and SOD may be more involved with the removal of ROS produced during mild stress, or generated as a consequence of normal metabolic processes like photosynthesis and respiration.

**Acknowledgments**

We gratefully acknowledge the National Research Foundation and the University of Natal Research Fund for financial assistance.

REFERENCES

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44**: 276–287.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248–254.

Calatayud, A., Deltoro, V.I., Barreno, E., and Del Valle-Tascon, S. 1997. Changes in *in vivo* chlorophyll fluorescence in lichen thalli as a function of water content and suggestion of zeaxanthin-associated photoprotection. *Physiologia Plantarum* **101**: 93–102.

Chakir, S., and Jensen, M. 1999. How does *Lobaria pulmonaria* regulate photosystem II during progressive desiccation and osmotic water stress? A chlorophyll fluorescence study at room temperature and at 77 K. *Physiologia Plantarum* **105**: 257–256.

Chance, B. and Maehly, A.C. 1955. Assay of catalase and peroxidase. In: *Methods in Enzymology*, 2. S.P. Colwich and M.O. Kaplan, eds. Academic Press, New York, pp. 764–775.

Close, T.J. 1997. Dehydrins: A commonalty in the response of plants to dehydration and low temperature. *Physiologia Plantarum* **100**: 291–296.

Elstner, E.F. and Osswald, W. 1994. Mechanisms of oxygen activation during plant stress. *Proceeding of the Royal Society of Edinburgh* Series B **102**: 131–154.

Foyer, C.H. and Halliwell, B. 1976. The presence of glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* **133**: 21– 25.

Fridovich, I. 1984. Overview: biological sources of $O_2^{\cdot -}$. *Methods in Enzymology* **105**: 59–61.

Gaff, D.F. 1997. Mechanisms of desiccation tolerance in resurrection vascular plants. In: *Mechanisms of Environmental Stress Resistance in Plants*. A.S. Basra and R.K. Basra, eds. Harwood Academic Publishers, Netherlands, pp. 43–58.

Halliwell, B. 1987. Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. *Chemistry and Physics of Lipids* **44**: 327–340.

Hunt, R. and Parsons, I.T. 1974. A computer program for deriving growth functions in plant growth analysis. *Journal of Applied Ecology* **11**: 297–307.

Kranner, I. and Lutzoni, F. 1999. Evolutionary consequence of transition to a lichen symbiotic state and physiological adaptation to oxidative damage associated with poikilohydry. In: *Plant Responses to Environmental Stresses: From Phytohormones to Genome Reorganization*. H.R. Lerner, ed. Marcel Dekker, New York, pp. 591–628.

Kunert, K.J. and Foyer, C. 1993. Thiol/disulfide exchange in plants. In: *Sulfur Nutrition and Assimilation in Higher Plants*. L.J. DeKok, I. Stulen, H. Rennenberg, C.H. Brunold, and W.E. Rauser, eds. SPB Academic Publishing,The Hague, pp. 139–151.

McKersie, B.D. and Lesham, Y.Y. 1994. *Stress and Stress Coping in Cultivated Plants*. Kluwer Academic Publishers, Dordrecht.

Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* **22**: 867–880.

Oliver, M.J., Wood, A.J., and O'Mahony, P. 1998. 'To dryness and beyond': preparation for the dried state and rehydration in vegetative desiccation tolerant plants. *Plant Growth Regulation* **24**: 193–201.

Scott, P. 2000. Resurrection plants and the secrets of eternal leaf. *Annals of Botany* **85**: 159–166.

Seel, W.E., Hendry, G.A.F., and Lee, J.A. 1992. Effects of desiccation on some active oxygen processing enzymes and antioxidants in mosses. *Journal of Experimental Botany* **43**: 1031–1037.

Sherwin, H. and Farrant, J. 1998. Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. *Plant Growth Regulation* **24**: 203–210.

Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist* **125**: 27–58.