

Mechanisms of adaptation to physical and chemical factors in plants

1. INTRODUCTION

The mechanisms by which plants adapt to environmental conditions have been studied almost as long as plants themselves have been an object of scientific investigation. Early workers observed a very high frequency of stomata in the leaves of xerophytes and immediately wondered whether such a system could help the plant to survive a period of drought. Experimentation soon showed that under conditions of ample water supply xerophytes had a very high rate of transpiration (per unit leaf area) but under limitation of soil water the numerous stomata allowed the plant to regulate its water loss in a much more precise way than would be possible in leaves with fewer stomata (for a review of the older literature, see CRAFTS *et al.* 1949).

The experimental approach also proved valuable in studies on the frost resistance of plants and as early as 1912, MAXIMOV contributed experimental evidence supporting the hypothesis that a high sugar content of plant cells formed part of the protective mechanism against frost damage: epidermal strips of cabbage leaves floating on a sucrose solution were more resistant to sub-zero temperatures than strips on salt solutions or distilled water or the intact tissue itself. Slowly, the increasing knowledge about the "hardiness of plants", as LEVITT (1956) described this field of research, gave rise to specialized research areas according to the environmental factor under investigation, including resistance to freezing (cryobiology), chilling, high temperature, salinity and drought, to mention the most important areas. LEVITT (1972) gave a synthesis of the knowledge gained by this work in a book entitled "Responses of plants to environmental stresses", which reflects the specialization in this field in a time when the majority of the plant physiologists are working with plant material grown under controlled conditions guaranteeing optimal growth.

However, optimal conditions for growth are not necessarily the main factors to be investigated by ecophysiologists, because the conditions encountered are usually suboptimal. Therefore, the establishment and maintenance of populations of a given plant species in natural vegetations will depend on many other factors, related to the physiology of the individual plant besides optimal conditions for growth.

At this point it may be useful for the experimental approach of the problem how to investigate the adaptation mechanisms, to classify the vegetations composed of these plants into the following groups. It should be kept in mind here that such a classification is a prerequisite for any experimental approach.

There are three main types of vegetation:

Type 1. Vegetations consisting of plant species occurring under conditions in which a single environmental factor or a cluster of environmental factors are decisive and limiting for the establishment or maintenance of populations of the species concerned. Such vegetations may be relatively poor with respect to the number of species of (higher) plants and consequently may have a monotonous appearance. Examples of this type include vegetations of salt marshes (*Salicornia* spp.; the habitat being characterized by flooding with sea water); peat bogs (*Sphagnum* spp.; permanently wet, the water having a low pH value and a low mineral content); dry heath areas (*Calluna vulgaris*; habitat relatively dry with a low mineral level and exposed to grazing); and submerged aquatic vegetations (*Potamogeton* spp.; water with a high pH value and relatively strong movement). Many of the pioneer vegetations belong to this category.

Type 2. This is a variant of type 1, but the environmental factor (or cluster of factors) acting as key factor strongly promotes a high growth rate of the individual plants of the population concerned. This type of vegetation is often highly dependent upon human activities. Dutch arable fields with their high yields are examples of such vegetations, but vegetations less directly dependent on human interference also belong to this type for instance vegetations composed of reed (*Phragmites australis*) and reed-grass (*Glyceria maxima*) along the banks of eutrophic lakes or meadows with a cover of English ryegrass (*Lolium perenne*). The species concerned often have a relatively high tolerance for the effect of foreign substances (pollution), treading, tillage, weeding, and other human activities. It should be stressed that most of our knowledge about plant physiology concerns species originating from vegetations of this type.

Type 3. Vegetations of greater diversity than those of type 1, composed of several to many species often representing a wide variety of life forms. In well-developed situations the floristic composition of such vegetations is very rich and includes many rarely encountered species. The diversity of the vegetation is also expressed in a marked seasonal variation. Competition between the individual plants seems to be much lower in vegetations of type 3 than in those of type 2, which is undoubtedly due to the lower growth rate of the individual plants and the low or very low productivity of such vegetations.

Vegetations of type 3 are found in gradient situations of environmental conditions, e.g. on the borderline between areas differing in topography, soil type, hydrology, salinity, or the level of mineral nutrients. It has been suggested by VAN LEEUWEN (1966) and WESTHOFF *et al.* (1970) that the more complex the system of gradients in environmental conditions, the more intricate

the pattern of the existing vegetation, especially under typical conditions. Type 3 vegetations are often difficult to preserve in densely populated areas characterized by vegetations of type 2. The physiology of species characteristic for such vegetations has hardly been studied at all, but intuitively one would suggest that the individual plants in such an environment must have evolved mechanisms to maintain proper conditions for the physiological functioning of their tissues and organs under the fluctuating environmental conditions. If these three types of vegetation are considered in relation to GRIME's three primary strategies (see this volume) it is evident that the stress-tolerant strategy concerns vegetations of type 1 and 3 and the ruderal and competitive strategy concerns vegetations of type 2, distinguishing between productive vegetation types of severely disturbed and of relatively undisturbed environments.

2. METHODOLOGY

In the following sections examples will be presented of adaptations by plants to extreme environmental conditions (such as prevail in vegetations of type 1) as well as responses of plants to fluctuations in environmental conditions, i.e., responses possibly having adaptive value for species that are characteristic for more diverse vegetations (type 3). Because of the scarcity of experimental data on the latter subject, caution must be applied in the interpretation of the results and generalization is not permissible.

Adaptations of plants occur at several levels of organization, from the intact plant organ down to the cells and cell organelles. Photosynthesis provides the source of energy for the formation of all the structures required to cope with extreme or fluctuating environmental conditions. Measurements on respiration, and more specifically on the efficiency of respiration, can provide information on the utilization of metabolic energy in growth, for instance in species of high-yield vegetations (type 2) and in the formation of the structures needed to cope with extreme environmental conditions (type 1) or with conditions prevailing in the diverse vegetations (type 3). On the cellular and subcellular levels striking alterations are observed as a response to different environmental stresses such as prevail in vegetations of types 1 and 3. Examples of such alterations will be presented on the level of the biomembranes which regulate so many physiological reactions. Specifically, the effect of the environment on the lipid composition of biomembranes and on the functioning of lipid-dependent membrane enzymes will be discussed in more detail.

The role of membranes in responses to environmental conditions can be studied in several ways. In the first place, comparison can be made between species or varieties, preferably grown under identical environmental conditions. The species are arranged in order of adaptation to environmental factors or

stresses (e.g. salinity, low temperature), and correlation with membrane properties, such as membrane enzyme activity or membrane lipid composition is sought. Secondly, an environmental condition can be varied and species differing in ecological adaptation to this environmental factor can be compared. A particularly useful experimental approach is to introduce an abrupt environmental change and follow the ensuing changes in membrane properties as a function of time. Such observations may indicate, for instance, the specific role of a membrane enzyme or membrane lipid in relation to the structure and transport properties of the biomembrane in question, enabling it to function properly under the altered environmental conditions. Specifically, plant species that have to cope with fluctuating environmental conditions (type 3) may yield information on adaptation on the membrane level when the last of these experimental approaches is used.

3. PHOTOSYNTHESIS

3.1. ADAPTATION TO SUN AND SHADE

As is evident from a recent review on environmental control of photosynthesis (MARCELLE 1975), photosynthetic efficiency is highly dependent on environmental conditions. In a densely shaded environment it is the efficiency with which the plant is able to absorb and utilize light of low intensity together with a minimal investment in constituents of the chloroplasts that determines the efficacy of the adaptation to shade. In *Alocasia macrorrhiza*, a shade plant of rain forests, the chloroplast grana are extremely well developed and oriented in all directions, resulting in a very high chlorophyll concentration per chloroplast as well as per cell (BJÖRKMAN 1975). Such an extreme shade plant shows, on a chlorophyll basis, very low levels of chloroplast soluble protein, chloroplast ribulose diphosphate carboxylase, and several carriers of the electron transport chain such as plastoquinone, cytochrome f, and cytochrome b_6 (BJÖRKMAN *et al.* 1972).

The reverse holds for a species with a preference for the sun, *Atriplex hastata*, which is a beach plant (C_3) characterized by high levels of chloroplast soluble protein, chloroplast RuDP-carboxylase, and photosynthetic electron transport carriers, by less well developed grana, and by the occupation of a larger volume by the stroma fraction, thus permitting the plant to reach a much higher level of photosynthesis under light-saturation conditions. Other *Atriplex* species belonging to the same environment i.e., *Atriplex glabriuscula* (C_3) and *Atriplex sabulosa* (C_4), show photosynthesis curves with similar responses to temperature, even though the rate of photosynthesis of the C_4 species is consistently higher than that of the C_3 species. In both species photosynthesis decreases at temperatures above 35°C, whereas in *Tidestromia oblongifolia*, a C_4 species of the hot desert photosynthesis reaches its

temperature optimum above 45°C. With respect to photosynthesis, the C₄ species are clearly at an advantage in bright sunlight. In that situation ATP production by chloroplasts does not limit CO₂ reduction even though the number of ATP molecules required to reduce a CO₂ molecule is considerably higher than for the chloroplasts of C₃ plants (BLACK 1973).

It should be noted that physiological factors that may depend indirectly upon the C₄ mechanism of photosynthesis, may give C₄ plants an advantage over C₃ plants. HOFSTRA & STIENSTRA (1977) observed that in dry and open fields in Indonesia the C₄ grasses occurring there, *Axonopus compressus* and *Setaria plicata*, showed a higher initial growth rate and a lower shoot/root ratio than the investigated C₃ grass (*Oplismenus compositus*), which grows in the shade. These factors may have competitive value and explain why C₃ grasses were absent in the open fields.

3.2. PHOTOSYNTHESIS OF AQUATIC PLANTS

Another interesting adaptation of photosynthesis occurs in submerged aquatic plants, which may absorb a considerable quantity of bicarbonate in addition to dissolved carbon dioxide gas. The distribution of the carbon dioxide components (CO₂, HCO₃⁻, and CO₃²⁻) is pH-dependent, and at pH 9.5 calculation gives a saturation concentration of bicarbonate of 20 mM, corresponding to a saturated CO₂ concentration at pH 7.0. Such high concentrations will cause a flux of HCO₃⁻ into the leaf that is almost equal to the rate of uptake of CO₂, assuming a CO₂ concentration of 75 ppm inside the leaf (HELDER & ZANSTRA 1977). The absorbed bicarbonate is utilized in photosynthesis, and part of the solar energy is used to pump the split hydroxyl ions (HCO₃⁻ → CO₂ + OH⁻) back into the medium. The hydroxyl efflux system is located in peripheral bands on *Chara* cells (LUCAS 1975, 1976).

In *Potamogeton* leaves a polar transport occurs, bicarbonate being absorbed at the lower surface of the leaf and hydroxyl ions expelled at the upper surface (HELDER 1975).

4. RESPIRATION

4.1. MITOCHONDRIAL RESPIRATION

Dark respiration seems to be regulated by various factors. Several authors found dark respiration of photosynthetic tissue to be reduced or even completely inhibited in the light (MANGEL *et al.* 1974; CHEVALIER & DOUCE 1976; RAVEN 1972, 1976), whereas in other experiments no effect of light was measurable (RAVEN 1972; CHAPMAN & GRAHAM 1974).

HEICHEL (1971) compared two corn varieties with significantly different rates of dark respiration. The variety with the lowest dark respiration showed the highest growth rate. Thus, it seems that as far as growth is concerned, part of the dark respiration can be considered wasteful (ZELITCH 1975). Part

of the mitochondrial respiration occurs along an alternative pathway which produces only one-third as much ATP for each pair of hydrogen ions oxidized as does the conventional pathway, and sometimes no ATP is produced at all. The alternative pathway is insensitive to cyanide and antimycin but is specifically inhibited by the salicylhydroxamic acid (SCHONBAUM *et al.* 1971), a compound which does not affect the conventional pathway. In leaves, at least half of the mitochondrial respiration is cyanide-insensitive (BONNER & WILDMAN 1946), but insensitivity to cyanide has also been observed in potato tubers (VAN DER PLAS 1977) and in the sunk cabbage spadix (BAHR & BONNER 1973). It has been suggested that in the latter organ cyanide-insensitive respiration might contribute by heat production to the well-known elevated temperature characteristic for the developing spadix of *Araceae*.

The alternative pathway may function as a regulator of the redox state of the cell under conditions of excess production of reducing power (NADH), e.g. when the plant is exposed to conditions limiting growth of the plant. It can be important that respiration continues under such "stress" conditions, which limit growth, because at the same time the plant may need respiratory activity for the synthesis of cell material needed to cope with the newly developed "stress" condition (LAMBERS, personal communication). In this connection it is important to note that during ageing the phospholipid level in sweet potato tuber mitochondria is lowered, while concomittantly the cyanide-insensitive respiration of these mitochondria is increased due to a change in the mitochondrial membrane structure (NAKAMURA & ASAHI 1976). Regulation of respiration and its response to various environmental conditions is still a highly neglected field of research.

The alternative pathway is easily inhibited by exposure of plant roots to anaerobiosis (SOLOMOS 1977). It is conceivable that the stimulation of growth of the flooding-insensitive *Senecio aquaticus* (LAMBERS 1976) and other swamp plants observed under anaerobiosis is attributable to inhibition of non-phosphorylating oxidase (LAMBERS & SMAKMAN 1977). Under anaerobic conditions growth respiration of the roots of this *Senecio* species is only one-third of that of aerobically grown plants, a phenomenon which again underlines the importance of inhibition of this "wasteful" respiration by flooding (LAMBERS & STEINGRÖVER 1977). The significance of this oxidase for environmental adaptations remains an open question, but the results obtained so far indicate a possible role of this enzyme in adaptation to flooding.

5. PHYSIOLOGY OF MEMBRANES: EFFECTS OF FREEZING AND LOW TEMPERATURES

5.1. SUPERCOOLING

Freezing resistance in higher plants has been discussed in relation to many cellular characteristics. A first prerequisite for resistance is supercooling

of the intracellular water (GEORGE *et al.* 1974) to prevent intracellular damage due to freezing. The role of supercooling and nucleation of ice was studied by RASMUSSEN *et al.* (1975) in single cells, whereas BERVAES *et al.* (1977) attempted to study this phenomenon in the more complicated system of higher-plant tissues. To this end, the kinetics of freezing damage were investigated in apple and pine trees. As expected, the killing rate associated with freezing was lowest in cold-acclimated trees. When this rate is plotted against the physical supercooling parameter, $1/T^3 \cdot (\Delta T)^2$, the results indicate that in cold-acclimated trees supercooling is indeed part of the mechanism of frost protection.

GEORGE *et al.* (1974), BURKE *et al.* (1975), and GEORGE & BURKE (1977) had suggested that deep supercooling has an important effect in the xylem of many trees. It is of interest that in North America many tree species have a northern distribution limit which is characterized by the rarity of minimum temperatures below -40°C in any year. This temperature limit has physical significance, since the limit for the supercooling of water is about -41°C , and calorimetric and nuclear magnetic resonance studies have indeed shown that in such species ice formation in the xylem starts in the region of -30°C to -40°C (GEORGE & BURKE 1977).

5.2. THE PLASMA MEMBRANE

Besides supercooling, the chemical and physical condition of the plasma membrane is crucial for frost resistance: after thawing, damage due to frost becomes visible as a loss of turgor caused by destruction of the plasma membrane structure. Upon lowering of the temperature, the lipid matrix of the plasma membrane starts to crystallize, saturated lipid molecules first, followed by less saturated lipid molecules at lower temperatures. The membrane proteins tend to aggregate in the remaining area of non-crystalline lipids (called the liquid-crystalline phase), and finally denaturation of membrane proteins takes place by oxidation of sulphhydryl groups of protein molecules in such close contact that disulfide bridges are formed and restoration of the original state of the plasma membrane after the temperature rises is no longer possible (LEVITT 1969).

5.3. LIPIDS AND FROST RESISTANCE

Plants whose winter hardiness is increased by low temperatures during growth are characterized by a high total lipid content. Under these conditions there is an appreciable increase in the level of two lipids viz. phosphatidyl choline and phosphatidyl ethanolamine (alfalfa leaves, KUIPER 1970; poplar bark, YOSHIDA 1974; black locust bark, SIMINOVITCH *et al.* 1975; wheat seedlings, DE LA ROCHE *et al.* 1972, 1973, 1975, and WILLEMOT 1975; rape leaves, SMOLENSKA & KUIPER 1977). The elevated level of these lipid fractions is accompanied by a depressed level of phosphatidyl glycerol. When the plant

tissue is damaged by freezing, the level of phosphatidic acid rises sharply, even if the tissue remains frozen (WILSON & RINNE 1976).

In many studies on frost hardiness an increase in lipid unsaturation, and more specifically an increased level of linolenic acid, has been found. From a study done in wheat varieties differing in their ability to harden to cold, DE LA ROCHE *et al.* (1975) concluded that the observed elevated levels of linolenic acid in plants grown at low temperature (2°C) reflected only a response to the low temperature growth condition without a direct connection with frost-hardening itself. In agreement with this conclusion is the observation made in the bark of poplar and black locust trees that no change in unsaturation occurred during the entire year, even though the frost sensitivity of the bark tissue in the winter differs widely from that in the summer. The assumption that an elevated level of linolenic acid is a factor in the functioning of plants at low temperatures is supported by the finding that the survival of cotton seedlings at 8°C was strongly reduced when the seedlings had been treated with a specific inhibitor of linolenic acid synthesis (HILTON *et al.* 1971; ST. JOHN & CHRISTIANSEN 1976).

5.4. FLEXIBILITY OF MEMBRANES AND CYCLIC ACIDS

Mitochondria rich in unsaturated lipids show a higher degree of flexibility and permeability to water than mitochondria containing a higher proportion of saturated lipids (LYONS & RAISON 1970). In pine trees whose winter hardiness can be increased by a low temperature treatment as well as by a short-day treatment, specific effects of these environmental factors on lipid composition are noteworthy: behenic acid was exclusively synthesized upon transfer of the trees to low temperature, and a cyclic acid was suggested upon exposure of the plants to short-day conditions (BERVAES *et al.* in preparation). Such cyclic fatty acids were found in early spring plants (KUIPER & STUIVER 1972). Like polyunsaturated fatty acids, they guarantee a high flexibility of the involved membrane at low temperatures. Large quantities of cyclopropane fatty acids were observed in the sulfolipid fraction of the snow drop and of *Anthriscus sylvestris*, provided the plants were collected early in the spring. Cyclic acids were absent in flowering *Anthriscus* plants at the end of May. Compared with polyunsaturated acids, cyclic acids are less susceptible to photo-oxidation at (high) day-time temperatures and thus the production of cyclic acids might be a mechanism used by species exposed to extreme daily temperature fluctuations. Plant species of widely different habitats occurring in The Netherlands were screened for cyclic acids and, interestingly enough, two grasses from inland sand dunes (*Ammophila arenaria* and *Corynephorus canescens*) showed cyclopropane fatty acids in the phosphatidyl choline fraction of the leaves (KUIPER & STUIVER 1972).

A similar observation was made by DERTIEN *et al.* (1977) in lichens of sand-dune areas. Tree-growing species like *Evernia prunastri*, *Parmelia saxatilis*,

and *Hypogymnia physodes* were characterized by high levels of the polyunsaturated linoleic and linolenic acids, whereas the terrestrial species from the sand-dune area, *Cetraria islandica* and *Cladonia impexa*, contained large quantities of cyclic acids (Table 1). In the latter species an analogue of nephromopsic acid containing a lactone ring was indicated by mass spectrometry.

TABLE 1. Fatty acid composition of tree-growing and terrestrial lichens expressed as percentage of total fatty acids. The numbers of the fatty acids refer to the number of C-atoms and of double bonds, respectively (after DERTIEN et al. 1977)

Fatty acids	Tree-growing			Terrestrial	
	<i>Evernia prunastri</i>	<i>Parmelia saxatilis</i>	<i>Hypogymnia physodes</i>	<i>Cetraria islandica</i>	<i>Cladonia impexa</i>
Saturated (16:0 + 18:0)	13.0	20.4	17.2	12.9	15.6
Monoenoic (18:1)	14.3	17.8	20.6	20.8	17.4
Dienoic (18:2)	20.3	34.5	34.9	25.2	19.1
Trienoic (18:3)	43.9	18.3	12.9	9.9	9.2
20 or more C-atoms*	7.0	13.8	14.8	26.3	38.1

* including cyclic lichen acids

Species adapted to extremely high day-time temperatures may contain large quantities of non-oxidizable lipids, and the organ-pipe cactus (*Lemaireocereus thurberii*) contains sterols in levels up to 50% of the dry weight of the plant (KIRCHER & BIRD 1976).

5.5. PHOTO-OXIDATION AT LOW TEMPERATURES

Damage of chloroplasts by photo-oxidation at low temperatures (1°C) is sometimes observed in chilling-sensitive plants. In *Cucumis* leaves the chloroplast envelope ruptured and vesicles formed in the thylakoids at 1°C in the light (VAN HASSELT 1974a). Under the same conditions *Cucumis* leaves showed rapid degradation of linolenic acid (VAN HASSELT 1974b). The linolenic acid level of the dark control at 1°C was not affected. Blue light was especially effective for the photo-oxidation of unsaturated fatty acids, which indicates that in addition to chlorophyll, carotenoids contributed to the photo-oxidation of unsaturated fatty acids. DE KOK & KUIPER (1977) showed that specifically monogalactose diglyceride, which lipid complexes with chlorophyll, was degraded in the photo-oxidative process. In all probability, photo-oxidation of monogalactose diglyceride -which is mainly esterified with linolenic acid- can be prevented by tocopherol, and the level of this compound was found to be

very low in *Cucumis* (VAN HASSELT, in preparation). This anti-oxidant was virtually absent in the lichen species studied by DERTIEN *et al.* (1977). Lichens with a large quantity of polyunsaturated fatty acids may therefore be sensitive to photo-oxidation. In this connection it is of interest that lichen species with the highest sensitivity to SO₂ pollution show visible symptoms of photo-oxidation (bleaching) (see also Table 2). SO₂ stimulates photo-oxidation, because at low pH the undissociated H₂SO₄ functions as a rather strong oxidator.

TABLE 2. Fatty acid composition of tree-growing lichens (expressed as % of total fatty acids) as related to their sensitivity to air pollution. High figures (BARKMAN 1958; HAWKWORTH & ROSE 1970) refer to high sensitivity of the lichens; the latter increases also from A upwards (DE WIT 1976)

Species	Fatty acids			Sensitivity to air pollution		
	dienoic (mainly linoleic acid)	trienoic (mainly linolenic acid)	20 or more C-atoms (saturated and cyclic acids)	BARKMAN (1958)	DE WIT (1976)	HAWKWORTH & ROSE (1970)
<i>Ramalina fastigiata</i>	11.8	54.9	10.4	8	F	7
<i>Evernia prunastri</i>	20.3	43.9	7.0	8	C	5
<i>Parmelia saxatilis</i>	34.5	18.3	13.8	10	F	5
<i>Ramalina farinacea</i>	31.1	15.0	18.3	7	D	5
<i>Parmelia sulcata</i>	20.9	17.0	34.2	6	B	4

A preliminary survey of species of *Ramalina*, *Evernia*, and *Parmelia*, which differ greatly in sensitivity to SO₂, indeed showed that the level of polyunsaturated fatty acid was directly related to the SO₂ sensitivity of the species (DE KOK, unpublished experiments).

5.6. TERPENES

A very interesting problem is presented by the chloroplasts of pine needles, which are known to continue photosynthesis even at sub-zero temperatures. Unlike the situation in most other higher plants, the monogalactose diglyceride fraction of these chloroplasts is esterified not with linolenic acid but with more saturated fatty acids. BERVAES *et al.* (1972) showed that upon dehardening of pine trees, terpene components (denoted as "extra-long-chain-fatty acids") in the chloroplasts moved from the digalactosyl diglyceride fraction to the monogalactosyl diglyceride fraction, as showed by DEAE-cellulose column chromatography. Extraction from the above fractions by

thin-layer chromatography, followed by n.m.r. and mass-spectrometry, indicated that the chemical structure of these components was that of a monomethylester of a cyclic diterpene dicarboxylic acid: pinifolic acid (BERVAES, in preparation). Interconversion of the two forms takes place by internal rearrangement of the molecule, i.e., by ring opening and closing, the open ring being characteristic for hardened pine trees (Fig. 1). The chemical nature of pinifolic acid is also very intriguing. Like sterols, this compound

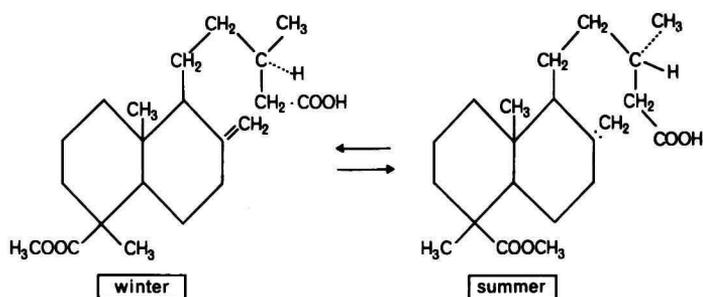


FIG. 1. *Interconversion of two forms of pinifolic acid monomethyl ester which was extracted from pine needles in various times of the year (BERVAES, unpublished data). The winter form is characterized by a vinyl group in the ring structure. By charge transfer within the molecule the summer form attains a higher degree of electron mobility in the ring structure*

functions as a membrane stabilizer and reduced temperature effects: the melting point of the relatively unsaturated egg phosphatidyl choline rises from 14° to 20°C (cholesterol: 18°C) and the melting point of the saturated dipalmitoyl phosphatidyl choline, drops from 41° to 24°C (cholesterol: 32°C), which provides a uniform flexibility of the lipid matrix of the biomembrane over a wide range of temperatures (STULEN & BERVAES, in preparation).

5.7. ATP-ASES AND COLD-SENSITIVITY

Membrane ATPases and soluble ATPases may be sensitive to cold or freezing temperatures (RACKER 1959; McCARTY & RACKER 1966; KUIPER 1971). Sensitivity of the membrane protein to frost depends on the lipid environment. LIVNE & RACKER (1969) showed that addition of lipids, with sulfolipid as the most efficient type, are among the factors that give stability to the chloroplast ATPase. ATPases of plant tissues required phosphatidyl choline and sulfolipid for proper functioning (KUIPER 1972), but many other membrane enzymes require

a lipid matrix as well. For example, LYONS & RAISON (1970) observed that in plants sensitive to chilling succinate oxidation of mitochondria showed a non-uniform temperature dependence resulting in broken curves when the logarithm of the rate of oxidation was plotted against temperature. At low temperatures the rate of oxidation is strongly temperature dependent, whereas at higher temperatures a much smaller temperature response was observed. The break in such temperature curves can be attributed to a phase shift of the mitochondrial membrane lipid matrix from the crystalline phase to the liquid-crystalline phase at rising temperatures. Such breaks are absent when the mitochondria originate from chilling-resistant plants, because the increased degree of unsaturation of the lipid matrix in such mitochondria lowers the temperature threshold at which crystallization occurs. Similar responses have also been seen in chloroplasts (NOBEL 1974).

5.8. CONCLUDING REMARKS

The foregoing illustrates that with respect to temperature, not only membrane structure but also physiological functions as dependent on that structure may show adaptations to low temperatures (polyunsaturated fatty acids), freezing (zwitterionic phospholipids), high temperatures (sterols), and extreme daily temperature variations (aliphatic cyclic acids) in sand dune grasses and terrestrial lichens, as well as a more uniform temperature response over a wide temperature range (terpenes in pine needle chloroplasts) and the need of an anti-oxidant (tocopherol) to prevent photo-oxidation when the enzymatic reactions are reduced at unfavourable temperatures. A careful evaluation of the environmental temperature regime to which an individual plant is exposed is needed to recognize the "solution" the species has selected to cope with the different demands put by various temperature regimes on the organism, either for survival alone or for functioning in general.

6. PHYSIOLOGY OF MEMBRANES: EFFECTS OF SALINITY

6.1. OSMOREGULATION

Osmoregulation is obligatory for the maintenance of turgor pressure under saline conditions. Most higher plants utilize ion transport to build up the required osmotic pressure, i.e. by pumping ions into the vacuole. Others excrete accumulated salts from the leaves or the roots back into the environment. To protect the cytoplasm from salt damage, high levels of non-electrolytes are often observed in the cells: polyalcohols (glycerol, galactoglycerol, and sugars) and amino acids (mainly proline). For further details the reader is referred to POLJAKOFF-MAYBER & GALE (1975).

6.2. BIOMEMBRANES AND SALINITY

As regards the effects of salinity on membranes, energy is needed for the active transport of ions across the ectoplast and tonoplast to build up the required turgor pressure by the pumping of ions into the vacuole; cation-specific ATPases indicate the presence of such ion pumps. Secondly, in salt-sensitive species the membrane structure may be damaged by salt, resulting in the release of protein and the suppression of the active uptake mechanism for phosphate and glucose (NIEMAN & WILLIS 1971). Furthermore, under such conditions membrane-stabilizing divalent cations such as Mg^{2+} and Ca^{2+} are replaced by Na^+ . Damage done by high salt concentrations is clearly visible under the electron microscope (POLJAKOFF-MAYBER 1975). Thirdly, sensitivity to chloride and other anions depends on the anion permeability of the lipid matrix of the biomembranes. Biomembranes containing (acidic) phospholipids tend to be virtually impermeable to cations and relatively more permeable to anions. For this reason, a distinction should be made between the effects of cations (Na^+) and of anions (Cl^-) as factors in salinity.

6.3. SENSITIVITY TO Na^+

A distinction can be made between natrophilic and natrophobic species. In natrophilic species Na^+ is taken up readily and is uniformly distributed in the plant. In some of these species a ($Na^+ + K^+$)-activated ATPase has been isolated and correlated with transport of these ions as a response to a saline environment (sugar beet, HANSSON & KYLIN 1969; *Avicennia*, KYLIN & GEE 1970). Stimulation of the enzyme by $Na^+ + K^+$ proved to be dependent on the sulfolipid level, and the removal of phosphatidyl choline -the other lipid found to be effective in the reconstitution of the activity of lipid-depleted plant-root ATPases (KUIPER 1972)- did not result in loss of activity. A similar ($Na^+ + K^+$)-ATPase preparation of animal origin, the salt gland of the duck has also been related to the level of an acidic sulfur-containing lipid, viz. sulphatide (KARLSSON *et al.* 1971). Roots of salt-tolerant *Plantago* species (*P. maritima* and *P. coronopus*) show higher levels of sulfolipid than roots of species from non-saline habitats (D.KUIPER, unpublished results). This acidic lipid might play an important role in the regulation of Na^+ transport in natrophilic plants.

A natrophobic species like *Phaseolus vulgaris* shows limited uptake of Na^+ by the roots together with effective excretion mechanisms that prevent any accumulation of Na^+ in the leaves (MARSCHNER 1974). When leaf segments of bean plants are exposed to NaCl, Na^+ accumulation can no longer be prevented, and this results in leakiness of membranes, loss of K^+ , and damage to the chloroplasts. Sugar beet leaf discs exposed to NaCl do not show any of these symptoms, which indicates differences in membrane structure between bean and sugar beet leaves. In the sugar beet and cotton, growth is even stimulated by NaCl and in the latter species, under saline conditions phosphate is preferentially incorporated into phospholipid (TWERSKY & FELHENDLER 1973).

6.4. SENSITIVITY TO Cl^-

KUIPER (1968a) compared the lipids of the roots of five varieties of grapes differing markedly in the translocation of Cl^- to the leaves. When these roots were exposed to moderate salt stress, the most salt-sensitive variety accumulated 15 times more Cl^- in the leaves than the most resistant variety. The monogalactosyl diglyceride content was directly related to Cl^- transport to the leaves, and this lipid was found to be the most efficient of the Cl^- transporters tested in a transport model (KUIPER 1968b). The phosphatidyl ethanolamine and phosphatidyl choline levels were inversely related to Cl^- accumulation. The roots of the most salt-sensitive variety had a very low sterol content. The charged phospholipids contributed strongly to the low Cl^- transport to the leaves. The effect on Cl^- transport of the addition of lipid to the root environment was also studied (KUIPER 1969). Galactolipids added to the root environment increased Cl^- transport to the roots, stem, and leaves of bean plants, whereas similarly supplied phosphatidyl choline was only absorbed by the roots; no transport to the stem and leaves could be detected. When glycerophosphoryl choline, a precursor of phosphatidyl choline, was added to the root environment, this substance was transported to the stem and leaves and incorporated into these tissues as phosphatidyl choline. The addition of glycerophosphoryl choline to the roots greatly reduced Cl^- transport to the leaves, thus demonstrating the importance of phosphatidyl choline in the regulation of Cl^- transport to the leaves when the plant is exposed to saline conditions.

7. PHYSIOLOGY OF MEMBRANES: RELATION TO MINERAL NUTRITION

7.1. INTRODUCTION

Higher plants have developed various mechanisms for regulating the uptake of mineral nutrients. Under limitation of the supply, species may develop a specific mechanism to guarantee sufficient uptake of an essential nutrient. MARSCHNER (1975) gives the example that under iron deficiency, sunflower roots lower the pH of the root environment. This results in leakage of reducing substances from the roots, which in turn reduce the local Fe^{3+} ion, which is not available for the plant, to Fe^{2+} . The reduced Fe^{2+} ions guarantee iron nutrition of the plants until the pH value of the root environment rises again, an iron deficiency develops, and the whole cycle is repeated.

A relationship between adaptation to variations in the availability of nutrients on the one hand and membrane properties on the other hand seems appropriate for plants, which unlike animals are unable to move away when changes occur in the environmental conditions of the habitat. The plasma membrane of the outer root cells is exposed to every fluctuation of the soil conditions and, to guarantee the required levels of uptake of essential

nutrients, plants have had to adapt the properties of their cell membranes to a considerable extent. As an example, English ryegrass roots were found to have four different isozymes of ATPase that were stimulated by various combinations of concentrations of Na^+ and K^+ . The activity of these isozymes varied along the root axis and was strongly affected by the level of the available nutrients. When the salt concentration was lowered from 35 to 0.75 mM, an isozyme specifically stimulated by Na^+ was detected that could have a function in osmotic adaptation by regulating the efflux of Na^+ (NELSON & KUIPER 1975). There are many other examples, but this discussion will be restricted to two crop plants (wheat and oat) and to species of *Plantago*, which will be discussed with respect to Ca^{2+} and Mg^{2+} ions.

7.2. Ca^{2+} AND Mg^{2+} IN WHEAT AND OAT

Wheat roots showed a high proportion of Ca^{2+} -stimulated ATPase activity, whereas in oat roots ATPase stimulation by Mg^{2+} was dominant (KYLIN & KÄHR 1973; KÄHR & KYLIN 1974; KÄHR & MAX MØLLER 1976). These species also differed in their response to the nutritional level of the substrate on which the plants were grown. Low-salt roots of oats showed a higher activity of divalent cation-stimulated ATPase than did high-salt roots. The reverse was found for wheat roots (KYLIN & KÄHR 1973; KÄHR & KYLIN 1974). These observations on the ATPase activity correlated well with field observations on the nutrient demands of these species. Oat is a crop of acid soils with a low mineral content, whereas wheat prefers calcium-rich soils with a high content of mineral nutrients.

The lipids of oat roots are more unsaturated than those of wheat roots, which is consistent with the habitat of these crops, oat having a preference for lower soil temperatures than wheat (KÄHR *et al.* 1976). When wheat and oat plants were grown at 18° and 25°C and different levels of nutrition, the highest activity of oat roots (in the presence of Mg^{2+}) was found in plants grown at 18°C and a low salt level (KÄHR & MAX MØLLER 1976).

7.3. Ca^{2+} AND Mg^{2+} IN *PLANTAGO*

The ATPase activity of the microsomal membrane fractions of the roots of several plantain species of various habitats was tested (D. KUIPER in preparation). The highest Ca^{2+} - and Mg^{2+} -stimulated ATPase activities were observed in species from relatively nutrient-poor environments (*Plantago lanceolata*, *Plantago coronopus*, and *Plantago media*), and species from relatively nutrient-rich environments (*Plantago major* and *Plantago maritima*) showed much lower activities (Fig. 2). A similar distinction between the plantain species could be made for the affinity of the ATPases for Mg^{2+} and Ca^{2+} . *Plantago major* and *Plantago maritima* shared other characteristics of their Ca^{2+} - and Mg^{2+} -stimulated ATPases. Besides an optimum in activity at pH 6.5 observed in all of the *Plantago* species tested, a second optimum was

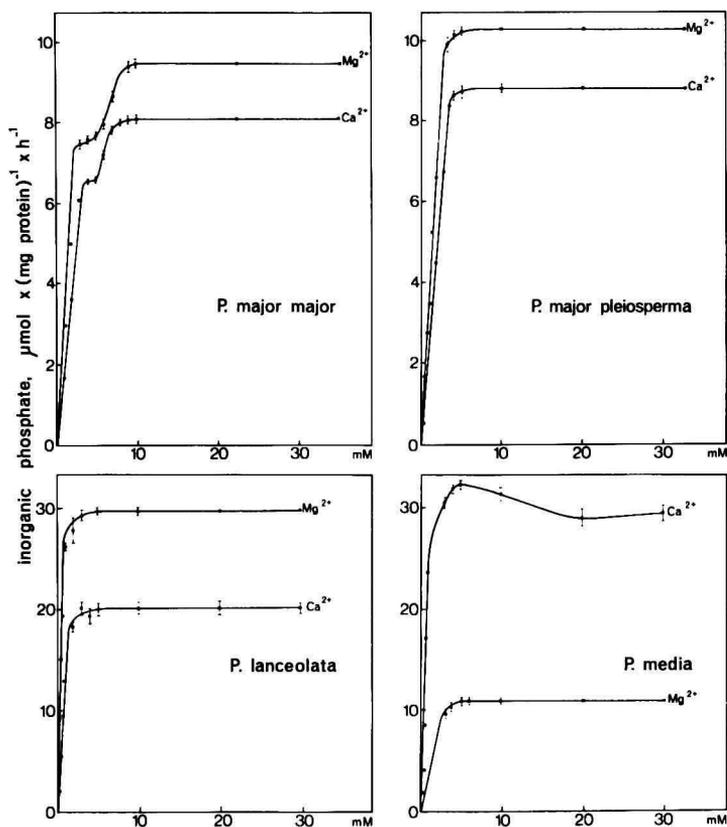


FIG. 2. Effect of Ca^{2+} and Mg^{2+} ions on the ATPase activity of microsomal fraction from the roots of various *Plantago* species from different ecological habitats: *Plantago major* spp. major and *P. major* spp. pleiosperma, relatively nutrient-rich habitats; *P. lanceolata* and *P. media*, relatively nutrient-poor habitats and from rather acidic and alkaline soils respectively (unpublished data of D. KUIPER)

observed at pH 8.0. Also, the Mg^{2+} - and Ca^{2+} -stimulated ATPase activities in both species showed a biphasic response when activity was plotted against ionic concentration, but only in plants grown on nutrient-rich solution. For this reason it is suggested that the high-affinity ATPase system of *Plantago major* and *Plantago maritima* is located at the plasma membrane and the low-affinity system at the tonoplast. Biphasic ion-uptake curves have been reported by PITMAN (1976) for Ca^{2+} (corn, cotton) and Mg^{2+} (barley).

In *Plantago lanceolata*, Mg^{2+} stimulated the ATPase activity more than Ca^{2+} did; in *Plantago major* and *Plantago maritima* the effect of these ions was virtually equal; and in the species occurring on calcareous soil (*Plantago*

media) Ca^{2+} had a much higher stimulatory effect than Mg^{2+} . In this respect *Plantago lanceolata* resembles oat and *Plantago media* wheat, except that in both *Plantago* species stimulation by Mg^{2+} (*Plantago lanceolata*) and by Ca^{2+} (*Plantago media*) is most dramatic in the roots of plants grown under low-salt conditions.

With respect to the ATPase activity of the entire root system, *Plantago coronopus* showed almost no response to fluctuations in the level of nutrients, a slight response was detectable in *Plantago lanceolata* and *Plantago media*, and *Plantago major* and *Plantago maritima* were severely affected by a drop in the nutrient level. The affinity of the Ca^{2+} - and Mg^{2+} -stimulated ATPase in the roots increased after transfer of the plants to low-salt conditions, which partly compensated for the lowered capacity of the ATPase. The well-regulated ATPase activity in *Plantago coronopus* is consistent with an ecological adaptation of the species, which enables it to grow under conditions fluctuating between saline and nutrient-poor.

7.4. CONCLUDING REMARKS

For salinity as well as for mineral nutrition, parallels can be drawn between biochemical properties of root-cell membranes and the specific demands the root environment makes on the individual plant. The various examples concerning salinity and membrane properties as well as mineral nutrition and ATPase activity in plants from different habitats clearly show the usefulness of this approach. As already mentioned, the right connection must be made between the levels of energy production and utilization on the one hand and the responses of the membranes involved on the other hand, and it is obvious that only an approach making use of several methods can provide a sound basis for an understanding of the relationship between membrane properties, physiological responses, and ecological adaptations. The *Plantago* research mentioned above forms part of such a project which was initiated by the Institute for Ecological Research and various university departments in The Netherlands.

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10. DISCUSSION

QUESTION: Is there a correlation between the rate of fatty acid substitution and the unpredictability of the environment in which species live?

ANSWER: I think the category "fluctuations of the environment" will more or less fit your "unpredictability". Terrestrial lichens, for instance, have very widely fluctuating rates of synthesis and breakdown of lipids and lichen acids, whereas the tree-growing lichens maintain a much more constant level

in these respects.

QUESTION: Halophytes, which are succulent, have a very high elasticity of the cells. Would you expect this phenomenon to be correlated with a biochemical change in the membrane?

ANSWER: For the sugar beet, some results are available. Nyctostatin, a compound which removes sterols from biomembranes and make them leaky to K^+ , is ineffective in the sugar beet, which indicates that the sterols present in the sugar beet biomembranes are inaccessible to this compound, unlike the membranes of glycophytes. Work done by D. KUIPER in our laboratory showed that sulpholipid, which is essential for functioning of the $(Na^+ + K^+)$ -ATPase observed in halophytes, is present in higher levels in *Plantago maritima* and *Plantago coronopus* than in the glycophytic *Plantago* species. Furthermore, a halophyte like *Plantago coronopus* is much better equipped than *Plantago major* to maintain a constant sterol level in its membranes, because the latter lacks a regulatory mechanism.

QUESTION: You started your lecture by mentioning that Professor MAXIMOV found that increased resistance to frost was correlated with increased sugar content in the plant. Do the sugars have other functions besides that of increasing the osmotic pressure of the cell?

ANSWER: Sugars are needed to prevent intracellular freezing damage to the cytoplasm.