9 Desiccation Stress and Damage

Christina Walters,1 Jilf M. Farrant,2 Norman W. Pammenter3 and Patricia Berjak3

1 USDA-ARS National Seed Storage Laboratory, 1111 South Mason Street, Fort Collins, CO 80521, USA; 2 Department of Molecular and Cellular Biology, University of Cape Town, 7700, South Africa; 3 School of Life and Environmental Sciences, University of Natal, Durban 4041, South Africa

9.1. Introduction 263
9.2. Water Stress 264
9.2.1 Drought vs. desiccation 264
9.2.2. Exacerbating stresses 265
9.2.3. Degrees of stress 265
9.3. Desiccation Damage 269
9.3.1. Mechanical strains and structural damage 269
9.3.1.1. Cellular and subcellular scales 269
9.3.1.2. Molecular scale 273
9.3.2. Metabolically derived damage 278
9.4. Perspectives on the Kinetics of Desiccation Damage 280
9.5. Conclusion 281
9.6. Acknowledgements 282
9.7. References 282

9.1. Introduction

Terrestrial plants became established in the Silurian Period (450–409 million years ago), a few hundred million years after the first appearance of multicellular organisms on earth (Late Precambrian Period: 900–545 million years ago) (Strickberger, 2000). The time required for the necessary adaptations to arise attests to the harshness of a water-limited environment. The two major challenges were maintaining cell and organism structures when water was not available to provide physical support and acquiring nutrients when the lack of water limited the movement of both organisms and the necessary resources. The loss of mobility also required plants to develop mechanisms to tolerate a spectrum of other stresses associated with life on land, particularly temperature extremes and high levels of radiation. The requirements for water are so fundamental (and obvious) that most research has focused on the strategies used
to address the challenges of life in non-aquatic environments (i.e. protective mechanisms) rather than the physical evidence of failure - collapse and starvation.

Studies of cellular responses to water stress mostly focus on what cells need to tolerate or resist water loss. Direct evidence concerning the damaging process is sparse, with the mechanisms of damage often made by inference from the presence of putative protectants. Often it is unclear whether a change in morphology, ultrastructure or metabolism is a simple consequence of drying, a protective strategy or a sign of damage. For example, cessation of metabolism is considered a component of all three possibilities (Vertucci and Leopold, 1984; Leprince et al., 1999, 2000; Salman Espindola et al., 1994, respectively). Damage by desiccation is often measured by an irreversible change or a failure of the organism to revive once water is plentiful again. These rather crude assays do not detect damage that is repairable, though the suite of repair enzymes produced de novo upon rehydration attests to the turnover of cellular constituents (Oliver et al., 1998). A better understanding of the nature of desiccation stress and the resulting strains is required if we are to understand fully the nature of protection and repair and, ultimately, exploit millions of years of evolutionary adaptation to produce plants more capable of withstanding the basic challenges of life in a water-limited environment.

9.2. Water Stress

9.2.1. Drought vs. desiccation

Most terrestrial organisms can grow (by mitotic divisions and cell expansion) at water potentials greater than about \(-1\) MPa (Levitt, 1980; Vertucci and Farrant, 1995, and references therein). Organisms that successfully deal with lower water potentials can either cope with limited water availability while maintaining high internal water concentration, or cope with water loss. The former class constitutes what are generally considered to be ‘drought-tolerant’ organisms, which usually resist water loss by having impermeable outer coverings and reducing surface area-to-volume ratios. Drought-stressed organisms may grow relatively slowly, perhaps because of the reduced turgor pressure for cell expansion, but also because of the tremendous metabolic costs of maintaining structures that block water loss to the environment (e.g. Pimenta-Barrion and Nobel, 1998), supporting root structures that seek water, and accumulating compatible solutes that keep osmotic potentials low (Jones and Gorham, 1983). The degree of drought tolerance can be based on how much the organism resists water loss (i.e. the minimum water potential sustained), the duration that the organism sustains low water potentials, or the productivity (growth) of the organism during the stress. When drought-tolerance mechanisms fail, the organism either loses water essential for structure or compromises metabolism to an unsupportable level. Either of these consequences is considered a subset of the strains associated with desiccation damage.

The distinction between drought and desiccation tolerance lies in the protection mechanisms - mechanisms conferring tolerance of drought avoid water removal while mechanisms conferring tolerance of desiccation enable the organism to survive in spite of the water loss.

In the above context, drought tolerance is really desiccation avoidance. Because the mechanisms required to scavenge and sequester water may differ from those that enable the organism to exist without it, tolerance of drought does not necessarily imply tolerance of desiccation. None the less, both desiccation- and drought-tolerant organisms accommodate life at low water potentials (\(< -1\) MPa). Mild drops in water potential (from \(-1\) to about \(-3\) MPa) coincide with a series of metabolic changes that make cells more tolerant of the water stress (Ingram and Bartels, 1996; Bray, 1997; Oliver et al., 1998; see Chapter 11). The products of these metabolic changes (antioxidants, low-molecular-weight carbohydrates, late embryogenesis abundant
(LEA)-like proteins, heat-shock proteins) are putative protectants for both drought and desiccation, even though the mechanism of protection is quite different for the two types of stress. Future research should be directed towards resolving this apparent contradiction.

### 9.2.2. Exacerbating stresses

Water-stressed plants ($\psi_w \leq -1$ MPa) are predisposed to damage by other stresses. Free-radical production appears to be a common effect of numerous stresses including drought and desiccation, ageing, freezing, pollution, temperature extremes and radiation (Elstner et al., 1988; McKersie et al., 1988; Puntarulo et al., 1991; Hendry, 1993; Leprince et al., 1993; Foyer et al., 1994; Wise, 1995; Bowler and Fluhr, 2000), and so it is likely that these stresses may be cooperative or synergistic. Stressed plants are particularly susceptible to photo-oxidative damage (Elstner et al., 1988; Foyer et al., 1994; Wise, 1995). Light energy, which was efficiently harvested, transduced and assimilated in non-water-stressed cells, may be absorbed by the photosynthetic apparatus and dissipated as reactive oxygen molecules that damage cellular constituents (Bewley and Krochko, 1982; Vertucci et al., 1985; Kaiser, 1987; Elstner et al., 1988; McKersie et al., 1988; Smirnoff, 1993; Foyer et al., 1994; Tuba et al., 1996, 1998; Sherwin and Farrant, 1998; Caintalan et al., 1998; Farrant, 2000; Vander Willigen et al., 2001). Desiccation-tolerant plants initiate many processes that are considered to be protective against photochemical damage. These processes include dismantling of the photosynthetic apparatus (Gaff and Hallam, 1974; Hetherington et al., 1982a,b; Öquist and Strand, 1986; Gaff, 1989; Demmig-Adams and Adams, 1992; Vertucci and Farrant, 1995; Tuba et al., 1996, 1998; Sherwin and Farrant, 1998; Farrant et al., 1999; Farrant, 2000), chlorophyll shading by leaf folding or rolling (Dalla Vecchia et al., 1998; Sherwin and Farrant, 1998; Farrant et al., 1999; Farrant, 2000), accumulation of protective pigments such as anthocyanins (Smirnoff, 1993; Foyer et al., 1994; Sherwin and Farrant, 1998; Farrant, 2000; Vander Willigen et al., 2001), increases in xanthophyll pools and conversion to the de-epoxide forms (Smirnoff, 1993; Foyer et al., 1994; Kranz and Grill, 1997) and production of free-radical-scavenging enzymes (Foyer et al., 1994; Wise, 1995; Pammelnet and Berjak, 1999).

Low temperatures tend to intensify water stress. The classic case describes temperatures at which water freezes extracellularly, thereby creating a water potential gradient and forcing intracellular water to migrate out of the cell (Meryman, 1974; Steponkus, 1979). Lowering the temperature requires an increase in the water content of cells to maintain a constant $\psi_w$ ($\psi_w \leq -10$ MPa). This requirement for more water at lower temperatures is related to the exothermic nature of water condensation on macromolecular surfaces at low water potentials (Walters, 1998). Consistently, critical water contents that lead to desiccation damage are greater at lower temperatures (Kovach and Bradford, 1992; Vertucci et al., 1995; Eira et al., 1999a). Indeed, the moisture content giving rise to changes in membrane phase behaviour, the most often cited consequence of desiccation stress in model systems, increases with decreasing temperature (Crowe et al., 1989; Crowe and Crowe, 1992; Hoekstra and Golovina, 1999; Hoekstra et al., 1999; Bryant et al., 2001).

### 9.2.3. Degrees of stress

There are many ways to measure water loss in cells. Water content (absolute or relative) (e.g. Berjak et al., 1992; Sun et al., 1994; Farrant, 2000), water potential and related functions (Roberts and Ellis, 1989; Vertucci and Roos, 1990; Tompsett and Pritchard, 1993; Vertucci and Farrant, 1995; Vertucci et al., 1995; Farrant and Walters, 1998), cell volume (Meryman, 1974; Steponkus, 1979; Mura and Yoshida, 1988a,b), intracellular viscosity (Vertucci and Roos, 1990; Koster, 1991; Williams et al., 1993; Leopold et al.,
1994; Buitink et al., 1998b; Leprince and Hoeckstra, 1998; Bryant et al., 2001) inter-
molecular proximity (Lis et al., 1982; Steponkus et al., 1995; Wolfe and Bryant, 1999; Bryant et al., 2001) and structural water (Ladbrooke and Chapman, 1969; Vertucci and Leopold, 1984, 1987; Crowe et al., 1990; Pammenter et al., 1991) all change with desiccation (Fig. 9.1; see Chapter 2). Each of these parameters has been used to define the level of water stress, but it is unclear which parameter(s) causes the stress and which is merely a correlate of the stress. The distinction is important as it reveals the nature of the strain and the damage. A better understanding of the nature of desiccation stress and strain will also reveal whether damage accrues continuously, whether it occurs when the stress or strain reaches a threshold, and whether numerous different strains result from the removal of water from the cell. In other words, is damage by desiccation a single event, a continuous event or a series of insults to the cell or organism?

Desiccation tolerance/sensitivity has traditionally been regarded as a qualitative feature: cells either do or do not survive drying. The definition of 'dry' varies among laboratories or experiments (i.e. 90% water loss; water contents < 10% (0.10 g H₂O g⁻¹ dry weight or fresh weight); water contents in equilibrium with 75% or maybe even 15% relative humidity (RH); water contents achieved after a material has been freeze-dried or held in a laminar flow hood for some period of time), and most studies use just one drying level. The binary approach suggested that damage was a single event that either happened or did not happen. Seeds were assigned to one of two categories, orthodox or recalcitrant, to distinguish between those that survived or did not survive drying (Roberts, 1973; see Chapter 5). Classification of seeds of certain species as recalcitrant has been disputed as laboratories around the world have demonstrated variable success in drying them. For example, some groups report survival of *Zizania palustris* at water contents as low as 0.07 g H₂O g⁻¹ dw (Kovach and Bradford, 1992) while other laboratories show detrimental effects of drying at much higher water contents (Probert and Longley, 1989; Vertucci et al., 1995). Similar discrepancies are reported for coffee (*Coffee arabica*), lemon (*Citrus limon*), neem (*Azadirachta indica*) and tea (*Camellia sinensis*) (e.g. Ezumah, 1986; Ellis et al., 1990; Chaudhury et al., 1991; Berjak et al., 1993; Hong and Ellis, 1995; Dussert et al., 1999; Eira et al., 1999a; Sacandé et al., 2000). Even when the recalcitrant category is undisputed, there are differences among species in how much water can be removed and how long a water-stressed seed can survive (Berjak et al., 1989, 1990; Farrant et al., 1989, 1997; Vertucci and Farrant, 1995; Pammenter and Berjak, 1999). To accommodate the variability in desiccation tolerances observed among species, the categories of seed behaviour were further divided to distinguish highly recalcitrant, recalcitrant, intermediate, sub-orthodox and orthodox, and Pammenter and Berjak [1999] have suggested that, in reality, a continuum exists among seed species, based on desiccation response.

Not only are there differences among species in response to desiccation, but there are also differences for seeds of the same species and among tissues as a function of developmental stage. Studies of the acquisition and loss of desiccation tolerance during embryogenesis and germination have demonstrated that tolerance to desiccation progressively increases with seed maturation (Berjak et al., 1990, 1992, 1993; Finch-Savage, 1992b; Farrant et al., 1993; Sun and Leopold, 1993; Sun et al., 1994; Tompsett and Pritchard, 1993; Vertucci et al., 1995; Farrant and Walters, 1998; reviewed by Vertucci and Farrant, 1995; Pammenter and Berjak, 1998; see Chapter 5) and decreases with germination (Sargent et al., 1981; Senaratna and McKersie, 1983; Leprince et al., 1990; Reisdorph and Koster, 1999). Correlative evidence suggests that there are similar stages of tolerance in vegetative tissues of desiccation-tolerant angiosperms (Farrant, 2000; Vander Willigen et al., 2001; J.M.
Fig. 9.1. Scales of water stress (water potential and relative humidity (RH)), water loss (water content) and strain (volume and viscosity changes) as they relate to conceptual models of hydration (shaded rectangles) and projected damage to cells (white box). The figure is modified from a similar figure by Wolfe and Leopold (Leopold, 1986). Water contents as a function of water potential are described for mature pea axes (Vertucci and Leopold, 1987; Walters et al., 2001) and immature *Aesculus hippocastanum* axes (Farrant and Walters, 1998). Changes in volume are calculated for immature and mature bean axes from the amount of water lost (assuming water density is 1 g ml⁻¹) and the proportion of space occupied by cell organelles (Farrant et al., 1997) at full hydration and an assumption that 8% of the volume of the cytoplasmic matrix at full hydration is dry matter; the effect of positive turgor pressure on cell volume is not accounted for. Measures of cytoplasmic viscosity are taken from Leprince and Hoekstra (1998) and Buitink et al. (1998b, 2000). Conceptual models of hydration are taken from Rupley et al. (1983), Vertucci (1990) and Vertucci and Farrant (1995). Different mechanisms of damage are as described in the text.
Vegetative tissues from resurrection and cold-tolerant angiosperms require time to adapt to water-stress situations (Steponkus et al., 1995; Oliver et al., 1998; Farrant et al., 1999; see Chapter 7), presumably to induce protective mechanisms. It is surmised that in vegetative tissues of angiosperms there is also a developmental programme in response to stress that leads to progressively greater desiccation tolerance. The variability in critical water contents among different species, during maturation or germination of embryos and during adjustment of vegetative tissues, leads to the general conclusion that tolerance/sensitivity is a quantitative feature.

The quantitative nature of desiccation tolerance/sensitivity suggests two broad possibilities for the mechanism(s) of desiccation damage in organisms. Desiccation damage may occur by a single mechanism with species and tissue types differing in how much damage they can accrue or how much stress they can endure. Alternatively, damage by different mechanisms may occur at multiple levels of stress and species and tissue types differ in which stresses, or combinations of stresses, they can withstand. The former possibility suggests that a desiccation-tolerant organism requires only a simple cadre of protectants, while the latter situation suggests that a complex suite of protectants, each with a different function, is required for an organism to be truly tolerant of dehydration.

Evidence is accumulating to suggest that desiccation damages cells and organs by many mechanisms and that different types of damage occur at different levels of stress. When expressed in terms of water potential, developing embryos acquire tolerance stepwise (Vertucci and Farrant, 1995; Farrant and Walters, 1998). During histodifferentiation, embryos are damaged by water potentials less than about −1.2 to −2 MPa (Vertucci and Farrant, 1995, and references therein). During dry matter accumulation, embryos tolerate water potentials as low as about −4 to −5 MPa (Farrant et al., 1992, 1993; Farrant and Walters, 1998). Following vascular separation, the water potential at which damage is first measured (i.e. critical ψ_r) in most embryos (Avicennia marina, and perhaps other highly recalcitrant seeds, excepted (Farrant et al., 1992, 1993)) was found to decline to about −10 to −15 MPa (Pritchard, 1991; Finch-Savage, 1992a; Tompsett and Pritchard, 1993; Vertucci and Farrant, 1995; Farrant and Walters, 1998). Embryos that are recalcitrant cannot be dried below this level. During the final stages of maturation, species defined as having intermediate postharvest physiology (e.g. coffee, citrus and papaya) acquire the ability to tolerate between about −60 and −80 MPa (Dussert et al., 1999; Eira et al., 1999a; Sacandé et al., 2000) (neem is considered to be in the intermediate category as it has diminishing longevity at lower water potentials (see Ellis et al., 1990)). Truly orthodox species survive the immediate effects of complete water loss, but succumb more rapidly if they are dried below about −190 to −250 MPa (Vertucci and Leopold, 1987; Vertucci and Roos, 1990; Walters, 1998). Vegetative tissues of resurrection plants acquire the same degree of extreme tolerance (Bewley, 1979; Gaff, 1989; Oliver et al., 1998). Cells that have the genetic capacity to induce tolerance mechanisms progress towards tolerance and, with sufficient time, complete the developmental programme leading to full tolerance of desiccation (e.g. Finch-Savage, 1992b).

The differing levels of desiccation sensitivity among developmental stages and seed categories appear to correspond to levels of physiological activity documented in desiccation-tolerant organisms with studies of metabolic activity and properties of the aqueous medium (Vertucci and Farrant, 1995). Five hydration levels designate the cells' ability to support growth (Level V, 0 to −1.5 MPa), to photosynthesize and effect stress-related metabolism (Level IV, −1.8 to −4 MPa), to respire (Level III, −5 to about −12 MPa), to carry out catabolic reactions (Level II, about −15 to −190 MPa), and to be almost in stasis (Level I, < −220 MPa) (Fig. 9.1) (Clegg, 1986; Roberts and Ellis, 1989; reviewed by Vertucci and Farrant, 1995; Farrant, 2000; Vander Willigen et al., 2001). These hydration levels correspond to
other parameters that measure the extent of desiccation (Fig 9.1) (Chapters 2 and 4). In
Hydration Level V, turgor pressure is positive and water behaves as it would in a
dilute solution. At lower hydration levels, cells shrink (Maryman, 1974; Steponkus,
1979; Steponkus et al., 1995), the properties of water change (Rupley et al., 1983;
Vertucci, 1990) and the aqueous matrix becomes more viscous having properties of
syrups (Level IV), rubbers (Level III) and leathers and glasses (Level II) (Vertucci and
Roos, 1990; Slade and Levine, 1991; Williams et al., 1993; Leopold et al., 1994;
Buitink et al., 1998b; Leprince and Hoekstra, 1998). Viscosity is minimized at
the transition from Level II to I (Vertucci and Roos, 1990; Buitink et al., 1998b), a
moisture level that also corresponds to a discrete change in the heat capacity of
water (Rupley et al., 1983; Vertucci, 1990; Buitink et al., 1998; M.T.S. Eira, unpub-
lished data) and poorly understood characteristics of water sorption (Vertucci and
Leopold, 1987; Vertucci and Roos, 1990; Vertucci et al., 1994; Buitink et al., 1998a,b;
Walters, 1998; Eira et al., 1999b). With the exception of Hydration Level I (Vertucci
and Leopold, 1987; Eira et al., 1999b; M.T.S. Eira, L.S. Candalas and C. Walters
unpublished data), the relationships between physical properties of water and
water potential appear similar among diverse cells (Fig. 9.1), perhaps with only
subtle differences to distinguish desiccation-tolerant from less tolerant materials (Koster,
1991; Berjak et al., 1993; Parratt and Walters, 1998; Leprince et al., 1999). If desic-
cation stress occurs when cells traverse critical water potentials or hydration levels,
then the stresses experienced by desiccating cells are similar among organisms, but
the responses to those stresses (e.g. damage versus protection) differ with desiccation
tolerance (Pammenter et al., 1991).

9.3. Desiccation Damage

As water is removed from cells, the physical and physiological properties of the cells
change. These changes, often characterized by a reduced cell size or lack of integrated
metabolism, do not in themselves imply damage. They may be purely consequences
of water removal and may be completely reversible once water is added back to the
system. Therefore, damage from desiccation is not indicated by differences
between the hydrated and dry state, but rather by the resumption of normal activity
upon rehydration.

The number of different stresses that can be associated with removal of water
from cells can be attributed to the multiple roles that water plays in supporting life.
Water plays a structural role: at the cellular scale, water fills spaces and provides turgor,
while, at the molecular scale, water provides hydrophilic and hydrophobic
associations and controls intermolecular distances that determine the conformation of
proteins, polar lipids and the partitioning of molecules within organelles. With
water present, reactive surfaces of metals or molecules are not as exposed, and this
limits reactivity among molecules. Water also plays a role in controlling metabolism,
as it is a reactant and product of many reactions. As a diluant, water affects the
chemical potential of other molecules, potentially shifting the likelihood of reactions.
Water also provides the fluid matrix that allows diffusion of substances to reac-
tive sites. Changes in water concentration affect viscosity of the matrix and the overall
mobility of dissolved or suspended molecules. The drier the medium becomes, the
more viscous it becomes, until it is essentially a solid matrix, trapping molecules
(Slade and Levine, 1991; Williams et al., 1993; Leopold et al., 1994; Buitink et al.,
1998b; Wolfe and Bryant, 1999). As one would expect from all the roles of water,
there will be a number of strains that the tissues undergo when water is removed.

9.3.1. Mechanical strains and structural damage

9.3.1.1. Cellular and subcellular scales

The first sign of desiccation/drought stress is the loss of turgor pressure. This occurs at
water potentials of about \(-1\) to \(-2\) MPa, coinciding with the water potential range designated as 'permanent wilting point' for non-transpiring vegetative tissue (Levitt, 1980). At lower water potentials, cells lose water and shrink (Meryman, 1974; Steponkus, 1979; Levitt, 1980; Steponkus and Lynch, 1989; Steponkus et al., 1995). Osmotic adjustments, which lessen the water potential difference between cells and the environment and augment the amount of dry matter in cells, can prevent water loss and cell contraction at water potentials between \(-1\) and \(-2.5\) MPa (Levitt, 1980; Jones and Gorham, 1983). Osmotic adjustments are fairly ineffective at reducing strains when cells are exposed to lower water potentials (Wolfe and Bryant, 1999). In slow-freezing experiments, believed to mimic dehydration stress, protoplasts can undergo reversible contraction–expansion cycles, or 'osmotic excursions', when slowly cooled and warmed from \(0^\circ\text{C} (\psi_w = -0.5 \text{ MPa})\) to temperatures of \(-2\) to \(-5^\circ\text{C} (\psi_w \approx -6 \text{ MPa})\) (Meryman, 1974; Steponkus, 1979; Steponkus and Lynch, 1989). A 60–80% reduction in cell volume occurs when the water potential of cells decreases from about \(-0.5\) MPa to about \(-4.5\) to \(-6\) MPa \((-4\) to \(-5^\circ\text{C})\) (Meryman, 1974; Steponkus, 1979; Steponkus and Lynch, 1989). Similar contraction was calculated for immature embryo cells in which 88% of the cell volume was occupied by water (Fig. 9.1). However, cells filled with dry matter reserves (mature embryos in Fig. 9.1) do not contract as much as highly vacuolated cells (immature embryos in Fig. 9.1). For a similar reduction in water potential to \(-5\) MPa, the cells of fully mature bean axes contract only by about 18%, and complete desiccation only causes a 24% reduction in volume in these cells (Fig. 9.1). When cells that have not been acclimatized to the water stress shrink by 50–80%, they burst when returned to the original water potential. This observation led to the concept of 'minimum critical volume' (Meryman, 1974), which describes the limits to which a cell can contract in a reversible osmotic excursion. As seen for mature embryos (Fig. 9.1), this strain of cell contraction can be avoided by accumulating dry matter.

Differences in the degree to which cell walls contract compared with protoplasm may cause mechanical stress and damage to the plasmalemma or plant cells during dehydration. The tight attachment of the plasmalemma to the cell wall is believed to create tension to the membrane in shrinking cells (e.g. Murai and Yoshida, 1998b), which is most profound at the cell wall–plasmalemma attachments near the plasmodesmata (Iljin, 1957; Bewley and Krochko, 1982). Plasmolysis, where the plasma membrane separates from the cell wall, appears to mitigate damage to whole cells during severe water stress (Murai and Yoshida, 1998b), and there is some evidence to suggest that cells in desiccation-tolerant seeds are slightly plasmolysed (Perner, 1965). Observations of plasmolysis may be an artefact of the aqueous fixatives used to study dry organisms (Opik, 1985; Platt et al., 1997; Wesley-Smith, 2001). In studies using anhydrous chemical fixation (Opik, 1985) or freeze substitution (Wesley-Smith, 2001, Wesley-Smith et al., 2001), the plasma membrane remained closely appressed to the cell walls, and both the cell wall and the plasmalemma became highly convoluted during desiccation of tolerant cells. Opik (1985) demonstrated that the plasmalemma separated from the cell wall during rehydration as a result of differential swelling or weakening of the cell wall–plasmalemma association caused by detergents such as dimethylsulphoxide.

The mechanical properties of the cell wall, including its elasticity, ability to fold and associations with plasmodesmata, influence the degree of plasma membrane disruption consequent upon contraction or expansion (Webb and Arnott, 1982; Opik, 1985; Murai and Yoshida, 1998b; Vicré et al., 1999).

Cell membranes must fold or vesiculate to accommodate the volume changes during cell contractions. Conservation of membrane surface area during contraction is critical for successful rehydration. If the surface area of the plasmalemma is
reduced too much, the cell bursts upon rehydration, suggesting that there is a critical minimum surface area, rather than a critical minimum volume, to which cells can survive (Steponkus, 1979; Steponkus and Lynch, 1988; Steponkus et al., 1995). Protoplasts from cells that are not acclimated to the cold contract through invaginations of the plasma membrane, which eventually form endocytotic vesicles that cannot be reincorporated into the plasmalemma upon warming (Steponkus and Lynch, 1988; Steponkus et al., 1995). The plasma membrane of protoplasts from cells more tolerant of water stress (i.e. acclimated by low temperatures) contracts through exocytotic extrusions which remain continuous with the plasma membrane and help to conserve the membrane surface area (Steponkus and Lynch, 1988; Steponkus et al., 1995). High phospholipid:sterol ratios and high amounts of diunsaturated fatty acids in the plasmalemma appear to facilitate exocytotic folding in shrinking protoplasts and greater elasticity of the expanding membranes (Steponkus and Lynch, 1988; Steponkus et al., 1995). Protoplasts with these properties tend to survive to lower water potentials (Steponkus et al., 1995).

The mechanism by which membrane surface area is conserved in intact cells is largely unknown. There are some studies of the effect of dehydration on cell volume and membrane configuration in cells from plant embryos, but these are often confounded by problems associated with using aqueous fixatives (Platt et al., 1997; Wesley-Smith et al., 2001). In addition, the studies often use mature embryos (recalcitrant or orthodox) where >50% of the cell volume is occupied by dry matter (e.g. Farrant et al., 1997). These cells will not experience the same degree of shrinkage as highly vacuolated cells (Fig. 9.1), and so the need for conserving membrane surface area is not as critical. Circumventing the problem of cell shrinkage may explain why most orthodox and recalcitrant embryos (except for A. marina and other recalcitrant seeds with highly vacuolated cells (Farrant et al., 1992, 1993)) are fairly tolerant of water stress, surviving to water potentials of ~12 MPa or less, compared with the benchmark of ~5 MPa described above for protoplasts from non-acclimatized cells. None the less, drying results in some degree of cell contraction, which is mostly completed when the water potential of the cell is reduced to ~12 MPa (Fig. 9.1). In cells that survive water potentials of about ~12 MPa but not lower, both endo- and exocytotic vesicles have been observed (P. Berjak and N.W. Pammenter, unpublished date). These observations are not reported in extremely dried embryos, perhaps because of technical problems of fixation. In severely dried cells of fully desiccation-tolerant seeds, the plasmalemma stays intact and closely attached to the cell wall as this folds, suggesting that the membrane surface area remains relatively constant during drying even though the cell volume is diminished (Opik, 1985). Some membrane constituents may be removed during cell contraction as evidenced by whorls of membrane close to the plasmalemma in seed cells (Webster and Leopold, 1977; Opik, 1985; Wesley-Smith et al., 2001) and circular membrane structures and plastoglobuli within chloroplasts in sections of leaf tissue from desiccation-tolerant angiosperms (Farrant et al., 1999; Farrant 2000; Mundree et al., 2000). These membrane bodies have been proposed to provide additional membrane reserves upon rehydration (Webster and Leopold, 1977; Farrant et al., 1999; Mundree et al., 2000), although mechanisms by which they would be reinserted are not clear and their very presence may be artefacts of aqueous fixation. Alternatively, these membrane abnormalities may arise from other organelles, such as endoplasmic reticula, and may participate in autophagy or vacuole formation (Wesley-Smith et al., 2001). The shapes of nuclei, mitochondria and plastids in dried cells of desiccation-tolerant seeds are irregular and convoluted, suggesting that the surface area of the membranes of these organelles are also conserved simply by folding (Opik, 1985).

The membranes of cell vacuoles are likely to experience tensions similar to
those described for protoplast membranes during osmotic excursions, and so are prone to rupture, with lethal consequences, following exposure to water potentials of −2.5 to −5 MPa (Muni and Yoshida, 1998a). Highly vacuolated cells of immature seeds (Berjak et al., 1984, 1994; Farrant et al., 1989, 1997) and desiccation-sensitive vegetative tissue (Farrant and Sherwin, 1997; Farrant, 2000) are particularly sensitive to tonoplast dissolution. Replacing the water in vacuoles with solid material reduces the degree to which vacuoles must contract, thereby lessening the tension on tonoplast membranes during drying. Dry matter reserves naturally accumulate during embryogenesis in orthodox and some recalcitrant seeds, and may explain the progressive tolerance to low water contents in developing embryos (Vertucci and Farrant, 1995; Farrant et al., 1997; Farrant and Walters, 1998). There is also accumulation of dry matter in vacuoles of vegetative tissues in many of the desiccation-tolerant angiosperm species during acclimatization to water stress (Farrant, 2000).

Water loss results in a general contraction of cell volume. The plasmalemmata of plant cells can be damaged if they are sheared from cell walls, which contract less than protoplasm, or if contraction results in an irreversible loss of membrane surface area. In addition to protection by filling cells with dry matter (described above), the consequences of volume changes can also be lessened by initial high surface area-to-volume ratios of cells and vacuoles (Ulijn, 1957; Bewley, 1979) and may explain why cells from non-vascular plants, which usually have small vacuoles and lack plasmodesmata, do not appear to suffer physical damage upon contraction (reviewed by Bewley and Krochko, 1982). Damaging effects of cell contraction are usually manifested during rehydration, suggesting that the stress and damage are not direct effects of desiccation, but rather indications of rehydration stress and mechanical failure.

A dismantling of mitochondria and chloroplasts is associated with severe water stress. Mitochondria observed in mature orthodox seeds lack defined cristae (Bergstrom et al., 1982; Thomson and Platt-Aloia, 1984; Farrant et al., 1997) and mitochondrial proteins are easily extractable from dried pollen (Hoekstra and van Roekel, 1983). Conversely, mitochondria from immature embryos and recalcitrant seeds are more defined, and the greater differentiation has been linked to greater sensitivity to desiccation (Farrant et al., 1997). Chloroplast structure also degrades during water stress. Dried leaves of the desiccation-tolerant grasses Borya nitida and Xerophyta humilis become yellow, concurrent with the loss of grana stacks in the chloroplasts (Gaff and Hallam, 1974; Farrant, 2000). Using fluorescence-induction kinetics to study partial processes of photosynthesis, researchers found a decrease in the efficiency of photosystem II at water potentials between −3 and −4 MPa (Wiltens et al., 1978; Hetherington, et al., 1982b; Vertucci et al., 1985; Sherwin and Farrant, 1998; Tuba et al., 1998; Coúlom et al., 1999) or during acclimatization to winter (Oquist and Strand, 1986). This decline could be a consequence of photochemical damage, but is more likely to be a reflection of protective dismantling of photosystem II (Demmig-Adams and Adams, 1992; Farrant, 2000). Indeed, the dismantling of the photosynthetic apparatus during drying of B. nitida and X. humilis is required for survival: plants dried too rapidly stay green and do not recover (Gaff and Hallam, 1974; Farrant et al., 1999).

Slight water stress (−1 ≥ Ψw ≥ −3 MPa) enhances the protein synthesis that is believed to be important for conferring tolerance (Ried and Walker-Simmons, 1993; Vertucci and Farrant, 1995; Ingram and Bartels, 1996; Oliver et al., 1998; Mundree et al., 2000; Whittaker et al., 2001). Further drying reduces the rate of protein synthesis in both tolerant and sensitive cells (Bewley and Krochko, 1982; Salmen Espindola et al., 1994; Ingram and Bartels, 1996; Oliver et al., 1998; Mundree et al., 2000; Whittaker et al., 2001), perhaps because of a dismantling of endoplasmic reticulum, dictyosomes and polysomes (Webster and...
Leopold, 1977; Thomson and Platt-Aloia, 1984; Farrant et al., 1997; Wesley-Smith et al., 2001).

Indirect evidence from recalcitrant seeds suggests that, during dehydration, the cytoskeleton is disrupted at fairly high water potentials (−3.8 MPa for Trichilia dregana and −3.5 MPa for Quercus robur) leading to an abnormal distribution of organelles within cells (Berjak et al., 1999; Mycock et al., 2000). Although it is tacitly assumed that cytoskeletal disassembly must occur during dehydration in desiccation-tolerant seeds (and vegetative tissues), it is its failure to reconstitute that characterizes this aspect of dehydration-related injury in recalcitrant material (Mycock et al., 2000).

There is clearly a general trend towards contraction or disassembly of cellular machinery during water stress to about −5 MPa. In most desiccation experiments, plant materials are stressed further and cell survival is assayed by whether or not organelles reassemble upon rehydration. In desiccation-sensitive cells that do not rupture, the protein-synthesizing machinery does not recover, nor do mitochondria and chloroplasts resume normal function; organelles become irregularly shaped and disorganized (reviewed by Bewley and Krochko, 1982; Farrant et al., 1989; Berjak et al., 1990; Mycock et al., 2000). The contraction and dismantling of organelles described above are clearly signs of water stress, but it is unclear whether these changes are symptoms of damage occurring at −5 MPa, or means of protection when water stress intensifies. It is also unclear whether the failure to reconstitute organelles indicates a primary site of damage or a general debilitation when cells die. These cause and effect arguments have led researchers to study the primary effects of dehydration on the structure of macromolecules.

9.3.1.2. Molecular scale

Removing water from cells pushes cellular constituents together, causing them to interact in ways that might not otherwise occur. A consequence of these molecular aggregations is an increased ordering of molecular structures, and it may seem ironic that primary lesions during drying are directly attributed to order rather than to loss of it. Drying-induced compaction of molecules requires greater packing efficiency, resulting in localized enrichments of similar-type molecules in a process known as demixing (Lis et al., 1982; Bryant and Wolfe, 1989; Rand and Parsegian, 1989; Bryant et al., 1992). Molecules remix upon rehydration, but the reactions that occurred in the desiccated state may have irreversible consequences.

Intermolecular associations of polar lipids are intrinsically linked to the water content of the medium. Under aqueous conditions, polar lipids spontaneously align to form micelles or bilayer structures depending on the polar head group of the lipid. Acyl chains within bilayers are more-or-less mobile, giving considerable fluidity to the structure and allowing proteins and other constituents to be inserted. Drying brings membrane bilayers into close proximity and causes membrane constituents to segregate laterally into different domains enriched with particular lipid classes or proteins (Lis et al., 1982; Bryant and Wolfe, 1989; Rand and Parsegian, 1989; Bryant et al., 1992; Crowe and Crowe, 1992; Steponkus et al., 1995; Hoekstra and Golovina, 1999) (Fig. 9.2). The closer packing between membranes and among membrane constituents results in greater rigidity of the fatty acid domain within the bilayer. There are two mechanisms, based on either intra- or interlamellar events, used to explain why fatty acid domains become more rigid. If water molecules are removed from between adjacent polar head groups, the associated fatty acids compress because of the increased strength of van der Waals attractions (Crowe et al., 1990; Crowe and Crowe, 1992; Hoekstra and Golovina, 1999). Alternatively, as different bilayers come into close apposition, strong repulsive hydration forces keep them separate, but create isotropic tensions that lead to lateral compression within the acyl domain (Lis et al., 1982; Wolfe, 1987;
Rand and Parsegian, 1989; Bryant and Wolfe, 1992; Wolfe and Bryant, 1999). Increased rigidity eventually leads to phase transitions within the membrane from a fluid to a gel state (Ladbrooke and Chapman, 1989; Cullis and de Kruijff, 1979). While these phase transitions are completely reversible, they are believed to interfere with the semi-permeable properties of membranes. Permanent damage comes from the exclusion of proteins from parts of the bilayer (Rand and Parsegian, 1989; Bryant and Wolfe, 1992; Crowe and Crowe, 1992; Hoekstra and Golovina, 1999) (Fig. 9.2). Transient damage occurs upon hydration: the rush of water on to an inelastic membrane may cause it to rupture (Murphy and Noland, 1982; Steponkus et al., 1995; Hoekstra et al., 1999) or imperfect packing among different domains may cause leakage of cellular constituents (Crowe and Crowe, 1992; Hoekstra et al., 1999).

The close approach of membrane systems and the lateral demixing of membrane components can lead to an even greater threat to membranes than lamellar fluid-to-gel transitions. Membranes can fuse together, causing the complete loss of compartmentation within the cell (Crowe et al., 1986; Crowe and Crowe, 1982; Steponkus et al., 1995) (Fig. 9.2). Fusion is known to occur among liposomes and native membrane fractions, although the mechanism that causes polar lipids to cross over to a different bilayer is unclear. In principle, the hydration characteristics of individual lipids and lipids in a mixture, the intrinsic curvature of different head groups, the water content and the temperature allow the formation of inverted micelles within closely appressed bilayers (Cullis and de Kruijff, 1979; Crowe et al., 1986; Steponkus et al., 1995) (Fig. 9.2). In domains enriched with non-bilayer-forming lipids such as phosphatidylethanolamine-diglycerides or monogalactosyl-diglycerides, the polar head groups coalesce into rings and the acyl chains extend radially outwards in what is known as a hexagonal phase (Cullis and de Kruijff, 1979; Siegel et al., 1994; Steponkus et al., 1995). Fusion via hexagonal-phase changes is rare in native membranes, but has been demonstrated in cells from non-acclimatized leaves that were lethally cooled to −5°C (osm, ψw = −6 MPa) or −10°C (rye, ψw = −12 MPa) (Steponkus et al., 1995) and more frequently in animal cells (Cullis and de Kruijff, 1979; Crowe and Crowe, 1992). Evidence of cell fusion, but not via hexagonal-phase changes, is common in desiccation-damaged cells, protoplasts and liposomes (e.g. Crowe et al., 1986; Steponkus et al., 1995). In oat and rye leaves acclimatized to cold (but clearly not fully desiccation-tolerant), fusion of plasmalemma and endomembrane systems is suggested at temperatures between −10 and −40°C (−12 ≥ ψw ≥ −48 MPa), depending on the level of cold tolerance achieved (Steponkus et al., 1995). Upon rehydration, improperly fused membranes produce vesicles that exclude cell constituents or are combinations of different membrane systems (e.g. the plasmalemma fuses with chloroplast outer membrane or with endoplasmic reticulum) (Fig. 9.2). Because the osmotic balance inside and outside the cells has been completely disrupted, vesicles produced from membrane fusions are identified by their inability to expand during rehydration (Steponkus et al., 1995).

Most of our understanding of how polar lipids behave in water-stressed situations comes from model studies of liposomes with known composition. In these systems, phase transitions are usually studied, even though they may only be harbingers of real damage. Phase transitions of prepared membrane systems occur at a range of water contents and temperatures depending on the saturation of the acyl chains and the presence of non-phospholipids (e.g. Ladbrooke and Chapman, 1989; Cullis and de Kruijff, 1979; Crowe et al., 1989; Steponkus et al., 1995). A water potential of about −12 MPa is often cited as critical. It has been suggested that structural water needed for the proper spacing of polar head groups is removed at ψw ≤ −12 MPa (Ladbrooke and Chapman, 1989; Crowe et al., 1990). Also, at ψw = −12 MPa, large, potentially deforming hydration forces result from the close approach of molecules (Wolfe, 1987).
Fig. 9.2. Schematic drawing of the effect of dehydration on cellular membranes. Different membrane systems may become closely appressed, leading to demixing of lipids and proteins and the loss of proteins from parts of the bilayer. Closely appressed membranes may then form non-bilayer structures that lead to fusion between different membrane systems. Upon rehydration, cellular contents leak out and fused membrane particles do not swell (i.e. they are ‘osmotically unresponsive’). (Adapted from Steponkus et al. (1993), with permission.)
There is little information for comparing membrane phase behaviour among orthodox and recalcitrant embryos, maturing embryos as they become more tolerant of desiccation, or leaves from desiccation-tolerant angiosperms as they adjust to low water potentials. Changes in bilayer spacings or lamellar fluid-to-gel transitions have been detected in both desiccation-tolerant and sensitive plant cells during dehydration, with little difference in behaviour detected with degree of tolerance (McKersie and Stinson, 1980; Seewaldt et al., 1981; Priestley and de Kruijff, 1982; Singh et al., 1984; Kerhoas et al., 1987; Crowe et al., 1989; Hoekstra et al., 1991, 1992; Sun et al., 1994; Hoekstra and Golovina, 1999). In tolerant soybean cotyledons, a gel-like transition occurred when seeds were dried to less than 0.2 g H₂O g⁻¹ dry mass (Seewaldt et al., 1981), a water content that corresponds to a water potential of about -12 MPa (e.g., Vertucci and Roos, 1990) (Fig. 9.1). Water potentials between -10 and -15 MPa also mark the survival limit for recalcitrant seeds (described above). A membrane-mediated mechanism is often invoked to explain damage in desiccation-sensitive embryos and pollen because the membrane integrity of these cells appears to be compromised upon rehydration (McKersie and Stinson, 1980; Senarathna and McKersie, 1983; Vertucci and Leopold, 1987; Berjak et al., 1992, 1993; Poulson and Eriksen, 1992; Sun and Leopold, 1993; Sun et al., 1994; Wolkers et al., 1998a).

The different views of dehydration stress (i.e., removal of structural water versus enhancement of hydration forces) have promoted different ideas for the mechanisms of protection. According to the 'Water Replacement Hypothesis', if structural water is removed, small hydrophilic molecules such as sugars must be inserted between polar lipid head groups to maintain proper intermolecular spacings and membrane integrity (Clegg, 1986; Crowe et al., 1990; Crowe and Crowe, 1992). An alternative, but not mutually exclusive, model suggests that high concentrations of compatible solutes can help resist water loss between molecular surfaces, relieving the size of hydration forces (Wolfe and Bryant, 1999; Koster et al., 2000; Bryant et al., 2001). As dehydration proceeds, the concentration within the interfaces increases, with a concomitant increase in viscosity (Fig. 9.2). The high viscosity of these interfacial solutions provides mechanical resistance to the further compression of macromolecules (Wolfe and Bryant, 1999; Koster et al., 2000; Bryant et al., 2001). In both protective models, the goal is to keep molecules separated so that harmful interactions are prevented. Sugars accomplish this capably in model membrane systems (Crowe et al., 1986, 1989; Crowe and Crowe, 1992; Wolfe and Bryant, 1999; Koster et al., 2000; Bryant et al., 2001). However, the presence of adequate quantities of sugars in cells and the vitrification of cellular constituents do not appear to prevent polar lipid phase changes in desiccation-tolerant cells (Seewaldt et al., 1981; Priestley and de Kruijff, 1982; Crowe et al., 1989; Hoekstra et al., 1989, 1992, 1999; Leopold et al., 1994) or damage in desiccation-sensitive cells (Berjak et al., 1992, 1993; Sun and Leopold, 1993; Still et al., 1994; Sun et al., 1994; Vertucci and Farrant, 1995; Vertucci et al., 1995; Farrant and Walters, 1998; Wolkers et al., 1998a; Hoekstra and Golovina, 1999; see Chapter 10). Changing the composition of membranes (reviewed by Supponkus et al., 1995) and reducing their surface area by dismantling endomembrane systems (described above) may be the important tools for maintaining compartmentation in drying cells.

Structural changes of proteins with hydration have received wide attention in the literature. Early work using a variety of proteins showed that protein structure was conserved during drying to extremely low levels (Schneider and Schneider, 1972; Kuntz and Kauzmann, 1974; Rugg and Hani, 1975; Rugg et al., 1975; Fujita and Noda, 1978; Carrer et al., 1980; Takahashi et al., 1980; Jamieson, 1981; Rupley et al., 1983). In parallel studies, it was demonstrated that some proteins even maintained functional activity (albeit at low levels)
when dry (Acker, 1969; Potthast, 1978; Labuza, 1980; Rupley et al., 1983). Secondary structure of cytoplasmic proteins (extracted from desiccation-tolerant pollen) was conservsed upon drying in the absence of protectant sugars, demonstrating innate stability perhaps because of the high degree of α-helical structures (Wolters and Hockstra, 1995). The reversibility of sorption-desorption isotherms of numerous proteins supported the idea that conformational changes of proteins during hydration were slight and reversible, making proteins an ideal model for studying hydration properties of biological materials (Bull, 1944; D’Arcy and Watt, 1970). Slight, reversible changes in protein structure, particularly secondary structure, have been attributed to volumetric changes from the loss of water rather than to changes in the native structure of proteins. These changes occur at fairly low moisture levels (between 0.2 and 0.1 g H₂O g⁻¹ dry mass or ~70 to ~200 MPa) (Ruegg and Hani, 1975; Griebnag and Klibanov, 1995). Drying, in fact, stabilizes protein structures, making them particularly resistant to ageing (Franks et al., 1991; Costantinno et al., 1998) and heat denaturation (e.g. Einocio et al., 1966; Ruegg et al., 1975; Fujita and Noda, 1978; Takahashi et al., 1980; Jaenicke, 1981; Leopold and Vertucci, 1986; Wolters and Hockstra, 1997). The extreme stability of protein structure with low hydration may be attributed to stronger intramolecular associations compared with the situation of polar lipids. Such interactions would reduce the need for hydrogen bonding with water to maintain structural integrity (obviating the need for water replacement by sugars as suggested by Crowe and co-workers (e.g. Crowe and Crowe, 1992]) and/or provide mechanical strength that resists deformation when molecules are compressed (obviating the need for mechanical barriers to compression as suggested by Wolfe and co-workers (e.g. Wolfe and Bryant, 1999)).

The conformations of some proteins and polypeptides are irreversibly damaged by drying or freeze-drying in the absence of protectants (Hansfuss, 1969; Carpenter et al., 1987, 1990; Franks et al., 1991; Prestrelski et al., 1993). Enzymes such as lactate dehydrogenase and polypeptides such as poly-lysine are particularly labile (Prestrelski et al., 1993), and damage is exacerbated if molecules are freeze-dried rather than air-dried (Franks et al., 1991). Rate of drying also has a large effect on the conservation of protein structure, with greater preservation achieved by rapid drying conditions (Wolters et al., 1998a, b). Often, desiccation-labile proteins are used to study the effects of protectants (Carpenter et al., 1987, 1990; Prestrelski et al., 1993). Clearly, these studies are essential to the pharmaceutical industry, but similar mechanisms of protection must not be presumed to apply in vivo in dehydrating plants. A tremendous amount of work has demonstrated that proteins are rather robust; thus, a need for protection must be demonstrated before a protective mechanism is implied. Studies must show that desiccation-labile enzymes exist in vivo, that they are not produced de novo during rehydration and that they are irreversibly damaged in desiccation-sensitive cells.

The structure and activity of proteins are compromised if they are stored under extremely dry conditions of approximately 0.1 g H₂O g⁻¹ dry matter or about ~200 MPa or less (Kuntz and Kauzmann, 1974; Luscher-Mattli and Ruegg, 1982; Sanches et al., 1986; Labruide et al., 1987). Substantial deterioration of the lattice of protein crystals was attributed to the refolding of polypeptide chains to increase packing efficiency (Kuntz and Kauzmann, 1974; Luscher-Mattli and Ruegg, 1982). Other studies have shown that severe drying exposes amine groups on proteins, promoting free radical production (Sanches et al., 1986; Labruide et al., 1987). At such low water contents, proton exchanges among charged amino acids could be measured, suggesting that these sites were exposed (Careri et al., 1980; Rupley et al., 1983). Deterioration at similar water potentials and in similar time frames is observed in stored seeds and pollen (e.g. Vertucci and Leopold, 1987; Vertucci and Rocs, 1990; Bultink et al., 1998). Although these organisms survive the initial stress of
complete water removal, they age progressively more rapidly when stored at \( \psi_w \leq -220 \text{ MPa} \) ( \( < 20\% \) RH). Perhaps mechanisms suggested to cause damage in proteins at low water contents (e.g., exposure of reactive sites on the proteins, increased relaxation of molecular structures as they fill voids left by water, or relaxation of the glassy matrix that embeds the proteins) are responsible for the deterioration of stored seeds and pollen. Protein structure is stable in seeds stored at about 30\% RH (Golovina et al., 1997), but stability of protein structures in seeds stored at lower humidities has not been documented. Increased ageing rates of seeds and pollen stored below a critical water content have also been attributed to reduced viscosity of the aqueous medium in cells that are almost completely dry (Buïtink et al., 1999b).

Upon dehydration, the same destabilizing forces that perturb lipid and some protein structures may also affect nucleic acid structure (Rau et al., 1984). DNA is a particularly stable molecule (Wayne et al., 1999) which maintains its structure in the absence of water and reversibly unfolds at high temperatures (Bonner and Klíbanov, 2000). The intermolecular distances of dehydrating DNA strands are comparable to those of condensed DNA in hydrated nuclei (Rau et al., 1984), suggesting that DNA structures are resistant to perturbations resulting from dense packing. When DNA is replicated and so is decondensed during germination, the cells concomitantly become susceptible to desiccation injury (Deltour and Jacquinard, 1974; Crèvecoeur et al., 1988) and rapidly dividing cells during embryogenesis also appear to be sensitive to desiccation (Myers et al., 1992). Desiccation did not affect the structure of condensed or decondensed chromatin in desiccation-tolerant or sensitive maize embryos, respectively (Leprince et al., 1995a). However, in those studies, the chelation of Ca\(^{2+}\) (and other divalent cations) by the ethylenediamine tetra-acetic acid (EDTA) present in the medium used for chromatin spreading, may have relaxed previously condensed chromatin, possibly accounting for the reportedly similar results from desiccation-tolerant and sensitive material (Pammenter and Berjak, 1999) (see also Chapter 12).

When unprotected cells are dried, organelles and macromolecules experience mechanical or structural damage. This type of desiccation damage is termed sensu stricto because the primary stress is water removal (Pammenter and Berjak, 1999; Walters et al., 2001). Membrane structures appear more prone to desiccation damage sensu stricto than do proteins or DNA, perhaps because of the intense hydrogen bonding within proteins and nucleic acid structures. Protection from damage often lies in the ability of the structure of the surrounding medium to offer mechanical resistance to the stress or to accommodate the stress through enhanced elasticity.

### 9.3.2. Metabolically derived damage

Loss of turgor precipitates a number of changes in metabolic pathways of plant cells. Assimilation of CO\(_2\) (if the tissue is photosynthetic) and growth are impaired. Often protein synthesis is temporarily stimulated during mild water stress (reviewed by Farrant et al., 1989; Ingram and Bartels, 1996; Oliver et al., 1998), with a switch in metabolism believed to lead to the production of putative protection mechanisms (reviewed by Verucci and Farrant, 1995; Ingram and Bartels, 1996; Oliver et al., 1998; Chapters 1, 5 and 11). Observations of increased polysomes and rough endoplasmic reticulum in slightly water-stressed recalcitrant embryos suggest that certain (possibly similar) metabolic pathways may also be induced in seeds that do not acquire full tolerance of desiccation (Berjak et al., 1984; Farrant et al., 1989; Pammenter et al., 1998). These changes in metabolism do not indicate that cells have already experienced damage; when briefly stressed, most organisms resume normal metabolism once the water stress is relieved. However, prolonged mild stress (which could be considered akin to drought) is deleterious to both vegetative and embryonic tissues. Many recalcitrant seeds lose viability if maintained for long
periods at constant high water contents (e.g. Chin and Roberts, 1980; Berjak et al., 1989; Pannemeyer et al., 1994; Walters et al., 2001) and similar damage is observed in orthodox seeds (Walters et al., 2001). The loss of viability has been associated with the continuation of metabolism (including cell division) (Farrant et al., 1989), which will ultimately lead to a greater demand for water to maintain high water potentials (Berjak et al., 1989; Pannemeyer et al., 1994).

Metabolism slows at water potentials less than about −2 MPa, but not all reactions are affected by dehydration in the same way. Protein synthesis slows down at relatively high water potentials (reviewed by Bewley and Krochko, 1982; Clegg, 1986; Salmen Espindola et al., 1994; Ingram and Bartels, 1996; Mundree et al., 2000; Whittaker et al., 2001), while respiration continues to much lower levels (Vertucci and Leopold, 1984; Vertucci and Roos, 1990; Salmen Espindola et al., 1994; Leprince and Hoekstra, 1998; Leprince et al., 1999; Farrant, 2000; Walters et al., 2001). Various reactions within photosynthetic (Witwens et al., 1978; Hetherington et al., 1982b; Vertucci et al., 1985; Vertucci and Leopold, 1986; Farrant, 2000) and respiratory (Vertucci and Leopold, 1986; Leprince and Hoekstra, 1998; Leprince et al., 2000) pathways respond differently to low water contents. The differing responses to water stress among and within metabolic pathways can lead to imbalances in metabolism. Metabolic imbalances may be compounded by the respiration of fungi that occurs at water potentials as low as −20 MPa in orthodox and recalcitrant seed tissues (Mycock and Berjak, 1990; Goodman, 1994; Calistro et al., 2000). Damage by metabolic stress is most pronounced in cells at water potentials between 2 and −5 MPa with a diminishing effect as cells are dried to −12 MPa (Leprince et al., 2000; Walters et al., 2001). Both desiccation-sensitive and -tolerant organisms are damaged when stored at intermediate water potentials, though the time-dependency of the damage varies considerably among species and tissues (Walters et al., 2001).

A by-product of continued respiration and light harvesting when other metabolic processes are shut off is the accumulation of high-energy intermediates that leak out of mitochondria and plastids and form reactive oxygen species (ROS) and free radicals (Puntarulo et al., 1991; Dean et al., 1993; Hendry, 1993; Leprince et al., 1993, 1994, 1995b; Smirnoff, 1993; Foyer et al., 1994; Halliwell and Gutteridge, 1999). Reactive oxygen species and free radicals react with proteins, lipids and nucleic acids, causing permanent damage to enzymes (Wolff et al., 1986; Dean et al., 1993; Halliwell and Gutteridge, 1999), membranes (Senaratna and McKersie, 1983, 1986; Chan, 1987; McKersie et al., 1988, 1989; Finch-Savage et al., 1996; Halliwell and Gutteridge, 1999; Leprince et al., 2000) and chromosomes (Dizdaroglu, 1994). Peroxidation of lipids decreases the fluidity within membranes (McKersie et al., 1988, 1989), interfering with their selective permeability upon rehydration (as described above). Upon dehydration, high levels of free radicals have been detected in desiccation-sensitive embryos (Senaratna and McKersie, 1983, 1986; McKersie et al., 1988; Hendry et al., 1992; Leprince et al., 1993, 1994, 1995b, 1999, 2000; reviewed by Vertucci and Farrant, 1995; Pannemeyer and Berjak, 1999). The origin and sequence of events following the appearance of these toxic compounds are still unclear. They may be produced by the water-stressed cell (Leprince et al., 1994, 1995b, 1999, 2000; Leprince and Hoekstra, 1998) or as a result of the associated fungi (Goodman, 1994; Finch-Savage, 1999), and they may precede (or precipitate) damage (Finch-Savage et al., 1996; Leprince et al., 2000) or arise after the cell has already died (Finch-Savage, 1999).

There are several ways that cells can protect themselves from metabolic imbalance and ROS-mediated damage. At higher moisture levels, free-radical-scavenging enzymes efficiently detoxify ROS (Bewley, 1979; Dhindsa, 1987; Hendry, 1993; Smirnoff, 1993; Foyer et al., 1994; Kranmer and Grill, 1997; Sherwin and Farrant, 1998; Pannemeyer and Berjak, 1999; Farrant,
2000]. These enzymes appear ineffective at low water contents, and tocopherol and ascorbic acid may be more effective (reviewed by McKersie et al., 1988; Pammenter and Berjak, 1999). Amphiphatic molecules such as tocopherol can partition between aqueous and lipid domains according to the water content of the cell and polarity of the molecule (Golovina et al., 1998). A controlled shut-down of metabolism upon drying may also mitigate the consequences of unbalanced metabolism (as reviewed by Leprince et al., 1993; Vertucci and Farrant, 1995; Hand and Hardewig, 1996; Pammenter and Berjak, 1999). Cells with more organelles and greater definition of organelle structure appear to be more sensitive to desiccation (Bewley, 1979; Hetherington, 1982a; Gaff, 1989; Berjak et al., 1990; Farrant et al., 1997; Farrant and Walters, 1998; Farrant, 2000), either because there are more membrane structures to protect (described above) or because the higher metabolism leads to greater ROS production. Conditions that reduce metabolism such as low temperature (Leprince et al., 1995b) or highly complex substrates (Leprince et al., 1990) also tend to reduce sensitivity to desiccation. Desiccation-sensitive cells require at comparatively greater rates than tolerant cells at the same water content (Leprince et al., 1999; Walters et al., 2001), which may reflect properties of the mitochondria themselves or of the cellular matrix. It has been suggested that changes in viscosity with dehydration are not as marked in desiccation-sensitive cells, and so metabolism is not as restricted (Leprince and Hoekstra, 1998). It has also been suggested that the packing of macromolecules during dehydration of desiccation-sensitive cells is not as dense (Wolkers et al., 1999a,b), and this might facilitate the diffusion of oxygen through the cell matrix.

9.4. Perspectives on the Kinetics of Desiccation Damage

This chapter has described how desiccation damage, incurred from structural changes of cellular constituents, may lead to metabolically derived damage and vice versa. Most model studies suggest that changes in molecular conformations with dehydration are reversible. Dehydration slows chemical reactions and so organisms that are dried sufficiently rapidly should experience few, if any, changes in the chemistry of their cells. Thus, it seems likely that the primary lesions resulting from water stress, whether they are physical or chemical, are minor. It also appears that the primary lesions are not exclusive to desiccation-sensitive cells. Gel-phase lipid transitions occur in both tolerant and sensitive pollens (e.g. Hoekstra and Golovina, 1999; Hoekstra et al., 1999); metabolic imbalances occur in both desiccation-tolerant pea and desiccation-sensitive tea (Walters et al., 2001); changes in the secondary structures of proteins are comparable in both mature and immature maize embryos (Wolkers et al., 1999a). Irreparable desiccation damage must then result from a cascade of reactions, initiated by primary but subtle lesions, which perturb organization within the cell and ultimately lead to cell death. The extent of desiccation damage can then be viewed as a function of the rapidity at which the cascade of deleterious reactions occurs. From this perspective, desiccation damage is a time-dependent process — an ageing phenomenon.

Dehydration has contrasting effects on the kinetics of both physical and chemical deteriorative reactions. Removing water from cells increases the concentrations of reactants but slows the molecular motions necessary for reactions to occur. The degree to which cells are damaged by desiccation, and by extension the critical moisture level to which they survive drying, is determined by the treatment duration, the concentration of the reactants and the physical barriers (e.g. viscosity and compartmentation) to thermodynamically favourable reactions. Given the same experimental time, cells that tolerate more stress have either fewer reacting substances or greater barriers to harmful reactions. For example, according to the Water Replacement
9.6. Acknowledgements

The authors gratefully acknowledge Drs Peter L. Steponkus (Cornell University), Folkert Hoekstra (Wageningen University) and, especially, Karen L. Koster (The University of South Dakota) for helpful and thought-provoking discussions.

9.7. References


Dean, R.T., Gissinger, S. and Davies, M.J. (1993) Reactive species and their accumulation on radical- 
Deltour, R. and Jaccard, A. (1974) Relation between water stress and DNA synthesis during germi-
Dhindsa, R.S. (1987) Protein synthesis during rehydration of rapidly dried Tortula ruralis. Plant 
Physiology 85, 1094–1098.
Dizdaroglu, M. (1994) Chemical determination of oxidative DNA damage by gas-chromatography 
desiccation sensitivity using a quantal response model: application to nine species of the genus 
Coffeea L. Seed Science Research 9, 135–144.
Echigo, A., Fujita, T. and Koga, S. (1986) Relationship between biological and physical properties of 
Tolerance of Coffea spp. seeds to desiccation and low temperature. Revista Brasileira de 
Fisiologia Vegetal 11, 97–105 (in English).
Eira, M.T.S., Walters, C. and Caldas, L.S. (1999b) Water sorption properties in Coffeea spp. seeds and 
Science and Technology 14, 593–600.
Farrant, J.M. (2000) A comparison of patterns of desiccation tolerance among three angiosperm resur-
Communication Services of the New York State Agricultural Experimental Station, Geneva, New 
York, pp. 109–120.
Farrant, J.M. and Walters, C. (1998) Ultrastructural and biophysical changes in developing embryos of 
Aesculus hippocastanum in relation to the acquisition of tolerance to drying. Physiologia 
Plantarum 104, 513–524.
Farrant, J.M., Pannenger, N.W. and Berjak, P. (1992) Development of the recalcitrant (homoiohy-
drous) seeds of Aveneina marina: anatomical, ultrastructural and biochemical events associated 
with development from histodifferentiation to maturation. Annals of Botany 70, 75–86.
Farrant, J.M., Pannenger, N.W. and Berjak, P. (1992) Seed development in relation to desiccation tol-
erance: a comparison between desiccation-sensitive (recalcitrant) seeds of Aveneina marina and 
Farrant, J.M., Pannenger, N.W., Berjak, P. and Walters, C. (1997) Subcellular organization and meta-
abolic activity during the development of seeds that attain different levels of desiccation toler-
ance. Seed Science Research 7, 135–144.
Finch-Savage, W.E. (1992a) Seed water status and survival in the recalcitrant species Quercus robur 
Finch-Savage, W.E. (1992b) Seed development in the recalcitrant species Quercus robur L.: develop-
ment of germinability and desiccation tolerance. Seed Science Research 2, 17–22.
Edwards, D.G.W. and Neithani, S.C. (eds) Innovations in Forest Tree Seed Science and Nursery 


Leprince, O., Buitink, J. and Hoekstra, F.A. (1999) Axes and cotyledons of recalcitrant seeds of


Roberts, E.H. (1973) Predicting the storage life of seeds. Seed Science and Technology 1, 499–514.


