FIRST JOINT MEETING OF THE BELGIAN FEDERATION FOR PLANT PHYSIOLOGY AND THE SECTION FOR PLANT PHYSIOLOGY, ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS ON 8 MAY 1987

This special issue of Acta Botanica Neerlandica comprises the abstracts of oral presentations and posters of the first joint meeting of the Belgian and Dutch societies for Plant Physiology.

Contacts between the Belgian and Dutch plant physiologists, though close neighbours, have been scarce. It was therefore a good Belgian proposition to have a combined meeting of our Societies.

We are happy to welcome our Belgian colleagues and hope the meeting will enable the exchange of ideas, strengthen the existing contacts and help to initiate projects of collaboration.

A.J.E. van Bel
Chairman Section for Plant Physiology
Royal Botanical Society of The Netherlands

The meeting has been organized by A.J.E. van Bel, by whom the abstracts were reviewed with the help of G.W.M. Barendse, A.C. Borstlap, A.M.C. Emons, H.W. Groeneveld, P.R. van Hasselt, H. Konings, F. Lambein, J.C. Lambers, A. Musgrave, H.A. van Onckelen, J.-M. Stassart, I. Stulen, H. Veen, J.-P. Verbelen, D. Vreugdenhil and P. Wolswinkel.

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Poster abstracts are numbered (in alphabetical order) to facilitate identification at the poster session, where posters are grouped according to the subject.

SPORE GERMINATION IN PHYCOMYCES

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Germination of fungal spores is a relatively simple model system of eukaryotic development and differentiation. Indeed, spores are completely different from growing hyphae in composition (cell wall, reserve, ..) and metabolism. This is certainly true for the constitutively dormant sporangiospores of the zygomycete *Phycomyces blakesleeanus*. These spores have a very low metabolic activity in a standard culture medium and will only germinate, with a concomitant dramatic increase in metabolic activity, after a suitable chemical or physical treatment.

A heat treatment of 5 min at 50°C induces irreversibly the capacity to germinate in a medium containing glucose, asparagine and some minerals. Heating for shorter time periods or at lower temperature induces a reversible activation: in this case the capacity to germinate in culture medium is lost by a preincubation in water (Van Laere et al., 1980). Also an incubation for 15 to 60 min in a solution of monocarboxylic acids induces a variable degree of spore germination depending on the acid used, the pH and the counterion in the solution (Van Mulders and Van Laere, 1986). Incubation for 15 min in 0.1 M ammonium acetate e.g. will induce more than 95% of the spores to germinate.

The quantitatively most important early event in germinating spores is a rapid breakdown of the large trehalose reserve (up to 30% of the spore dry weight). This breakdown is mediated by a tenfold increase in trehalase activity immediately after activation (Van Assche et al., 1972). The increase in trehalase activity is probably mediated by a cAMP-dependent phosphorylation since:

a). the same effect is found in the presence of cycloheximide indicating that no neosynthesis is involved (Van Assche, 1973).

b). the activation of trehalase can be duplicated *in vitro* (Van Laere and Hendrix, 1983) by incubation of a crude extract in the presence of cAMP and MgATP;

c). immediately after activation the cellular content of cAMP increases severalfold;

d). the degree of trehalase activation (and spore activation) is proportional to the rise in cAMP content when the spores are activated at different temperatures (Van Mulders and Van Laere, 1984).

The importance of cAMP in spore germination is further corroborated by the fact that:

a). a variety of physical and chemical treatments, known to induce spore germination, all cause a transient increase in cAMP content (Van Mulders and Van Laere, 1984);

b). germination of the spores can be induced by the phosphodiesterase inhibitor 3-isobutyl-1-methyl xanthine (Van Laere, 1986[a]);

c). a mutant that cannot be activated by a heat treatment does not show the increase in cAMP content (Van Laere and Rivero, 1986);

d). short-term transient changes in cAMP content are also found in germinating spores of other zygomycetes such as *Mucor rouxii* (Dewerchin and Van Laere, 1984) or *Pilobolus longipes* (Bourret, 1986) and even in ascospores of *Saccharomyces cerevisiae* (Thevelein, 1984).

The increase in cAMP-content in heat-activated spores is correlated with a decreese in phosphodiesterase activity (Van Laere, 1986[a]). Since no change in phosphodiesterase activity is found after acid induced activation, these treatments perhaps stimulate cAMP synthesis by an acidification of the cytoplasm which might favour adenylate cyclase (with a low pH optimum) over phosphodiesterase (with a high pH optimum).

Whether the breakdown of trehalose to glucose provides the necessary substrate for metabolism and in doing so triggers spore germination is questionable (Van Laere, 1986[b]) since:

a). dormant spores can take up glucose but instead of metabolizing it, they synthesize even more thehalose;

b). activated spores are still dependent on the presence of exogenous glucose for germination;

c). trehalose breakdown is stimulated by its product glucose, a property rather unusual for a reserve substance (Van Mulders and Van Laere, 1984);

d). the breakdown of trehalose is to sudden and disproportionate to be useful as a metabolic substrate. Moreover, from the trehalose broken down about 50% is converted to glycerol (Van Schaftingen and Van Laere, 1985) which rather demands than produces energy for the spores.

The spores have a very efficient system to convert triosephosphates to glycerol using both NADH from glycolysis (glycerol-3-phosphate dehydrogenase) and NADPH from the pentose phosphate pathway (dihydroxyacetone reductase (Van Laere, 1985)) as reducing equivalents. Glycolysis in the spores is stimulated by a transient increase in fructose 2,6-biphosphate concentration (Van Laere et al., 1983). This compound has a dramatic stimulatory effect on phosphofructokinase in micromolar concentrations (Van Laere, 1983). Synthesis of glycerol is probably also controlled by the transient rise in cAMP content. Indeed, when measured under physiological conditions (1 mM phosphate), the activity of glycerol-3-phosphatase increases about tenfold during heat- or acetate-induced germination (Van Schaftingen and Van Laere, 1985). Like for trehalase, this activation can also be duplicated *in vitro* in the presence of cAMP and MgATP. Since the enzyme attacks dihydroxyacetone-phosphate almost equally efficiently it will also generate dihydroxyacetone which can be reduced by NADPH from the pentose phosphate pathway.

The finely tuned conversion of trehalose to glycerol raises the question of the meaning of this bulk change in the spores. A close inspection of the glycerol data reveals that glycerol is initially withheld in the spores and starts to leak out of the spores after some 15 min of germination. Around the same critical point of time the spores suddenly swell as indicated by a doubling of their water content, a decrease in their phase contrast halo and an increase in their cross-sectional area (Van Laere and Hulsmans, 1987). These data suggest that glycerol might have a function in lowering the water potential of the spores thereby stimulating water uptake and swelling of the spores. Glycerol is known to have an important role as a compatible solute in a variety of orgamisms under water stress. Such an osmotic role is also indicated in *Phycomyces* spores since much higher internal glycerol concentrations are reached before glycerol starts to leak out, if the spores are incubated in a medium supplemented with 0.5 or 1 M sucrose (Van Laere and Hulsmans, 1987). Dormant spores have a low water content and the mobility of their cytoplasmic water is very restricted as shown by the spin-spin relaxation times (T2) in proton NMR. Glycerol-induced swelling dramatically increases cytoplasmic water content and mobility and, in doing so, it might cause a general stimulation of metabolism (Van Laere et al., 1987). Especially the hydration and activity of mitochondria and other trehalose-impermeable organelles could be greatly stimulated. Changes in mitochondrial structure during early germination are indeed indicated by electron microscopy (Pambor, 1978).

The role of the large amount of trehalose in the spores is probably to protect the spores from desiccation-induced damage. Indeed, trehalose is known to accumulate in various anhydrobiotic organism ranging from fungi to nematodes and *Artemia* cysts (Crowe et al., 1984[a]). Trehalose is also far superior to other carbohydrates in protecting membranes from dehydration-induced damage (Crowe et al., 1984[b]). There is also a direct correlation between the trehalose content of germinating spores and their survival after drying (Van Laere, 1986[c]). Apparently trehalose is stockpiled in the spores to protect them against desiccation damage in such amounts that it hinders a sufficient hydration of the spores with their rigid wall. The rapid transformation of trehalose to glycerol, finely tuned by a cAMP-dependent phosphorylation cascade, overcomes this inhibition by increasing the osmotic pressure in the spores and hydration of cytoplasm and organelles.

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DETECTION AND TRANSDUCTION OF PLANT HORMONES

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In higher plants long-range cell-to-cell communication is largely dependent upon a complex hormonal system. Over the past circa 15 years attempts have been made to elucidate the mechanism of detection and transduction of the plant hormones. Thus far, high-affinity specific binding proteins have been identified for each class of plant hormone (Venis, 1985; Chadwick and Garrod, 1986), but only for a few of them there is circumstantial evidence that they are genuine receptors (Löbler and Klämbt, 1985[a,b]; Hornberg and Weiler, 1984). Indeed, at present the major problem is to establish a receptor function for these proteins; that is to show that they are detectors and primary transducers in a transduction route. In this connection, an intriguing question is whether plant cells possess a limited number of transduction routes which may be related to those established for animal systems.

With regard to possible trans-membrane transduction routes in plant cells, the following observations may have some relevance:

a). Plant-cell membranes contain specific high-affinity binding proteins for plant hormones. The best known binding proteins are those for auxin in maize coleoptiles and those for abscisic acid in guard cells from broad bean. Recent studies by Klämbt and co-workers (1985[a,b]) and Weiler and co-workers (1984) respectively, show that these binding proteins are probably located at the plasmamembrane with the binding moiety facing the apoplast.

b). Plant hormones have rapid effects on: the transmembrane transport of ions and alterations in $\Delta\Psi$ and ΔpH (Marré and Ciferri, 1977; Marmé et al., 1982) and the phosphorylation of proteins, including (plasma-)membrane proteins (Morré et al., 1984).

c). Plant cells possess components of the adenylate-cyclase system (cAMP, adenylate cyclase, cAMP phosphodiesterase) (Newton and Brown, 1980).

d). There is accumulating evidence showing that in plant cells phosphatidyl-inositol-phosphate turnover may have a regulatory function, including hormone transduction (Heim and Wagner, 1986; Zbell, 1983).

e). Plant cells possess a protein-kinase-C like enzyme (Schäfer et al., 1985).

f). Ca^{2+} and its binding protein calmodulin are important modulators in plant cells.

These and other observations, however, have not yet led to generally accepted models for trans-(plasma)membrane transduction routes of plant hormones.

Since plant hormones, like steroid hormones, are rapidly taken up by plant cells we cannot exclude the possibility that they, besides interacting with (plasma)membrane-bound receptors, may also interact with cytosolic receptors, which as hormone-modulated regulatory proteins are directly involved in the regulation of gene expression. Recent evidence shows that, for example, auxin can have very rapid effects (response times in minutes) on nuclear-gene transcription (Theologis, 1980). Moreover, it has been shown that cytosolic preparations and nuclear extracts can contain high-affinity auxin-binding proteins (Libbenga et al., 1986). In our laboratory, using a well characterized cell suspension system from tobacco, we are trying to link a high-affinity cytosolic/nuclear auxin-binding protein to auxin-induced gene transcription (reponse times in minutes) which we could also establish in these cells (Libbenga et al., 1987).

We intend to evaluate such observations as summarized above as to their relevance for plant-hormone detection and transduction.

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THE NEED FOR WHOLE-PLANT PHYSIOLOGY REVIVAL

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Contemporary biology is dramatically different from its antecedents only ten years ago. Skimming biological sciences from the past to the present it can be noticed that biology has traditionally been a descriptive science. In the second half of the XXth century when the pinnacle of biochemistry was reached and the molecular structure of genetics was unraveled, the main point of actual biology shifted largely to cell biology.

Botany research workers have attempted to uncover the regulatory systems of plant growth and development since before the time of Charles Darwin. In the book 'The power of movement in plants' (1880) one can find detailed descriptions of plant growth patterns. It is nonetheless only with the advent of new technologies in molecular biology and by means of a substantial increase in governmental funding for basic research that investigators have made significant progress in describing (but not explaining) the processes that underlie plant growth regulation. As a consequence classic plant physiology was more and more abandoned in the fifties and the preponderating ideas in plant sciences were focused on the chemistry of life at that time. Nowadays - thirty years later - there is a renewed interest in whole-plant physiology. Indeed, one can find several laboratories pursuing a specialty in this domain of botany. Amongst others dr. Michael L. Evans who obtained a Ph.D. from the University of California at Santa Cruz is developing a laboratory of whole plant physiology. At present he is professor of botany at Ohio State University. In a covering letter of one of his recent papers (1986) he writes: 'As important as the techniques of molecular biology are for the study of plant hormones, it is essential that we not lose sight of the integrated function of the plant as a whole'.

In general terms, we must avoid being unable to see the wood for the trees.

In the laboratory of plant physiology and biochemistry at the University of Antwerp we have been studying growth and development of higher plant systems since the early seventies. This research program is a continuation of the earlier concepts elaborated by scientists of the laboratory of plant physiology of the State University of Ghent studying photomorphogenesis of lower plants throughout the sixties. The cooperative research between both universities is still going on at the time being.

Searching for the effects of a physical cue such as light on the successive phases of the plant's life cycle we were able to corroborate what had already become known from other sources, namely that phytochrome is a photoreceptor for many red light mediated morphogenic and developmental responses in lower and higher plants. This photoreceptor is a blue-green chromoprotein existing in two interconvertible forms: a physiologically inactive red absorbing form (P_r) and a physiologically active far-red absorbing form (P_{fr}).

On the other hand, we have been involved to a large extent in studies of the effects of plant growth regulating substances. In our view the term 'growth' encompasses all activities leading to an irreversible increase in size which includes both cell enlargement prior to cell division and cell vacuolation. The term 'development' (e.g. changes in form which occur as growth proceeds) accents more the processes dealing with differentiation of cells, tissues and organs. It is common knowledge that each stage of the life cycle of higher plants, - together with the shift to the next stage - is regulated (e.g. promoted or inhibited) by endogenous growth regulators. There is also a firm belief that the availability and quantity of these endogenous growth substances are influenced in turn by environmental conditions such as light quality and intensity, day length, humidity, temperature, nutrients, ... Plant physiologists are well aware of the general experience that is often difficult or sometimes impossible to draw a clear distinction between the effects of individual parameters of the external environment on plant growth, nor between the various internal factors, nor again between external and internal factors. In other words: growth, differentiation and morphogenesis are controlled by very fine regulatory mechanisms of sequential adjustments through the interactions of intrinsic factors and extrinsic parameters. In this way, the orderly arranged expression of the intrinsic morphogenetic program in the successive phases of the life cycle of lower and higher plants may be due to a continuous and sensitive adjustment of each growth stage to outside stimuli. This means that the life cycle of plants is regular, though complex.

If the growth pattern of plants is regulated by environmentally controlled changes in growth substance concentrations, then the onset of a distinct developmental stage in the intact plant has to be correlated with a specified amount of growth substance(s). In this respect, there is no conclusive evidence in literature at present.

'There is generally a poor correlation between growth rate and endogenous phytohormone levels in developing roots, stems, leaves, cambial tissues and vegetative food storage organs' (Goodwin et al., 1978).

It is very easy to say that plant life needs a constant re-routing of metabolic activities under the influence of environmental and endogenous cues, but at the very moment it stays without direct prospect of success to come to grips with the problems of what these changes are at the molecular level.

Despite massive research efforts and a large body of literature dealing with the properties and the effects of plant growth regulatory molecules (such as light receptors and hormone-like substances) at

the level of different tissues in the plant's organs throughout its life cycle, there have been no breakthroughs in the quest for understanding the action and/or interaction of these modulator molecules throughout the last thirty years of intensive research in plant molecular biology. Scientists who are familiar with the recent advances in this field of plant physiology, are fully aware of a lot of confusion, puzzling interactions and apparent uncertainties concerning supposedly established facts. Nevertheless, it is useful, indeed essential if any study of the subject is intended, to identify the factors essential to the regulation of plant growth. Let us, however, always bear in mind that the effect(s) caused by one growth limiting factor cannot explain the processes involved in overall plant growth.

In broad outline we can say that each growth activity as a response to an external stimulus can be divided into three phases: perception, transduction and response. Transduction, - the intermediate and most mysterious phase - almost certainly involves some kind of communication between the receptor site and the plant part where the observed physiological response comes to its expression. In some cases, the exact nature of the interaction between the two regions is only now beginning to be deciphered. In other words: for each phenomenal event in plant morphogenesis there is a triangle of certainties: environmental signal, developmental stage, and physiological response. In between environment and plant development the mediating role of sensor molecules and growth regulators is still a matter of debate. At the level of possible interactions there remain many uncertainties which are mainly related with the nature of modulator-receptor interaction, the identification of the primary reactions and the nature and mechanism of stimulus-response coupling.

There are observations that light through daylength or phytochrome may change hormone concentrations or balances between hormones or between different forms of the same hormone, but there are no direct correlations made with responsiveness. When the interactions between light and levels of endogenous hormones are examined, the light action is often expressed by short- and long-term effects suggesting a continuity of light control throughout the completion of the photomorphogenic response. There are observations that hormone levels may vary with the developmental stage of the plant without any obvious change in environmental signal, but it is not clear what kind of specificity can be attributed to the hormonal activity for integrated plant growth. Light can increase the sensitivity of tissues to hormones, and, inversely, hormone application may enhance the sensitivity to light action. P_{fr} and hormones often appear to speed up reaction rates, on the one hand, and to synchronize mutually dependent processes, on the other. Here we are faced with the unsolved problem of the fate of applied hormones and the multiplicity of the effects of hormones and phytochrome. There are indications that phytochrome exerts its effect on membrane and/or membrane-associated structures. These alterations may intrinsically affect the sensitivity of the target tissues to endogenous hormone action. This suits the trend of thought that the spatial and temporal patterns of plant morphogenesis are dependent on changes in tissue sensitivity (Trewavas, 1981), i.e. the ability of a tissue to respond in function of receptor properties (amounts available and binding affinities).

Though remaining largely unexplored, there is circumstantial evidence that both kinds of signal molecules (photoreceptors and hormones) can selectively regulate gene transcription, either in an independent or in a consequential way. In addition, it cannot be ruled out that the membrane bound and gene-regulated expressions of signal molecule action might be interrelated. All such possibilities must be considered as idle speculation at the present since the way in which gene activity is regulated, remains to be established. Furthermore, our information on receptors of plant modulator molecules is still very limited compared with the animal field of hormone research. Inevitably, concepts from animal receptor work have influenced, perhapt constrained and, certainly, at times misdirected plant physiological research.

Taking into account all these data and viewpoints it seems rational to anticipate that successful results in plant molecular research of hormones and photoreceptors will be determined by the exact knowledge of the developmental stage of the plant as a whole.

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HORMONAL REGULATION OF GERMINATION: A MATTER OF SUPPLY AND DEMAND

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Recently, controversy has been growing in the plant growth substances area. Contrary to the classic concept that hormones are the major controlling factors of development, the view has been strongly advocated that regulation occurs through changes in sensitivity to hormones. In that view the presence of growth substances is certainly essential for development to occur, but they are never the limiting factors (Trewavas and Cleland, 1983).

Numerous studies on seeds have shown that exogenous growth regulators, applied singly or in combination, maintain, impose, or release dormancy. Convincing evidence that such effects reflect the action of naturally occurring hormones is hard to produce. Attempts to correlate changes in dormancy with changes in endogenous hormone levels mostly fail to present reliable evidence for a causal relationship. Mutants that are deficient for a certain hormone or a class of hormones offer unique tools to answer two questions: 1). are hormones indeed essential for the development and germination of seeds and 2). if so, does hormonal regulation occur through changes in hormone level or in sensivity to hormones (or neither or both)?

Studies in tomato and *Arabidopsis thaliana* in which wild-type seeds were compared with seeds of isogenic lines deficient for abscisic acid (ABA) and/or gibberellins (GA) proved that the role of each of these hormones is limited to only one, essential step in seed life. ABA only plays a crucial role during seed development. In both species an embryo-produced ABA-fraction is responsible for the induction of dormancy (Karssen et al., 1983; Groot, 1987). All other processes in developing seeds occur similarly in wild-type and ABA- and/or GA-deficient seeds. Mutant seeds contain the same reserve proteins, in similar quantities, and have the same or nearly the same fresh and dry weights as wild-type seeds. They also do not differ in morphological characteristics. The presence of ABA is also not essential to prevent precocious germination in unripe fruits. Most likely, limitation of water supply or osmotic inhibition are the major controlling factors here.

Both in tomato and *Arabidopsis* seed germination is absolutely dependent on GA. In tomato seeds it was shown that the requirement for GA is restricted to the stimulation of the synthesis or activity of some hydrolytic enzymes that, prior to visible germination, weaken the mechanical restraint of the endosperm layers opposing the radicle tip. When these layers are removed after a few hours of inhibition, GA-deficient seeds develop into dwarf plants without the application of any GA (Groot and Karssen, 1987). ABA has no direct function in the mature seeds. The maintenance of dormancy does not depend on the actual presence of ABA, but is the result of ABA action during seed development. In *Arabidopsis* it was clearly shown that such ABA action raised the GA requirement during germination (Karssen and Laçka, 1985).

Since the presence of GA is absolutely required for germination, favourable conditions may stimulate germination by increasing the level of endogenous GA. Germination in *Arabidopsis* is light-requiring, but incubation in GA causes full germination in darkness. Wild-type seeds require in darkness about 10-fold higher GA concentrations than GA-deficient seeds, a clear example of receptor-down-regulation by the high levels of GA during seed development in wild-type. Irradiation with light makes wild-type seeds independent of exogenous GA. GA-deficient seeds still require GA (Karssen et al., 1987). Tetcyclacis, an inhibitor of GA biosynthesis, prevents the light effect in wild-type. It is concluded that light promotes germination of wild-type seeds by stimulation of GA-biosynthesis, but light has also a second effect. In the absence of GA biosynthesis, it is seen that light strongly enhances the sensitivity of the seeds to exogenous GA. Similar dual action of light was observed in seeds of the related species *Sisymbrium officinale* (see Hilhorst and Karssen in this issue, Hilhorst et al., 1986).

GA sensivity of the seeds is also influenced by the temperature of a pre-incubation in darkness. In GA-deficient seeds of *Arabidopsis* pre-incubation at low temperature relieves dormancy and enhances the sensitivity to GA, whereas high temperature has the opposite effect (Karssen et al., 1987). In the field, seasonal patterns of dormancy are most likely regulated by changes in the sensitivity of seeds to promotors. Germination does only occur, when GA is synthesized due to light at the soil surface.

It is concluded that both the regulation of hormone levels ('supply') and of hormone sensivity ('demand') take part in the hormonal control of germination.

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(1) THE EFFECT OF SEEDING WITH ICE ON THE MEASUREMENT OF FROST HARDINESS OF SPINACH
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Frost hardiness of plants is commonly determined by subjecting tissue samples to a freeze-thaw cycle. Around -2°C the tissue samples are often seeded with ice crystals to induce simultaneous freezing. In this manner a high variability due to undercooling is avoided. But a possible protective effect by an increase of the undercooling ability of the tissue cannot be detected. In this study the effects of seeding on frost-induced membrane damage were measured during hardening in spinach leaf discs. Chlorophyll fluorescence induction capacity and ion leakage were used to study frost-induced damage of the chloroplast membrane and plasmalemma.

Without seeding, LT 50 (temperature at which 50% of the cells is dead) measured with the conductivity test as well as with chlorophyll-fluorescence, induction capacity shifted similarly to a lower temperature during hardening. When the samples were seeded at -2°C, LT 50 was hardly influenced by hardening. But during the hardening period the amount of leakage decreased and the capacity of chlorophyll fluorescence induction increased indicating that hardening affected the amount of cells which were damaged by the induced freezing.

Chlorophyll fluorescence measurement during the freeze-thaw cycle showed a fast shift of the freezing point of leaf discs from -3.5°C to -5°C in the first week of hardening. These results will be discussed in relation to the nature of the hardening mechanism of spinach.

(2) A GENETIC APPROACH TO THE STUDY OF PHOTOMORPHOGENESIS IN HIGHER PLANTS
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Study of photomorphogenesis is often complicated by the interaction of different photoreceptors regulating a given process or by the multiple effects induced by a single photoreceptor. Mutants in which components of the morphogenetic 'pathways' are eliminated provide the possibility of studying a more simplified form of photomorphogenesis. After partial characterization we distinguish between the following mutant types:

(a) Photoreceptor mutants: possible examples are hy-1 and hy-2 of *Arabidopsis*; and au and yg-2 of tomato (Koorneef et al., 1980; 1985). Etiolated seedlings of these mutants possess no or hardly any spectrophotometrically detectable phytochrome and in the au mutant immunology supports this conclusion (Parks et al., submitted).

(b) A photoresponse mutant would be expected to have photomorhogenetic processes reduced or absent, while retaining the photoreceptor itself. With respect to phytochrome, hy-3 and hy-5 of *Arabidopsis* (Koornneef et al., 1980) and the lh mutant of cucumber (Adamse et al., 1987) possibly fulfill this criterium. However, spectrophotometric measurements of light-grown tissue (flower petals and Norflurazon-bleached leaves) indicate a reduced phytochrome level in the lh mutant, suggesting it may be a photoreceptor mutant after all.

(c) While photoreceptor and photoresponse mutants are photo-insensitive, the third group of mutants is hypersensitive. A high pigment tomato mutant (hp) (Mochizuki and Kamimura, 1984) is a possible candidate for this category. The hp mutant is so sensitive to red light that phytochrome control of anthocyanin induction can be demonstrated without a blue light pretreatment, required in the case of wild type.

These mutants are useful to study: (i) the relative roles played by individual photoreceptors where interaction occurs; (ii) analysis of genetic defects will indicate essential parts of the photoreceptor molecule (e.g. phytochrome-deficient mutants can be used to study the effect of (*in vitro*-modified) phytochrome genes without interference from endogenous phytochrome levels after the genes are transferred into such a mutant).

(3) PLASMA MEMBRANE FUNCTIONS IN HIGHER PLANTS: ATPase ACTIVITY AND PROTON TRANSPORT

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Several physiological processes are dependent on the ability of the plasma membrane to maintain proton and charge gradient (e.g. IAA transport, blue light perception). A cation-stimulated proton transport-ATPase dependent on Mg^{2+}/ATP appears to be an essential element in formation of these gradients. This enzyme was characterized in a plasma membrane fraction obtained through partitioning in an aqueous two phase system. (Larsson, 1985; Caubergs et al., 1986).

Hydrolysis of Mg^{2+}/ATP showed a typical pH optimum at pH 7 and revealed a Michaelis-Menten relationship (Km = 1.37 mM). Specific inhibition by vanadate (Ki near 20 µM) further pointed to an ATPase incorporated in the plasma membrane and described earlier (Sze, 1985). In apparent contradiction with other reports (e.g. Dupont and Leonard, 1980), however, the enzyme was only slightly stimulated by monovalent cations. It has further been shown that this ATPase and a blue light receptor were associated with the same membrane fraction which also displayed NADH-dependent redox activity.

The ability of ATPases to induce transport of protons was studied by means of a spectrophotometric method to follow internal acidification in membrane vesicles (Hager and Helmle, 1981). A crude microsomal fraction of cauliflower and corn coleoptiles showed ATP-dependent proton uptake reversible by either CCCP or gramicidin. This activity, however, was presumably of tonoplast origin as demonstrated by its nitrate sensitivity. Purified plasma membranes, obtained by polymer partitioning, were less effective in proton accumulation possibly due to the orientation of the ATPase (right side-out vesicles).

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H. Asard is a NFWO research assistant.

(4) OSMOACCLIMATION IN THE HALOTOLERANT ALGAL GENUS DUNALIELLA

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The naked, unicellular algae of the genus *Dunaliella* are model systems to investigate the biochemical mechanisms of cellular adaptation to osmotic stress. These green flagellates can grow in media with salinities ranging from near zero to saturated salt solutions.

Osmoacclimation in these microalgae is mainly accomplished by adjustment of the intracellular water potential, by increasing the glycerol content, to the external water potential. The cells responded to a hyperosmotic shock by accumulating large amounts of glycerol at a rate of some 50 nmoles/min. mg protein during the first hour after the osmotic upshock. The increased rate of glycerol synthesis was independent of protein synthesis and occurred both in light and darkness. Measurement of the activity of enzymes involved in glycerol metabolism pointed to a glycerol cycle with a pivotal role for dihydroxyacetonephosphate, originating from photosynthesis or starch breakdown. A dramatic transient increase of the glycerol 3-phosphate level was found immediately after lowering the water potential of the medium. This suggested an increased *in vivo* activity of glycerol-3-P dehydrogenase.

During the period of glycerol production, starch was degraded to glucose at a rate of approximately 15 nmoles/min. mg protein. Both a soluble and a starch-bound α-amylase were detected in cell-free extracts. An α-glucanphosphorylase was also found, but it had a higher affinity for pyrophosphate (K_m = 0.365mM) than for phosphate (K_m = 1.9mM).

In vitro activities of the enzymes involved in the starch-glycerol interconversion remained the same after subjecting the cells to osmotic shock. Regulation of the altered metabolism during osmotic stress is most probably due to changes in the concentration of substrates and effectors of enzymes involved in the metabolism of glycerol and starch. The transient decrease in ATP content shortly after hyperosmotic shock, could well affect the activity of the glycerol-3-P dehydrogenase, since this enzyme is strongly inhibited by an ATP concentration of 1mM.

(5) THE FER GENE FOR FE-EFFICIENCY IN TOMATO

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Fe deficiency induces, in the roots of dicotyledons and non-grass monocotyledons, a number of morphological and biochemical changes: high root hair density, formation of rhizodermal transfer cells, strong proton extrusion and a high ferric chelate reduction capacity ('Turbo' reductase, Bienfait, 1985) in the epidermal plasma membranes. The roots can produce these responses autonomously, i.e. no signal from the leaves is necessary (Bienfait et al., 1987).

The tomato mutant T 3820 fer cannot develop all these Fe efficiency reactions (Brown et al., 1971). T. 3820 fer plants are fully capable to form transfer cells at other places in the plant, and it synthesizes the 'standard' electron transfer system in the plasma membrane, both of which are independent of the Fe-status.

The mutated gene obeys the Mendelian laws. The FER gene apparently determines the sensitivity of the root to lowered Fe levels and the concomitant development of a number of responses. Our present aim is to identify the FER gene product.

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(6) EFFICIENCY OF ROOT RESPIRATION OF PLANTAGO, GROWN WITH NH_4^+ OR NO_3^-

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Plantago lanceolata plants were grown in culture solutions with nitrate or ammonium as the N-source. The relative growth rate of the plants declined with age. With ammonium the shoot to root ratio was lower than with nitrate. The organic N concentration was higher with ammonium, especially in the roots.

Root respiration rate declined with age, predominantly due to a decrease in alternative path activity. The alternative path activity amounted to about 30 - 60% of total root respiration. Respiration via the cytochrome path was slightly increased by ammonium, whereas the activity of the alternative path was strongly enhanced. The concentration of soluble sugars in the roots was higher with nitrate, in contradiction with the overflow hypothesis for the alternative path.

The ATP yield of root respiration was calculated, using the P/O ratio calculated from the relative contributions of the cytochrome and alternative pathways. From the linear regression of RGR versus the ATP yield, the ATP consumption for growth (slope) and maintenance (intercept) were estimated. Growth respiration was higher with ammonium than with nitrate. The figures are compared with calculations based on the current knowledge of the energy costs of biochemical processes, and the actual chemical composition of the plants.

(7) THE UPTAKE OF NO_3^- BY LETTUCE PLANTS UNDER VARYING ENVIRONMENTAL FACTORS

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Uptake of NO_3^- by lettuce plants (*Lactuca sativa* L.) grown in 1 liter containers was studied in a computer-controlled system in which ion activity (H^+ and NO_3^-) in the medium can be measured and controlled.

The nitrate uptake of plants in a steady state, monitored during a 24-hours experiment, did not show a diurnal rhythm, which suggests that light does not play a direct role in nitrate uptake. Apparently, the energy supply for nitrate uptake is not limiting during the night. On a long-term basis nitrate uptake increases with increasing growth rate. The uptake rate shows a linear correlation with fresh weight production.

In the non-steady-state period after a reduction of the light intensity the correlation between nitrate uptake and growth did not hold. Fresh weight production declined with decreasing light intensity. Nitrate uptake decreased in proportion even more than the growth rate in spite of an increased want of nitrate as an osmotic with decreasing light intensity. Apparently, nitrate uptake has a lower priority than growth during a period of energy deficiency.

In another experiment the relative humidity was varied, while light intensity was kept constant. The uptake rate as a function of fresh weight production decreased with increasing relative humidity. As light intensity was low, the relative humidity may not have affected photosynthesis, suggesting that the decrease in nitrate uptake in this experiment was not caused by a lack of energy. Here, the release of nitrate into the xylem might have been inhibited by an increasing NO_3^- concentration in the xylem thus retarding the flux through the symplast, which might inhibit uptake.

(8) PLANT PHYSIOLOGY IN RELATION TO SOIL POLLUTION WITH VOLATILE ORGANIC XENOBIOTICS: PHYTOTOXICITY, CONTAMINATION AND BIOTRANSFORMATION

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By unjudicious use and as a consequence of accidents, soils get polluted by industrial chemicals such as volatile organic solvents. There is very little known about reactions of higher plants to soil contamination with organic xenobiotics. It is not clear, for instance, whether there are differences in toxic reactions between species, whether accumulation in specific plant tissues occurs and what plant tissues are able to transform absorbed organic xenobiotics.

It is plausible to assume that, as a result of chemo-physical processes, organic soil contaminations penetrate plants, whereafter transport and/or transformation takes place. Penetration can occur underground, but after volatilation or dispersion also overground.

After entrance, there are two possibilities:

- the compound remains unchanged: transport (including exitation) and accumulation in plant tissues will depend on the specific chemo-physical properties of the compound and the plant tissues, a.o. the lipid content;
- alternatively, the compound is transformed (for example degraded or metabolized by cytochrome P-450 and detoxified by conjugations with amino acids, sugars or glutathion). Due to metabolic conversions originally lipophilic compounds become - in general - more hydrophilic. On grounds of their chemo-physical properties it will be more difficult for these compounds to exit the plants, but transport under metabolic control - for example to storage organs - will be easier.

This research project mainly dealt with the effects of the chlorinated aliphates TCE (trichloroethylene) and PCE (per- or tetrachloroethylene) on lettuce growth. The concentrations of TCE and PCE in the soil were comparable to those found in polluted 'field' soils: 0,1 up to 9 mg/kg TCE or 0,1 up to 16 mg/kg PCE in preliminary experiments and over 20 mg/kg TCE or PCE in a more elaborated experiment.

It has become clear that both TCE and PCE can decrease yield by more than 40%.

Chemical analyses demonstrated that contamination of lettuce with these volatile organic solvents does occur. The preliminary experiments showed that the roots were contaminated with a concentration of 10% of that in the soil at harvest time. For whole crops, the percentages of contamination ranged between 0,1 and 1,5%, on the basis of fresh weight. In the more elaborated experiment the inner stem of the lettuce contained > 5000 mg/kg PCE, which might suggest that PCE can be concentrated in the vascular system. This would coincide with the considerable variations in the results of the 'whole crop' analyses in the preliminary experiments.

Transformation of TCE and PCE in plants remains to be investigated. Similar experiments will be done with other crops which have different harvest parts such as roots, tubers, seeds and fruits.

(9) CHARACTERISATION OF PLANT MATERIAL AND PLANT CELL WALL POLYMERS BY ON-LINE FLASH PYROLYSIS CAPILLARY GAS CHROMATOGRAPHY MASS SPECTROMETRY

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The bio- and geocycles of plant material and plant-derived organic matter fractions dominate the earth ecosystem and play a central role in agriculture, energy and food technology. Plant fibers are important for the paper and textile industry, food technology, timber industry and energy. Decomposed plant material contributes to sewage, soils and sediments and plays a role in the ecology of rivers, lakes, estuaries and oceans. Many problems remain, with respect to the analysis of plant-derived polymers and of partially decomposed plant-derived fractions in the environment.

For several years, we have explored the potential of mass spectrometry in studies of biogeochemical cycles of plant materials and also in studies of agricultural importance. The analysis of plant-tissue architecture on the molecular level and the evaluation of the state of development, senescence and decomposition of plant materials is the focus of our interest. We have choosen for an approach in which, in principle, all the chemical building blocks of the plant material contribute to the analytical signal (profiling). This is contrary to most strategies which concentrate on certain compounds or polymers in a macromolecular system (target analysis).

The analysis of solid organic material is performed in our instruments with microscale flash pyrolysis using our own FOM-3LX Curie point pyrolysis unit coupled on-line to a capillary gas-chromatograph double-focussing mass spectrometer (pygcms mode) or coupled directly to the ion source of a quadrupole mass spectrometer in the FOMautopyms (pyms mode). In a typical experiment, 10-50 microgram of plant matter sample placed on ferromagnetic wires will be heated by induction (T-rise time: 5000 K/s), the internal energy of the polymer systems is instantly increased which leads to the generation of depolymerisation products which reflect the original plant polymer composition. These dissociation products are identified my mass spectrometry and quantified.

In this way, we are exploring the depolymerisation products of plant polymers such as cellulose, the various polysaccharides in the hemicellulose group, the pectins, the polyphenolic ethers such as lignins and the polyesters such as the cutins and the suberins. The contributions of fragments of each of these polymers and their crosslinks are studied for example in woody tissue, leaf and stem material, and in endocarps. The poster will demonstrate the method of analysis and will show data from analyses on beech wood, tobacco leaves, rootlets from *Ericoceae*, corn stalk and barley straw.

(10) PHOTOMORPHOGENETIC RESPONSES OF ETIOLATED WHEAT PROTOPLASTS

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To study changes in membrane properties due to phytochrome, we have chosen mesophyll protoplasts isolated from the primary leaves of dark-grown wheat, as a model system. The influence of red (R) and far-red (FR) irradiation on the swelling of protoplasts was investigated by measuring their diameter. As a measure of the surface membrane potential, binding of the fluorescent probe 9-aminoacridine was investigated. Protoplasts were isolated under a green safe light and incubated at 22°C in a medium consisting of 0.5 M sorbitol, 5 mM MES/TRIS pH 6.0, 1 mM $CaCl_2$ and 1 mM KCl.

For protoplast population studies, samples were photographed, the diameter of a hundred protoplasts measured and the mean volume calculated. Protoplasts swelled 10-20% after 1 min R and this effect was negated by 5 min subsequent FR. The swelling response only occurred, when Ca^{2+} was present in the medium. K^+ did not enhance the response and Mg^{2+} could not replace Ca^{2+}. To investigate whether this swelling was the result of an increase in non-osmotic volume or an increase in osmotically active substances, protoplasts were transferred, directly after irradiation, to media with different osmotic values. Surprisingly, protoplasts maintained in darkness did not behave according the Boyle-Van't Hoff relation, although they did so after R. In darkness, protoplasts had an apparent negative non-osmotic volume. These results confirm and extend those of Blakeley et al. (1983).

For individual protoplast studies, protoplasts were held on a micro-suction pipette or embedded in agarose and volume changes were followed with time. Swelling after R showed a short lag (less than 1 min) and was completed within 10 min. The time-course of protoplast-swelling, upon changing the bathing solution from 0.5 M sorbitol to 0.4 M, was the same after R and FR. However, the end-volume was larger after R.

9-aminoacridine (9-AA) is a fluorescent probe which binds to membranes in an amount dependent on their net negative surface charge (Chow and Barber, 1980; Möller et al., 1984). Initial experiments indicate that this probe can be used to investigate possible changes in surface charge after R of FR irradiation.

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(11) SOME ASPECTS OF ALTERED STRUCTURE AND FUNCTIONING OF THE PHOTOSYNTHETIC APPARATUS IN PHYTOCHROME-LESS MUTANTS OF TOMATO

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The amplitude and kinetics of distinguishable components of the flash-induced P515 absorbance change very strongly, dependent upon the structure and functioning of the photosynthetic membrane. The availability of plant mutants with altered photomorphogenetic systems, serves as a means to study some aspects of energy conversion and coupling in relation to composition and functioning of the thylakoid membrane. In the present study we have used a phytochrome-less yellow-green mutant of tomato, called yg-2. The photosynthetic apparatus of wildtype and mutant leaves has been characterized with respect to pigment composition, photosynthetic rate, chlorophyll a-fluorescence induction and P515 reaction kinetics.

The mutant has a yellow-green colour due to a decreased chlorophyll and carotenoid content, together with a two-fold increase in the chlorophyll a/b ratio compared with the wildtype. The high chlorophyll a/b ratio indicates a decreased LHCP content. The photosynthetic activities (O_2-evolution and CO_2-fixation rate) of mutant and wildtype leaves were comparable at saturating light intensities (Buurmeijer et al., 1987).

Low temperature (77 K) chlorophyll a-fluorescence induction studies indicate a reduction of approximately 30% in energy transfer from PS-II to PS-I ('spill-over') as well as energy transfer between PS-II units ('grouping') in the mutant. The photochemical and energy-dependent fluorescence quenching during fluorescence induction of mutant and wildtype tomato leaves has been studied with a light-modulated fluorometer. The q_Q was higher for the mutant while there seemed to be no major difference in the q_E. The higher q_Q of the mutant indicates a more oxidized PQ-pool. This indicates an imbalanced turnover rate of PS-II and PS-I, with a slower turnover rate of PS-II due to its smaller chlorophyll antenna size.

The flash-induced P515 absorbance changes in the mutant and wildtype were comparable, when the plants were adapted to the dark, but were different after pre-illumination of the whole plants. The decay time of the signal after pre-illumination was much faster for the mutant, which indicates a higher passive membrane permeability of the energized thylakoid membrane (Buurmeijer et al., 1987).

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(12) EFFECTS OF HYDROGEN SULFIDE ON COMPOSITION OF THE FREE AMINO ACID POOL IN SPINACH LEAVES

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Exposure of spinach leaves to 0.25 $\mu l.l^{-1}$ H_2S in the atmosphere causes a reduction in growth rate. Early effects of H_2S fumigation include a rapid accumulation of the tripeptide glutathione (glu-cys-gly), which probably serves as a temporary storage compound of excess reduced sulfur in spinach shoots. Since glutathione synthesis draws on the amino acid pool, changes in amino acid metabolism can be expected. A method for separating dansyl derivatives of amino acids by reverse phase HPLC was developed. Low pressure gradient formation was controlled by an inexpensive home computer.

HPLC-analysis of the composition of the total free acid pool in fumigated and non-fumigated leaves showed that glutathione accumulation is accompanied by considerable changes in concentration of some amino acids.

These changes may reflect a disruption of nitrogen metabolism which could be the cause of the observed growth reduction.

(13) PLASMA MEMBRANE FUNCTIONS IN HIGHER PLANTS: BLUE LIGHT PERCEPTION AND REDOX FUNCTIONS

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Many physiological processes in higher and lower plants are potentiated by blue light. On the basis of the similarity in action spectra, these reactions are called cryptochrome responses (Senger, 1984). It is, however, not clear as yet whether the photoreceptor is identical for all blue light phenomena. Flavoproteins and carotenoproteins are the most likely candidates as blue light receptors. The finding that blue light is able to reduce a b-type cytochrome (LIAC) in mycelium of *Neurospora crassa* led to the conclusion that flavins are involved in the blue light perception (Munoz and Butler, 1975). A similar light-triggered redox system was discovered in higher plants (Goldsmith et al., 1980). Our localisation studies reveal the presence of a high potential b-type cytochrome and a flavoprotein in the plasma membrane of cauliflower inflorescences. The results of the characterization of the flavin cytochrome b-complex will be presented. The idea was prompted that the physiological action of blue light was expressed through a change in plasma membrane properties after light activation of the protein complex perhaps by modulating the existing proton gradient (Zeiger, 1984). The discovery of NADH-oxidase activity on the plasma membrane of cauliflower inflorescences raised the interesting question concerning a functional relationship between the mentioned redox systems (Asard et al., unpublished results). The subcellular distribution of this enzyme and some of its properties will be discussed.

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(14) IMPROVED EXTRACTION METHOD FOR 2D-PAGE ANALYSIS OF THE PROTEINS FROM FLORAL PARTS IN SINAPIS ALBA L.

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A method has been developed previously for the extraction of proteins from green plant tissues (Cremer and Van de Walle, 1985). In this method proteins were extracted from the apical bud of *Sinapis alba* at different moments of the floral transition and analysed by 2D-PAGE (Cremer et al., 1986). When the same procedure was applied in the study of proteins from floral parts or flower buds of the same species, the results were unsatisfactory. Critical examination of the patterns suggested that the poor reproducibility was most probably due to proteolytic degradation. Similar difficulties have been described recently by Colas des Francs et al. (1985).

The extraction procedure was therefore modified as follows. The plant material was ground in liquid nitrogen and transferred to 5 ml of extraction buffer (4% SDS, 5% saccharose, 5% β-mercaptoethanol, 0.25 M sodium ascorbate, 50 mM Tris-HCl pH 7.4 at 4°C) containing insoluble polyvinylpyrrolidone. After stirring for 1 min., the extract was heated for 3 min. in boiling water and centrifuged at 35.000 g for 30 min. at 4°C. The protein was precipitated from the supernatant with 4 volumes of cold acetone containing 10 mM β-mercaptoethanol for 1 h at -20°C. After centrifugation, the pellet was resuspended in lysis buffer (O'Farrel, 1975) and dialysed against 200 volumes of 0.5% SDS, 5% β-mercaptoethanol, 9.5 M urea. The protein content was determined by the modified Lowry assay (Peterson, 1977) and an aliquot of the sample containing 200 µg of protein was subjected to 2D-PAGE.

This method was used successfully to analyse floral parts from 4-mm flower buds and from flowers collected at anthesis. The results obtained from the stamen showed the best improvement. When stamen proteins were prepared with the previous method only a few dozen of spots were detected by electrophoresis. By use of the modified method we have been able to detect several hundreds of spots. The patterns for stamens collected at anthesis and for 4-mm buds will be presented and compared.

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(15) EXTRACTION AND ISOLATION OF HETEROCYST-TYPE GLYCOLIPIDS FROM CYANOBACTERIA

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The refractile, multilayered envelope surrounding the heterocyst cell wall of certain cyanobacteria provides a rich source of the long-chain (C_{26} - C_{28}) glycolipids (Lambein and Wolk, 1973) thought to be important for maintenance of the low internal O_2-tensions necessary for N_2-fixation. To examine such an idea, a protocol for the quantitative extraction and isolation of these heterocyst-type glycolipids (HG) has been developed.

Lipids from lyophilised cells of *Anabaena sphaerica* and *Nostoc linkia* were extracted, free of non-lipid contaminants with hot dilute aqueous ethanoic acid (Phillips and Privett, 1979). The extracts were then purified by a novel partitioning technique, where the lipids, dissolved in a small volume of CCL_4:MeOH (2:1,v/v), are thoroughly shaken with 2 volumes of 0.05% aq. $CaCl_2$. Following centrifugation the HGs are obtained at the interface with other glycosylated compounds in pellet form. Recovery appears quantitative after 4 such partitions, as judged by anthrone and dry-weight determinations. HGs are subsequently obtained as a group by preparative TLC, with $CHCl_3$:CH_3COOH:MeOH:H_2O (85:15:10:3.7, v/v) as the developing solvent. Separation of the individual components is achieved by silicic acid HPLC, on a linear gradient of 8 - 10% of a MeOH:CH_3COOH (1:1,v/v) mixture, containing 0.2 M H_3BO_3, in CH_2Cl_2 (1% 2-propanol), aliquots of the effluent being monitored by TLC, with H_2SO_4 charring.

For quantitative work the HGs are perbenzoylated to allow UV detection. Separations according to the number of -OH groups and differences in the lipid moieties are accomplished by silicic acid and C_{18}-reverse phase HPLC respectively. Detection is at 230 nm with a linear gradient of 0.5 - 5.0% 2-propanol in n-hexane for the silicic acid, while from the C_{18} column, derivatives are eluted isocratically in 95% aq. MeOH with detection at 220 nm.

* M.D. is recipient of a NATO fellowship.

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(16) METHOD FOR THE MEASUREMENT OF MEMBRANE PERMEABILITY FOR ^3H_2O IN PLANT TISSUES
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The permeability for ^3H_2O was measured of tissues saturated with tritiated water. Its efflux is followed as a function of time. From the exponential curve obtained the half-time was calculated (De Clerck, 1986). Ten 5-mm-segments were cut from etiolated mung bean hypocotyls, rinsed in water for 3 hours and then saturated with 3 µCi/ml ^3H_2O. We supposed that the in- and efflux of water mainly occurs through the cut surfaces, as the cuticula prohibits the water diffusion. After saturating the tissues, these were rinsed in cold medium for a few seconds to remove adsorbed ^3H_2O.

The efflux of ^3H_2O was measured by a flow-through cell, made of a 2-ml-disposible injection syringe, that was placed upside down with the needle bent downwards. The segments were placed in the syringe, of which the plunger was modified and connected to a reservoir containing non-radioactive medium and that was placed above the flow-through cell. This medium flew through the cell by gravity at a fixed flow-rate of 2 ml/min. The ^3H_2O in the segments was exchanged with the non-radioactive medium. During 30 min samples of 1 ml were collected every 30 s and the ^3H counted in a liquid scintillation spectrometer.

The radioactivity of the fractions plotted against time shows an exponential curve, a result to be expected from a passive diffusion of water. An exponential curve fit demonstrated that mainly the first 10 measurements did not fit very well with an exponential curve. This is also demonstrated by plotting the logarithm of the measured radioactivity against time. The first 10 points did not fit very well with a straight line. From the next points a straight line could be drawn. The first 10 measurements most probably demonstrated the diffusion from the 'apparent free space'. Thereafter the diffusion through membranes was measured. In experiments on diffusion of KCl Lüttge (1973) even considered 3 phases. He attributed this result to the efflux from the three compartments: 'apparent free space', cytoplasm, and vacuole.

As we are mostly interested in the efflux through the membranes, we eliminated the first 10 points and calculated the half-time from the remaining points. This half-time is a measure for the water permeability of the tissue. The coefficient of correlation between our measuring points and the theoretical exponential curve is better than 0.99. A computer program has been developed for calculating the half-times and the best fitting curves.

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(17) ATP PRODUCTION AND ENERGY CHARGE OF KALANCHOE SEEDS DURING IMBIBITION
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The pool of adenylates in dry *Kalanchoë blossfeldiana* seeds is composed of 70% AMP, 23% ADP and 7% ATP. The increase in ATP content after the start of imbibition showed a lag phase of about 40 minutes (phase 1). During this time ADP rose rather slowly parallel with ATP, but AMP-content increased very sharply and subsequently dropped to about its initial value at the moment that the ATP content began to rise exponentially (phase 2). During phase 2 the increase in ADP content slowed down and ADP reached a plateau after one hour. From that moment on, AMP stayed almost constant, somewhat below the ADP level while the ATP content was steadily increasing during the following five hours (phase 3). As the sum of adenine nucleotides increased 3 to 4 fold during the first hour after the onset of imbibition, *de novo* synthesis evidently occurred during this period. The energy charge (E.C.) remained constant at the low value of 0.2 in dry seeds during about 20 minutes after the start of imbibition but increased to 0.65 within one hour, indicating an important rise of metabolic activity.

ATP production during imbibition was strongly O_2-dependent: under N_2 the ATP content amounted 30% of that in air. O_2-uptake by Kalanchoë seeds was inhibited for 80% by 0.5 mM KCN after 3 hours of imbibition, indicating electron transfer coupled with cytochrome oxidase. On the ATP level, presence of KCN resulted in 50% inhibition of the ATP content during the first 3 hours. Thereafter, the inhibiting effect was less pronounced.

Involvement of the cyanide-sensitive pathway in the electron transport chain to O_2 was indicated by inhibition of the O_2-uptake by 1mM salicylhydroxamic acid (SHAM) after 3 hours of imbibition. The more pronounced effect of SHAM after 4 hours of imbibition seems to correspond with the decrease of the effect of KCN on the ATP content.

Our findings indicate that in Kalanchoë seeds, in addition to oxidative phosphorylation, fermentation activity and also alternative respiration are involved in the ATP synthesis during early imbibition.

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(18) SENSITIZATION OF KALANCHOE SEEDS BY GIBBERELLINS: STUDY OF THE LOW FLUENCE RESPONSE

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Fluence-response curves are presented for the effect of 3 R pulses, on days 1, 2 and 3 after sowing, both in presence and absence of GA_3. They demonstrate the identity of the LFR component in the presence of GA_3 with the germination response in the absence of GA_3. The biphasic fluence-response curves for phytochrome-mediated responses, described so far in the literature, including our own results with two R irradiations on days 7 and 8 after sowing, always display a LFR at ± 10,000 fold higher fluences than the VLFR.(Rethy et al., 1987) With 3 daily R irradiations given from the first day on after sowing, the difference in light-sensitivity between VLFR and LFR is clearly less than 4 decades. This is not due to the number of irradiations, as shown by fluence-response curves for the effect of 3 R pulses on days 7, 8 and 9 after sowing, but to the length of the dark incubation period before irradiation. Only the VLFR shifts to higher fluences, when the irradiations are given from the first day on.

No clear-cut LFR occurs with only one R irradiation in the presence of GA_3. (De Petter et al., 1985) An intervening dark period of 18 to 20 h between two equal R pulses of LFR-saturating total fluence is required for full expression of the LFR. A VLFR saturating R of FR pulse, given as first irradiation on day 7 after sowing, in combination with a LFR-saturating R pulse on day 8, however, also induce a LFR, indicating that not more than 2 to 3% P is required after the second irradiation and only for the first 7 to 8 hours. Indeed, reversion of the LFR component by a high FR fluence, given at different moments after the R irradiation on day 8, already disappears, when the dark interval between the R and FR irradiation exceeds 8 to 9 hours.

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(19) CONTINUOUS CULTURE OF GLUCOSE-LIMITED PETUNIA HYBRIDA CELLS IN AN AIR-LIFT FERMENTOR

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Chemostats have already proven their value in studying the cell metabolism. In a chemostat fresh nutrient solution, of which one component is present in a growth-limiting concentration, is added to a cell suspension, while an equal amount of the culture is removed. In such a continuous culture system steady states can be obtained in which the cells are kept in a constant and controllable metabolic state.

In contrast to bacterial cells many plant cell lines cannot be cultured in stirred-tank fermentors, because stirring causes high shear forces that can damage the cells. Therefore, plant cells often are cultured in so called air-lift fermentors. We used a Kurz air-lift fermentor in studying the relation between growth and (ATP-yielding) respiration in continuously cultured *Petunia hybrida* cells. These studies were seriously hampered by the fact that the cells invariably started to aggregate. Because of this aggregation the distribution of the biomass in the fermentor was no longer uniform, resulting in low overflow efficiencies. Furthermore, the cell aggregates regularly blocked the overflow-tube, which lowered the overflow efficiencies even more and disturbed steady states.

These problems prompted us to make some modifications in the fermentor design:

- The overflow-tube was broadened and the position changed.

Blocking of the overflow-tube by aggregates no longer occurred and the overflow efficiencies were improved.

- A glass tube was introduced in the centre of the fermentor. Aeration took place through this tube.

This modification allowed for a circulation of the biomass through the fermentor, thus improving the distribution of the biomass. Under certain conditions of aeration, the distribution of small aggregates (mean diameter 100 µm) in the original fermentor was not uniform, whereas under identical conditions of aeration the distribution of aggregates up to 250 µm was uniform in the modified fermentor.

Growth rate (0,011 per h) and growth yield (circa 0,5 g DW per g glucose) of *Petunia* cells grown in batch at 25° C in both types of fermentor were comparable, indicating that the circulation of the biomass in the fermentor did not give rise to serious cell damage.

Preliminary results indicate that continuous culture of *Petunia* cells in the modified fermentor is also possible. Steady states can be obtained at various dilution rates (0,003-0,007 per h) with glucose (less than 10 g per l) as the growth-limiting nutrient. Under these circumstances the growth yield (0,3-0,5 g DW per g glucose) is essentially the same as in the batch cultures.

Further data on the relationship between growth(yield) and respiration will be discussed.

(20) CHANGES IN BIOCHEMISTRY AND GENE EXPRESSION IN SENESCING SHOOTS OF ARABIDOPSIS THALIANA L.

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The levels of chlorophyll, carotenoids, soluble proteins, free amino acids and water-soluble non-protein SH-compounds in senescing shoots of *Arabidopsis thaliana* L. were examined. Likewise, gene expression in senescing shoots/rosettes was determined by *in vitro* translation analysis of isolated mRNA. The changes which occurred during dark-induced senescence of detached and attached shoots and during natural senescence of the rosette were compared. For all treatments senescence was characterized by a decrease in the content of chlorophyll, carotenoids and soluble proteins at the following rate: carotenoids $<$ soluble proteins $\leq$ chlorophyll. Dark-induced senescence occurred somewhat faster in attached shoots than in detached shoots. Senescence of detached shoots was characterized by a substantial increase of free amino acids. Such an increase was absent during dark-induced senescence of attached shoots and during natural senescence. The latter may indicate a redistribution of amino acids in the intact plant. The content of water-soluble non-protein SH-compounds (for the greater part glutathione) increased initially during senescence and later decreased at all treatments. This may indicate that in the intact plant sulfur-containing amino acid are slower remobilized during senescence than the other amino acids. Separation of ^{35}S-methionine-labelled *in vitro* translation products of isolated poly A^+ mRNA by polyacrylamide gel electrophoresis demonstrated that during senescence a number of changes in gene expression occurred. At least three mRNAs were synthetized *de novo*, while several others increased or decreased in quantity.

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(21) INFLUENCE OF THE MINERAL NUTRITION ON YIELD AND ALKALOID CONTENT IN DATURA STRAMONIUM L.

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Recently it has become clear that mineral nutrition not only has an important influence on crop yield, but may also have a considerable effect on the production of secondary metabolites in plants as well. For this reason we studied the influence of the mineral composition of the medium on the hyoscyamine and scopolamine production in *Datura stramonium* L. var. tatula.

In general, a higher crop yield (increase in dry matter) as well as an increase in hyoscyamine content was obtained when the interionic balance was shifted to NO_3^-. Until 16 weeks after sowing, the highest values (crop yield and hyoscyamine content) were obtained with the $NO_3^-Ca^{++}$ treatment (3.7 ± 0.6 mg hyoscyamine/ g. dr.wt. leaves), but at the end of the experiment (24 weeks after sowing), the highest hyoscyamine content was reached with the NO_3^--K^+ treatment (5.9 ± 0.3 mg/g. dr.wt. leaves). The latter treatment, however, caused a delay of 4 weeks in the development of the plants with regard to the other treatments. This also affected the scopolamine content, as the production of this alkaloid seems to be strongly related with the developmental stage. We assume that in this context flower formation plays an important role. It has probably a negative effect on the activity of the enzymes responsible for the epoxydation of hyoscyamine to scopolamine.

Within the tested range (pH=5-7) no influence of the pH (anion/ cation ratio) on the crop yield could be demonstrated. However, a significant lower hyoscyamine synthesis was observed with the lowest pH tested (pH=5: 2.6 ± 0.05; pH=6: 3.6 ± 0.1 and pH=7: 4.0 ± 0.2 mg hyoscyamine/g. dr.wt. leaves). This is in agreement with the observations that the 'pH tolerance' of *D. stramonium* is between 6.00 and 8.20.

As could be expected a higher mineral dose clearly led to a higher crop yield and to an increased hyoscyamine production. With the highest dose tested this increased production was only observed at the end of the experiment (20 weeks after sowing). Apparently, in younger plants, the precursors which serve at the same time for the synthesis of primary and of secondary metabolites, were used with the highest dose to a greater extent for the primary metabolism than with the two other treatments.

(22) THE ROLE OF ETHYLENE IN THE FLOWERING RESPONSE OF DUTCH IRISES

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Ethylene induces flower formation and stimulates flower-bud development of Dutch iris bulbs, when the apex is in the vegetative state. Due to the ethylene treatment the plants flower earlier, and the leaf and internode number is reduced.

Maximum responses have been found for cv. 'Ideal' after exposure to 5 ppm ethylene for 8 hours. Lower concentrations, shorter exposure periods and depending on seasonal conditions low temperatures during the gas treatment gave intermediate responses.

However, the flower-inducing effect is unwanted for the production of plant material. Only vegetative plants produce highly qualified round saleable bulbs. Problems presumably caused by endogenously formed ethylene arise, when the material is subjected to the usual temperature treatments. Storage of bulbs in an atmosphere containing 5% CO_2 during storage reduces flower formation. The anti-ethylene compound norbornadiene influences this process as well.

The use of gaseous compounds to control flowering of irises is very attractive because bulbs have closed structures which reduces the uptake of solutions and dissolved substances. Ethylene treatments enhance flowering results of sealable bulbs. CA-storage (prevention of ethylene contamination and high CO_2) prevents flowering which is required for planting stocks.

(23) T-DNA EXPRESSION IN PLANT CELLS: EXPRESSION SIGNALS OF THE T-CYT GENE

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Upon infection of dicotyledonous plants with *Agrobacterium tumefaciens*, T-DNA originating from the Ti (tumor-inducing) plasmid of this soil bacterium is transferred to plant cells, integrated in their chromosomal DNA and expressed into RNA and protein. T-DNA gene 4, also called T-cyt gene, codes in plant cells for an enzyme which catalyzes the first step in the biosynthesis of the plant hormone cytokinin. Its expression causes overproduction of cytokinins. We are studying the expression signals of the T-cyt gene.

Using the exonuclease Bal 31, deletions were made in the 5' and 3' non-coding regions of the T-cyt gene. The mutated derivatives and the wild type form of the gene were inserted in a wide host range plant vector and the resulting plasmids were conjugatively introduced into *Agrobacterium tumefaciens* strains carrying a Ti plasmid with an inactive T-cyt gene (LBA 4210) or a Ti plasmid lacking the T-cyt gene (LBA 1900). Several plant species were infected with these strains. By analyzing their tumorous response and the steady state T-cyt transcript level in the induced tumorous outgrowths, non-coding region sequences which are instrumental in the expression of the T-cyt gene were identified.

The following conclusions could be drawn with regard to the 5' non-coding region. (i) A region located 130-180 bp upstream from the ATG-codon appears to be absolutely essential for T-cyt gene expression. (ii) Putative CAAT-boxes further downstream are not essential. (iii) The two TATA-boxes are essential and one TATA-box is sufficient for expression. Primer extension experiments revealed that the TATA-boxes determine the 5' endpoints of the T-cyt mRNA. With regard to the 3' non-coding region it was found that the second poly (A) addition-box is not essential for T-cyt gene expression but that a region between the two poly (A) addition-boxes cannot be removed without affecting expression. This region contains a sequence with strong homology to the sequence PyGTGTTPyPy of which the significance in animal gene expression has been demonstrated.

(24) THE EFFECT OF REPEATED TREATMENTS IN THE STUDY OF VERY LOW FLUENCE, LOW FLUENCE AND HIGH IRRADIANCE GERMINATION RESPONSES: A MATHEMATICAL APPROACH

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Kalanchoë seeds are absolutely light-requiring for germination. Seed germination is the result of one out of three reactions, viz. the VLFR of very low fluence response, the LFR or low fluence response or the HIR or high intensity response. The germinating fraction for each of the response types is determined by the experimental conditions.

In order to explain the differences in the fluence-response curves (i.e. different shape and/or position), obtained for repeated light treatments, we define the percent germination induced by the first treatment as the responding proportion (p) of the total treated seed population. If consecutive light treatments are separated by a dark period long enough to enable fixation of the effect of the preceding treatment, theoretical calculation of the germination response is possible. The germination response induced by a second treatment should be relative to the proportion (q) of seeds non-responding to the first treatment ($q = 1 - p$) ('PQ' rule).

The fitting of these calculations with experimental data for the VLFR for germination of *Kalanchoë* seeds, induced by repeated light pulses, suggest the independency of the effect of each treatment i.e. the effect of the second treatment is neither positively nor negatively influenced by the first treatment.

This hypothesis is not valid for calculation of the LFR for germination of *Kalanchoë* seeds induced by repeated light pulses as the first light pulse does not result in a germination response.

At least two irradiations were needed for a LFR, while the third and following pulses increased the response much more than calculated with the proposed equation. It is suggested that for the LFR in *Kalanchoë*, in contrast with the VLFR, the involvement of some pre-existing far-red absorbing form of phytochrome (Pfr) and the involvement of dark reactions are to be considered.

The effect of long irradiation times (up to 200,000 s) resulting in a HIR for germination of *Kalanchoë* seeds is also discussed in terms of independently responding seed population fractions.

(25) THE INFLUENCE OF ADJUVANTS ON THE EFFECT OF HERBICIDES

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Adjuvants can enhance the biological activity of herbicides. Surfactants, mineral oils, vegetable oils and salts are commonly used as adjuvant. The precise mode of action of these compounds in relation to the plant-herbicide interaction is poorly understood. The retention, the absorption and the translocation of the herbicide can be influenced by these compounds. In our study we measured the influence of five adjuvants: Armoblen T25 (alkylamine ethoxylate), Agral LN (akylphenol ethoxylate), emulsified rape seed oil, Ulvapron (emulsified mineral oil) and ammonium sulphate. These compounds were combined with the systemic herbicides glyphosate (hydrophilic) and the R-enantiomer of fluazifop-butyl (lipophylic). The herbicide rates were 42 g active ingredient/ha (fluazifop-butyl) and 108 g active ingredient/ha (glyphosate). The concentrations of the adjuvants in the spray liquid were 0,5% (v/v) for the liquid compounds and 0,75% (w/v) for ammonium-sulphate. The spray volume was 450 l/ha. Winter-wheat was grown outside until the 3-leaf stage. Then the potted plants were placed into a growth cabinet and treated after 48 hours. After treatment, the plants remained in the cabinet until harvest, three weeks after treatment. Then the fresh and dry weights were measured. The surfactants and rapeseed oil enhanced the effect of the two herbicides. Ammonium sulphate enhanced the effect of glyphosate. Ulvapron showed a relatively small effect on the action of fluazifop-butyl. The retention data showed that surfactants at 0,5% (v/v) and the rapeseed oil at 5,0% (v/v) enhanced the retention by a factor 2 to 3. The mineral oil was not included in these measurements. The surface tension of the spray liquid was equal or somewhat lower than the surface tension of spray liquids with only the formulated herbicides: fluazifop-butyl ('Fusilade PP005') and glyphosate ('Roundup'). Thus the increase in retention does not result from a decreased surface tension. At present there is no simple explanation for the retention data. The concentration of surfactant used in our experiments exceeds the critical micelle concentration (CMC) which is much lower. These micelles or monomers which originate from these micelles may influence the interaction between spray liquid and leaf surface.

(26) TRANSFORMATION OF MONOHAPLOID AND DIPLOID POTATO GENOTYPES BY 'HAIRY ROOT'-INDUCING AGROBACTERIUM STRAINS

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Successful transformation is plant tissue with 'hairy root'-inducing *Agrobacterium* strains is indicated by the formation of hairy roots, and is proven by the production of opines or by the demonstration of integrated T-DNA in the resulting hairy roots. Isolated hairy roots can be subcultured as individual root clones.

Different genotypes ($H^{1}260$, 2xM9: diploid; and 839/79, 849/7, 851/23: monohaploid; all clones, except $H^{1}260$, were kindly supplied by ir. B.A. Uijtewaal, Dept. of Plant Breeding, Agricultural University, Wageningen), appeared to be suitable for genetic transformation by several 'hairy root'-inducing *Agrobacterium* strains). The induction and growth of the 'hairy roots' were much less dependent on the *Agrobacterium* strain applied than on the potato genotype: the diploid genotypes produced more and better growing root clones than the monohaploid ones. The growth of the root clones from the monohaploid genotypes could not be improved by addition to the growth medium of plant growth regulators, vitamins and/or organic compounds.

The transformed character of 11 out of 19 root clones investigated was demonstrated by agropine/mannopine tests. Root clone N° 13 showed spontaneous shoot-formation: the shoots appeared to have the same ploidy level as the source material ($H^{1}260$). In the root clone and shoot material, no opine-expression was found. However, Southern blotting showed the presence of integrated T-DNA.

(27) ISOLATION AND IDENTIFICATION OF A BIOLOGICALLY ACTIVE DITERPENE FROM COLEUS

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It is known since years that *Coleus* contains physiologically active compounds. Vendrig (1967) reported the isolation of a steroid-like component from *Coleus blumei*. This substance, the exact chemical structure of which was not determined, had strong auxin-like activity in the Avena curvature test.

While studying the medium-polar fraction of extracts of freeze-dried *Coleus* shoots, we found a fraction which stimulated the adventitious rooting of light-grown mung bean cuttings. After further purification, we obtained crystalline needles which were identified as Coleon O by means of UV, IR, NMR and GC-MS. Coleon O is an abietan which was first isolated from *Coleus somaliensis* (Arihara et al, 1975). The compound is localized in special glands on all aerial parts of *Coleus*. A physiological role had not been found yet (Eugster, 1975). Coleon O stimulated adventitious rooting on light-grown mung bean cuttings by more than 100% above the control in concentrations between 2×10^{-6} and 2×10^{-5} M. It is known that some other diterpenes as heliangine (Shibaoka et al., 1967) and portulal (Mitsuhashi et al, 1969) can stimulate adventitious root formation.

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(28) POTATO-PLANT-GROWTH STIMULATING PSEUDOMONAS SPP.

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Frequent cultivation of potato plants in the same field results in substantial yield decreases, due to an accumulation of deleterious, probably cyanide-producing, micro-organisms (Bakker and Schippers, 1987; Bakker et al., 1987). These yield losses can be reduced by prior bacterization of the seed potatoes with selected fluorescent *Pseudomonas* spp. The mechanism of the beneficial effect of these Pseudomonads on potato plant growth is being studied in a project in which the know-how of genetics, molecular microbiologists and phytopathologists is combined. The production of siderophores, iron chelating compounds, by the *Pseudomonas* strains appeared to be essential for plant-growth stimulation (Bakker et al., 1987; Marrug et al., 1985). Under the iron-limiting conditions present in most soils, the beneficial *Pseudomonas* strains are supposed to produce fluorescent siderophores (Bakker et al., 1986), which complex the limiting iron (III) ions, thereby making this essential element unavailable to other micro-organisms, including the deleterious ones. This may result in a decrease in the number or activities of the latter (Bakker and Schippers, 1987), thus providing a more favourable environment for the plant to grow. Moreover, in order to deliver the siderophores along the whole root system, efficient colonization of the potato root by the beneficial bacteria is supposed to be essential for plant-growth stimulation. Presently, the following topics are being investigated:

1) the nature of the proposed deleterious micro-organisms; 2) genetics and physiology of siderophore synthesis and uptake; 3) the structure of siderophores; 4) use of bacterial cell surface characteristics for identification of beneficial strains; 5) molecular analysis of bacterial factors involved in root colonization; 6) analysis of the ability of wild type and mutant *Pseudomonas* strains to increase root growth and tuber yield.

The results to be presented answer the questions whether the bacterial mobility is involved in potato root colonization and whether bacterial cell surface-characteristics can be used for identification of the root-colonizing fluorescent *Pseudomonas* spp (De Weger et al., 1986; De Weger et al., 1987).

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(29) THE INFLUENCE OF THE ROOT-KNOT NEMATODE MELOIDOGYNE INCOGNITA ON THE TRANSLOCATION OF MINERALS IN TOMATO ROOTS

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Changes in the mineral content of roots and shoots due to nematode infection has been demonstrated (Melakeberhan et al., 1985). Infective larvae of *Meloidogyne incognita* (Kofoid and White) generally penetrate the root near the apical meristem. Following a mild infection the root apex grows further and the gall becomes visible some distance from the root tip. The present work was conducted to determine the effect of nematode infection on the distribution of minerals within the root system.

Tomato seedlings (*Lycopersicon esculentum* Mill cv. Moneymaker) were grown from seeds in potting compost and transplanted into a sandy loam soil, when 2-3 true leaves had expanded. One week after transplantation half of the plants were inoculated with eggs and second-stage larvae of *Meloidogyne*. The other plants were used as controls. The plants were grown in a glasshouse for 12 weeks. Uninfected root tips, galls and uninfected root segments just above the infection site of infected plants were compared with similar root parts of control plants with respect to their Ca, Cu, Fe, Mg, Mn and Zn content on the basis of dry weight.

In infected plants, the uninfected root tips below the infection sites contained significantly higher contents of Fe, Mg, Mn and Zn than the infected parts of the roots (galls) or the root segments just above the infection site. The various root parts of uninfected controls showed no significant differences in their mineral contents. The overall contents in healthy and galled roots were similar for all elements analysed.

Light microscopic studies of transverse sections of infected roots showed that the development of the xylem was disturbed, resulting in a reduction of the cross-sectional area of the xylem near the site of infection. We suggest that a reduced xylem-volume at that site may account for a reduced translocation of water and minerals from the root tip to other parts of the plant. This reduced translocation may explain the accumulation of minerals in the root tip.

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(30) COMPARISON OF PROTON-SUBSTRATE STOICHIOMETRICS OF VALINE AND SUCROSE UPTAKE IN COMMELINA MESOPHYLL CELLS

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It has been implicitly assumed (Reinhold and Kaplan, 1984) that sugar and amino acid uptake are alike with regard to carrier operation and energization. Recently, it was proposed (Van Bel et al., 1986) that only the high-affinity systems for sucrose and valine uptake were similarly operating. Differential energization of uptake was postulated for the low-affinity uptake. We investigated therefore whether membrane depolarisation after substrate supply and concurrent proton fluxes reflected a differential behaviour of valine and sucrose uptake.

It appeared that the proton/substrate stoichiometry decreased with increasing concentrations. At low concentrations, valine uptake consumed more protons per substrate molecule than sucrose uptake. This in contrast to high concentrations were sucrose uptake consumed more protons than valine uptake per unit of substrate.

The results suggest that valine and sucrose are absorbed by similar proton co-transport systems at low concentrations. At concentrations, higher than 10 mol.m-3, the uptake of valine may occur mainly by diffusion, whereas the uptake of sucrose may be performed, at least partly, by a proton-consuming transfer. This concurs with the proposal of Maynard and Lucas (1982) and Van Bel et al. (1986) that sucrose uptake at high concentrations is carried out by a unique uptake system with an extremely high K_m.

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(31) DETECTION OF DIFFERENT 3'5'-cAMP PHOSPHODIESTERASE INHIBITORS IN PHASEOLUS VULGARIS L. CV. LIMBURG

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The presence in bean seedlings of a water-soluble, non-dialysable and heat-resistant 3'5'-cAMP phosphodiesterase (PDE) inhibitor was reported earlier (Dupon et al., 1984). In this report we present evidence for the occurrence of at least two distinct PDE-inhibitors in the bean seedling.

A lyophilised aqueous extract was refluxed with 100% MeOH yielding a MeOH-soluble, and a MeOH-insoluble fraction, both containing PDE-inhibiting activity. Precipitating the aqueous extract with 75% EtOH resulted in pelletable and a non-pelletable PDE-inhibitor. By HPLC-gel permeation it was shown that the former eluted at a higher molecular weight range than the latter. The existence of at least two distinct PDE- inhibitors was also shown by reversed phase chromotography on Wide Pore-Butyl-columns, using H_2O and acetonitrile batch elutions. Preliminary data suggesting the PDE-specificity of at least one inhibitor fraction (EtOH-pelletable) will be discussed.

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(32) EFFECT OF CO_2 CONCENTRATION DURING GROWTH ON THE BICARBONATE UTILIZATION MECHANISMS OF ELODEA CANADENSIS MICHX

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Under low free CO_2-concentrations the submerged angiosperm *Elodea canadensis* can utilize bicarbonate for its photosynthesis. The ability to do this is influenced by the CO_2-concentration of the culturing medium. In ^{14}C-fixation experiments, the rate of photosynthesis at pH 7 of high CO_2-grown leaves (H-CO_2) and leaves grown at ambient air levels of CO_2 (L-CO_2) proved to be the same. Photosynthesis at pH 9 (taken to be a measure for bicarbonate utilization) is 30 - 40% of that at pH 7 in L-CO_2 leaves but only 5 - 15% in H-CO_2 leaves. Adding 50 µM acetazolamide (AZ), a membrane impermeable inhibitor of carbonic anhydrase (CA), had no effect on the ^{14}C fixation in L-CO_2 leaves at pH 7 and inhibited 60 - 80% at pH 9. In H-CO_2 leaves, however, fixation was inhibited at both pH 7 and pH 9: 15 - 20%. It was concluded that extracellular conversion of bicarbonate into CO_2 catalyzed by CA plays a role in bicarbonate utilization. And that, assuming that the use of free CO_2 is the same in L-CO_2 and H-CO_2 leaves, L-CO_2 possesses a bicarbonate utilization mechanism, apart from CA-mediated conversion, which is less efficient or absent in H-CO_2 leaves.

(33) PHENOLIC ACIDS OF MATURING STEMS OF MAIZE CULTIVARS DIFFERING IN DIGESTIBILITY

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In plant cell walls phenolic acids occur as the polymerized phenolic acids, which may represent the true lignin content of the cell wall, and as the phenolic acids, which are attached to hemicelluloses. These phenolic cross-links of matrix polysaccharides are likely to be one of the factors determining cell wall digestibility (Fry, 1986). Therefore a study was undertaken to determine phenolic acid composition of maize stems of different ages of three cultivars with known differences in the *in vitro* dry matter digestibility (Cone et al. unpublished): Brown midrib (BM), LG11 and Eta Ipho. Plant were harvested in July and November. Internodes bearing the kernel were freeze-dried and ground in a mill equipped with a 1 mm screen. Alkaline extraction was carried out according to Salomonson et al., (1978). Phenolic acid composition was determined of the ethanol fraction and of the ethyl acetate fraction obtained after hydrolysis and subsequent acidification. Samples were analysed by HPLC*. The eluting solvents were 4% aqueous HAc and 4% HAc in methanol. Phenolic acids were detected with an UV detector at 280 nm. In the ethanol fraction mainly coumaric acid was present and an unidentified peak occurred, which was not present in the residue after ethanol extraction. Almost no ferulic acid was determined in this fraction. In the ethyl acetate fraction coumaric acid and ferulic acid were present as the main phenolic compounds. A number of yet unidentified phenolic acids was present in lesser amounts. Catechuic acid, chlorogenic acid, caffeic acid, epicatechuic acid, phloridzinic acid and quercitinic acid were not present in amounts detectable by the procedures used. In all three cultivars coumaric acid and ferulic acid content increased from July to November. During this time digestibility decreased (Cone et al., unpublished). The cell wall residue after hydrolysis was used for pyrolysis and mass spectroscopy* to determine lignin concent. In all three cultivars lignin content increased from July to November. At both ages lignin content of Brown midrib was lower than of LG11 and Eta Ipho. In vitro dry matter digestibility of young Brown midrib stems was higher, but the three cultivars did not differ in digestibility of full-grown stems (Cone et al. unpublished). Our preliminary results support the hypothesis that matrix-bound phenolic acids influence cell wall digestibility.

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(34) THE EFFECTS OF PCMBS ON PHOTOSYNTHESIS AND VEIN LOADING IN COMMELINA BENGHALENSIS LEAVES

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The -SH reagent PCMBS has been often employed in experiments on vein loading because it has no direct inhibitory effect on photosynthesis, whereas it strongly inhibits a presumptive direct uptake of photosynthate by minor veins (Giaquinta, 1976). Indirect effects of PCMBS on photosynthesis, possibly interfering with the loading process, were investigated. Furthermore, the mesophyll-to-vein displacement of $^{14}CO_2$-derived sucrose was monitored in the presence of PCMBS.

During pulse-labelling with $^{14}CO_2$, *Commelina Benghalensis* L. leaf discs were floating on liquid media with or without 2.5 mol m^{-3} PCMBS (parachloromercuribenzenesulfonic acid). After washing, the effect of PCMBS on stomata closure and vein loading was determined by monitoring the ^{14}C-leakage, measurement of the ^{14}C-content of the discs and examination of disc autoradiograms.

In discs with the stripped side floating on the medium, the manufacturing of assimilates was completely hampered by PCMBS. In discs, stripped at both sides, the ^{14}C-sucrose production was reduced to 72%. In isolated mesophyll cells, the reduction amounted 15%. It indicated that PCMBS has indeed a minor effect on photosynthesis itself, but a very drastic one on stomatal closure.

PCMBS also prevented the resorption of ^{14}C-assimilates. After a leakage period of 2 hours, the leakage from discs in PCMBS (7% of the disc content) exceeded by far that in the control discs (less than 1%). The autoradiograms did not show clearly whether this leakage occurred from mesophyll or veins. The relatively small leakage rate and the almost unhampered phloem loading in the presence of PCMBS suggested that, in contrast to Beta (Giaquinta, 1976), $^{14}CO_2$-derived assimilates are transported symplastically from mesophyll-to-vein (cf Van Bel, 1987).

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(35) CHARACTERISTICS OF A PROTON-GIBBERELLIC ACID SYMPORT

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Many plant hormones such as gibberellins are absorbed by plant roots. However, not much is known about their transport mechanisms. Incubation of barley roots (*Hordeum vulgare* L. cv. Union) in gibberellic acid (GA_3) causes a substantial membrane depolarization and a proton influx in different circumstances (François, 1985). Comparable results have been obtained with giant cells of *Chara corallina* (Homblé and François, 1985).

We suggest the existence of a proton symport system for the absorption of GA_3 by plants. The inward flux of one GA_3^--anion is coupled to the downhill flux of two protons. The proton-motive force is generated by the proton extrusion pump and has electrical and chemical components. A number of criteria proposed for the operation of such a system (Reinhold and Kaplan, 1984) seem to be fulfilled: the occurrence of a transmembrane proton flux, the electrogenicity of the transport resulting in membrane depolarization and the decrease in substrate uptake with increasing external pH values (François and Homblé, 1985).

The transient character of the induced phenomena is often considered as being typical for symport (Reinhold and Kaplan, 1984). We criticize this point of view. Indeed, in our experiments the induced changes were permanent. They only caused a minor decrease in electrochemical potential difference. We believe that the remaining part is sufficient to keep the proton-GA_3^--complex moving energetically downhill.

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(36) FRACTIONAL STOICHIOMETRIES FOR A PROTON-GIBBERELLIC ACID SYMPORT

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A symport system responsible for the uptake of gibberellic acid by plant cells implies the coupled transport of protons and substrate in a ratio n : 1 (François and Homblé, 1985). We have no certainty about the value of n, except that it should be larger than 1. Indeed, the carrier-H^+-GA_3^--complex induced a depolarization of the plasma membrane in *Hordeum vulgare* L. and in *Chara corallina* (François et al., 1984; Homblé and François, 1985). In principle, n could be a whole or a fractional number. In order to keep the discussion clear, we assume that n is not larger than 2. We describe 3 possible transport models (Grüneberg and Komor, 1976).

Model 1. The carrier molecule has 2 binding sites for protons. Both have access to both sides of the cytoplasmic membrane. Two protons can be transported during each cycle.

Model 2. The carrier has one binding site for a proton and 1 proton is transported together with 1 GA_3^--molecule. However, it is also possible that only the proton and not GA_3^- is released at the inner side of the membrane.

Model 3. The carrier has 1 binding site for protons. The ternary carrier-proton-gibberellic acid complex occasionally dissociates at the outer side of the membrane before migration occurs.

Only in model 1 the stoichiometric number is undoubtedly a whole number and equals 2. In model 2 the stoichiometry is fractional, if the proton is delivered at the cytoplasmic side with a probability which is different from 0% (n = 1) and 50% (n = 2). In model 3, n is a fractional number, if the probability of dissociation of the ternary complex at the outer side differs from 0% (n = 1) and 50% (n = 2).

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(37) CORRELATION BETWEEN THE ENDOGENOUS c-AMP LEVEL AND DEETIOLATION OF PHASEOLUS VULGARIS L. CV. LIMBURG SEEDLINGS?

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Previously we demonstrated the presence of c-AMP in *Phaseolus vulgaris L.* cv. Limburg seedlings (Van Onckelen et al., 1982).

In order to evaluate the physiological significance of this c-nucleotide during growth and development of higher plants, we investigated the possible correlation of the endogenous c-AMP level with deetiolation. This physiological process was chosen in view of some earlier reports on the correlations of endogenous c-AMP with chlorophyll synthesis in *Chlorella fusca* and with chloronema differentiation in *Funaria hygrometrica* (Berchtold and Bachofen, 1977; Handa and Johri, 1979).

Endogenous c-AMP quantifications were based on 'ion-paired' HPLC by on line UV-absorption (254 nm) after an extraction and purification procedure described earlier (Gadeyne et al., 1987). In this contribution we compare the endogenous c-AMP levels in different organs of etiolated and seedlings grown in white light. We also present a preliminary kinetic analysis of the endogenous c-AMP concentration during deetiolation of eight days old etiolated primary leaves. This kinetic analysis will be interpreted in view of a possible photoperiod dependent variation of the endogenous c-AMP concentration in eight days old primary leaves grown in white light (8h light - 16h dark).

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** Senior Research Associate

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(38) FAST HPLC ANALYSIS OF BENZOYLATED POLYAMINES

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Recently we published a fast isocratic HPLC analysis of dansylated polyamines (Geuns, 1986; Walter and Geuns, 1987). Now we tried to improve the analysis of benzoylated polyamines (Redmond and Tseng, 1979) to avoid a sample clean-up. About 200 mg tissue, frozen in N_2, was homogenized in 2 ml cold 4% $HClO_4$ containing 1,6-diaminohexane.2HCl as internal standard (4.7 mg/l). After 1 h at 4°C, the 'supernatant' was recovered by sucking through a plug of glass wool. Samples of 0.2 ml were benzoylated in 1 ml 2N NaOH to which 10 µl benzoylchloride were added. The mixture was shaken vigorously. After 20 min 1 ml 0.75M NH_4Cl was added. The benzoates were extracted with 5 ml methylene chloride. The water phase was pipetted off and the methylene chloride was carefully washed with 1 ml 0.75 M NH_4Cl and then with 1 ml H_2O. The methylene chloride (4 ml) was transferred into a sample vial and dried under a stream of N_2. HPLC analysis was performed on columns filled up with 5 µm ODS-silica (10 cm length, 3 mm diameter). The analysis was done at 50°C. The Van Deempter-curve allowed an isocratic solvent flow of 2 ml/min (MeOH: H_2O:isoBuOH : 45:55:2 v/v/v). Detection was at 225 nm. Straight calibration curves were obtained up to 100 µM (putrescine: y = 0.027x; spermidine: y=0.019x; spermine: y=0.01x; y being the peak height ratio of polyamine over internal standard and x being the concentration of polyamine in nmol/ml). The most retained compound (spermine-tetra-benzoate) is completely eluted within 5 min.

The advantages of our method are: 1). Very fast sample preparation. 2). The use of NH_4Cl gives a complete breakdown of the residual benzoylchloride, thus avoiding unwanted peaks in the chromatograms, e.g. of methylbenzoates that might arise from traces of benzoylchloride in contact with MeOH as sample preparation is very critical. 3). The use of an internal standard compensates for the loss of 1 ml of the methylene chloride fraction. 4). Very low cost per sample as the fast isocratic HPLC uses very cheap solvents of an ordinary brand, 55% being water. 5). As the home-made columns are packed at 750 bar, still higher solvent flows are possible without considerable loss of resolution. 6). Very reproducible results are obtained with a SE of about 5% between different experiments. 7). The use of 5 cm columns filled up with 3 µm ODS-silica will even halve the analysis time. 8). Short columns of 1 cm filled up with 2 µm ODS-silica and in combination with a steep gradient will perform the analysis within 1 min or less.

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(39) SYNERGISM BETWEEN CORTISOL AND IAA IN THE ADVENTITIOUS ROOT FORMATION

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Previously we demonstrated that cortisol stimulated the adventitious root formation on the hypocotyls of intact mung bean seedlings as well as on hypocotyl cuttings (Loeys and Geuns, 1978). Now we studied the interaction between cortisol and IAA during the adventitious root formation in mung bean cuttings prepared as described by Bassuk and Howard (1981). As under certain conditions the outgrowth of adventitious root initials decreased, the adventitious roots as well as the initials were recorded after clearing of the cuttings. IAA was applied at 5×10^{-5}M, cortisol at a concentration of 50 mg/l^{-1}. The adventitious roots were counted 6 d after the start of the experiment.

Cortisol applied together with IAA during the first 48 h enhanced the adventitious root formation by about 700%, against 115% stimulation obtained by application of IAA alone and 240% with cortisol alone. This synergistic effect was also obtained, when IAA and cortisol were applied together during the first 24 h only followed by an application of cortisol alone for the next 24 h.

In experiments in which we applied cortisol after a pretreatment of cuttings in IAA for different periods, we could demonstrate that IAA was active during the first 24 h after cuttings were made, whereas the effect of cortisol was mainly during the period between 24 and 48 h. IAA had to be applied for 8 h only during the first 24 h. Its application may be separated from the cortisol treatment by a 16 h incubation in H_2O. Further work is in progress to study the correlation with polyamine content.

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(40) CELL PROLIFERATION IN THE SHOOT APICAL MERISTEM OF SINAPIS ALBA DURING PLANT AGING

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Mitotic activity has been studied in the vegetative shoot apical meristem of many species but very few studies have been dealing with the effect of aging (Bernier, 1969; Nougarède and Rembur, 1979). Moreover, only mitotic index or DNA synthesis index were estimated. In this study, we investigated cell- cycle duration and growth fraction in the meristem of *Sinapis alba*, LDP, grown in SD on soil without any addition of nutrients. Cell proliferation was analysed, when plants were 17 or 60 days old. The durations of the cell cycle and its component phases in the meristem were measured by the pulse-labelled-mitoses method (Quastler and Sherman, 1959) using a 4-h treatment with [^{3}H] TdR (0.37 MBq/ml; spec. act. 910 GBq/nmol; Amersham, U.K.). The growth fraction was estimated by methods of Clowes (1976).

During aging of the plants, the cell-cycle duration lengthened from approximately 68 to 86h and the growth fraction shortened from 84 to 30-40%. Durations of the component phases were modified as follows: G2 + $\frac{M}{2}$ was considerably lengthened, from 10 to 31h; S was increased from 17 to 33h; but G1 + $\frac{M}{2}$ was reduced from 41 to 22h.

Different hypotheses can be proposed to explain this decrease of the cell proliferation in the shoot meristem of *Sinapis* at aging. Firstly, the shoot meristematic cells could be progressively deprived of essential nutrients to progress through the cell cycle, since the availability of mineral nutrients in the soil decreases with time. This view is supported by the results of Yun and Naylor (1973). Alternatively, the nutrient- deprived roots could reduce their production of cytokinins (Salama and Wareing, 1979) which are known to be a control factor of the cell proliferation in *Sinapis* (Bernier et al., 1977). Thirdly, the meristem itself could undergo aging but this possibility remains controversial (Woolhouse, 1972).

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(41) COPPER- AND ZINC-INDUCED ETHYLENE PRODUCTION BY PHASEOLUS VULGARIS L.

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A variety of stresses causes enhanced ethylene production. Interference of heavy metals with ethylene synthesis in intact plants (Fuhrer, 1982; Rodecap, 1981[a,b]) and isolated chloroplasts (Sandmann, 1980) is well documented.

Phaseolus vulgaris L. was cultivated in a day-night cycle of 12 hours on vermiculite in Hoagland's nutrient solution. Fifteen days old plants were carefully removed from the vermiculite and, after rinsing the roots, were placed in a 200 µM Cu^{2+}- or a 1 mM Zn^{2+}-solution. Control plants received water only. Propylgallate (10^{-3}M) was applied to de-rooted plants 16 h before heavy metal treatment. Ethylene and ethane production were measured in an open flow system. Malondialdehyde was determined by the thiobarbituric acid test (Asakawa, 1978). Large amounts of Cu and Zn accumulated in the leaves of intact plants within 22 h. In intact en de-rooted plants, ethylene production increased 4-fold within 7 h and declined within 22 h after Cu-application. With Zn the ethylene production doubled.

Although ethane evolution of intact and de-rooted plants did not change within 22 h, the stimulatory effect of Cu and Zn on ethylene production could be due to membrane peroxidation, since malondialdehyde, a quantitative marker of lipid peroxidation (Asakawa, 1978), rose significantly. This hypothesis is confirmed by the inhibitory effect of propylgallate, on Cu- and Zn-induced ethylene production of de-rooted plants. Propylgallate is a quencher of singlet oxygen. Heavy metal-stimulated lipid peroxidation might give rise to increased free oxygen radical formation and therefore promote the turnover of ACC to ethylene. One of the questions is whether lipoxygenase is involved in this process (Kacperska, 1985). Further research is in progress to elucidate to what extent ACC-content and -synthesis are affected by the heavy metals studied.

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(42) ENERGETIC ASPECTS OF TERPENOID SYNTHESIS IN EUPHORBIA

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In germinating seeds and young seedlings of *Euphorbia lathyris* up to 80% of the non-saponifiable lipids occur in the laticifers. These special cells are characterised by the synthesis of triterpenes, which in turn are finally secreted as solid particles in the vacuole.

During germination the endosperm can be removed from the seedling and a variety of substrates can be administered physiologically to the cotyledons. Most substrates were taken up rapidly and translocated in the growing seedling. Several amino acids (threonine, valine, leucine, isoleucine and alanine) were incorporated into the latex triterpenes, but sucrose (and to a lesser extent glucose and fructose) was found to be the most effective precursor. Tracer experiments with specifically labelled glucose showed glycolysis to be the major pathway in glucose catabolism inside the laticifer. The pyruvate produced is converted to acetyl-CoA, which in turn is used for the production of a triterpenol via mevalonate, isopentenyl- pyrophosphate, and squalene.

Transmission electron microscopy showed many mitochondria in the cytoplasm of the laticifers and with this ultrastructural information the energetics of terpenoid synthesis in the latex cells could be calculated. Assuming that the citrate shuttle in these mitochondria is involved in the conversion of pyruvate into acetyl-CoA we conclude:

a) 9 molecules of glucose are used for the synthesis of one molecule of triterpene;
b) this synthesis is self-supporting in ATP requirement;
c) this synthesis is self-supporting in NADPH + H^+ requirement;
d) there is a surplus of NADH + H^+/NADPH + H^+ produced in this synthesis which might be used to convert amino acids into triterpenes;
e) terpenoid synthesis in a latex vessel is not an anaerobic process;
f) the synthesis of a triterpene can be written as:

$$9\ C_6H_{12}O_6 + 4\ O_2 \rightarrow C_{30}H_{50}O + 24\ CO_2 + 32\ H + 13\ H_2O$$

or per isoprene unit:

$$1\tfrac{1}{2}\ C_6H_{12}O_6 + \tfrac{1}{2}\ O_2 \rightarrow C_5H_8 + 4\ CO_2 + 6\ H + 2\ H_2O$$

(43) THE ROLE OF ETHYLENE AND ABSCISIC ACID IN THE REGULATION OF RESPIRATION OF POTATO TUBER TISSUE

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Two types of potato tuber tissue discs were used to study the role of the phytohormones ethylene and abscisic acid (ABA) in the regulation of growth and respiration of potato tuber tissue: 1). Callus-forming discs, obtained by incubation of discs on a nutrient medium containing an auxin and a cytokinin. 2). Non- callus-forming discs, obtained by incubation on the same medium without callus-inducing hormones.

Ethylene specifically affected the induction of alternative oxidase. The presence of auxin and cytokinin in the nutrient medium of callus-forming discs caused an ethylene production that was 100-fold the amount produced by non-callus-forming discs. Incubation of callus-forming discs in an atmosphere with 4000 ppm NBD (2,5-norbornadiene, a competitive inhibitor of ethylene action) specifically inhibited the induction of alternative oxidase: mitochondria from these discs showed a CN-resistance of only 20% of the uninhibited respiration in the presence of ADP (state 3 respiration), compared to 40% in control discs. In the non-callus- forming discs (with a relatively low ethylene formation) the level of alternative oxidase could be increased by adding 10 µM ACC (1-aminocyclopropane-1-carboxylic acid, the precursor of ethylene) to the nutrient medium which caused an enhanced ethylene formation.

Addition of 40 µM ABA, the phytohormone functioning in dormancy induction in potato tubers, to the nutrient media cause a specific inhibition of alternative pathway capacity: from 33 to 23% (of state 3 respiration) in mitochondria from callus and from 25 to 10% in mitochondria from non-callus-forming discs.

These results indicate that the induction of the alternative pathway in potato tuber tissue is regulated by ethylene and abscisic acid. The contribution of the alternative pathway to *in vivo* respiration is also influenced by these phytohormones, but these effects are only secondary. They are the consequence of phytohormone-effects on cytochrome path activity and on supply of respiratory substrates.

(44) COMPARISON OF SOME PHYSIOLOGICAL PARAMETERS OF AGROBACTERIUM RHIZOGENES RI-TRANSFORMED AND NORMAL ROOTS OF POTATO

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Infection of wounded plant parts with *Agrobacterium rhizogenes* results in an excessive proliferation of transformed roots ('hairy roots'). From such Ri-transformed roots of the potato cultivar Bintje plants have been regenerated (Hänisch ten Cate et al., 1987). These Ri-transformed potato plants, in which a part of the bacterial DNA is inserted, grew *in vitro* more vigorously than normal Bintje. In the present study we compared some physiological parameters of normal and *A. rhizogenes* LBA 9402 transformed-root systems of cv. Bintje (Hänisch ten Cate et al., 1987). Such a comparison could yield valuable information on the feasibility of the use of Ri-transformed root systems to improve plant growth.

Plant growth and mineral uptake were investigated by grafting potato or tomato shoots on normal and transformed potato root systems. Bintje shoots on transformed root systems formed small callus-like structures on the leaves, two days after grafting. Moreover, less graftings survived on transformed than on normal root systems. Grafting of tomato shoots was more succesful, and did not differ between both types of roots. No callus-like structures were observed. However, with the transformed root systems severe chlorosis appeared in the young leaves after one month of growth. As the chlorosis was suspected to be caused by a difficultly remobilizable element, concentrations of Fe, Mn and Ca were measured. Concentrations in the shoots, however, did not differ significantly. Although at the time of grafting the transformed root systems outweighted the normal ones, the shoots and roots of the untransformed system had outgrown the transformed ones after one month of growth. Similar observations were made for normal and transformed potato plants not subjected to the grafting procedure.

Three weeks after the transfer from the *in vitro* culture to a nutrient-solution root respiration measurements were done according to the method of Lambers et al. (1983). The disturbing effect of the presence of some peroxidases was suppressed by using gentisic acid. In normal roots both the respiration and the activity of the alternative pathway were higher. At that moment the transformed roots still outweighted the normal ones. The capacity of the alternative pathway was at least 100% of the actual respiration in both cases. The reduction of the osmotic pressure in leaves of regenerated transformed potato-plants as described by Ooms et al. (1986) for the cultivar Desirée could not be confirmed for Bintje.

The present results indicate that the observed vigorous growth of transformed 'Bintje' plants *in vitro* did not persist *in vivo* in completely transformed and in plants grafted with a root system. The cause of the growth aberrations found for shoots grafted on transformed roots are not yet understood, but limit the prospects for the use of transformed potato root systems to enhance growth.

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(45) THE RELATIONSHIP BETWEEN EXOGENOUS AND ENDOGENOUS AMOUNTS OF GROWTH REGULATORS IN THIN CELL LAYER CULTURES OF NICOTIANA TABACUM L.

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Thin cell layers (epidermis and three to six subepidermal cell layers), obtained from floral branches of *Nicotiana tabacum* L., can be induced to express four different morphogenetic patterns: flower buds, vegetative buds, roots or callus formation. The initiation of each of these programs is determined by the auxin/cytokinin ratio, the sucrose concentration and the stage of development of the mother plant.

The formation of flower buds on the explant was optimal when the basal medium contained an auxin and a cytokinin, both in a concentration of 1 µM, sucrose (30g/l) and when the explants were excised from mother plants in which the terminal bud was in the green-fruit stage.

Under these conditions we have followed the endogenous contents of IAA and zeatin in the thin cell layers during the first eight days of the culture. Simultaneously the amounts of the growth regulators were determined in the medium. IAA was analysed after purification by HPLC and the cytokinin by a radio- immunoassay (Van Onckelen et al., 1984).

Van Onckelen, H. Rüdelsheim, R., Hermans, R., Horemans, S., Messens, E., Hernalsteens, J.P., Van Montagu, U. and De Greef, J. (1984), Plant Cell Physiol. 25, 1017-1025.

(46) THE INFLUENCE OF DIFFERENT CYTOKININS AND AUXINS ON FLOWER NEOFORMATION IN THIN CELL LAYERS OF NICOTIANA TABACUM L.

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With the method used by Tran Thanh Van (1973, 1974), neoformation of flower buds was studied in thin cell layers derived from floral branches of tobacco. It was found that in the presence of 1 μM IAA and 1 μM kinetin the explants formed only flower buds. However, our results demonstrated that organogenesis in this experimental system is also determined by the kind of auxin or cytokinin and not only by the concentrations of the applied growth regulators.

The natural auxins IAA and IBA seemed to have different effects on morphogenesis in these explants compared to CTA (α-(3-chloro-o-tolyl)acetic acid) and NAA, two synthetic auxins. The last group promoted, in combination with a cytokinin, the number of flower buds per explant and also the initiation of roots on the same explant.

Comparing different cytokinins, it can be stated that BA in combination with one of the auxins mentioned before, was the most active cytokinin in comparison with kinetin, zeatin or iso-pentenyl-adenine (IPA) for the initiation of flower buds and vegetative buds.

On the other hand, zeatin stimulated more the further development of the initiated flower buds and at same time the initiation of roots.

In contrast to the experience of other authors (Tran Thanh Van, 1973; 1974), we also found an effect of 2,4D on organogenesis in these thin cell layers.

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Tran Thanh Van, U., Chlyah, H. and Chlyah, A. (1974), Tissue culture and Plant Science, ed., H.E. Street, 101-138.

(47) TWO EFFECTS OF LIGHT ON THE GA- AND NITRATE-STIMULATED GERMINATION OF SEEDS OF SISYMBRIUM OFFICINALE AND ARABIDOPSIS THALIANA L.

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Red light stimulates seed germination of both species in two essentially different ways. Light-effect I is expressed in the presence of nitrate and light-effect II in the presence of gibberellins. Fluence-response experiments in the presence of different concentrations of the growth regulators and subsequent probit analysis of the suboptimal segments of the curves shows that the two light-effects can be separated with regard to their respective modes of co-action.

Light-effect I shows a multiplicative interaction with nitrate in the germination of *Sisymbrium* seeds which is absolutely dependent on the presence of both light and nitrate. The germination of wild-type seeds of *Arabidopsis* is nitrate-independent. However, application of nitrate gives an extra effect on the stimulation of germination which is also of the multiplicative type of interaction. Light-effect II appears to be additive to the effect of GA on dark germination, but is only expressed in the presence of GA. This type of co-action is clearly additive and is remarkably similar in both species.

From these and earlier observations we conclude that light-effect I stimulates germination through GA- biosynthesis whereby nitrate may act as an essential factor. Light-effect II is believed to act at the GA- receptor site where it enhances the sensitivity to GA.

(48) INDUCTION OF ENZYMES OF THE PHENYL PROPANOID PATHWAY IN PETUNIA HYBRIDA CELL SUSPENSIONS

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Secondary metabolite production in plant cell cultures can be of industrial importance. In growing cell cultures however, most energy is used for the growth and maintenance. It is important to understand the mechanisms that direct the energy stream either to primary or to secondary metabolism, in order to optimize the secondary metabolite production in a growing system.

To create a model system, in which the interrelation between the primary and the secondary metabolism can be studied, cell suspensions of *Petunia hybrida* were cultivated in a MS-medium. The induction of enzymes of the phenyl propanoid pathway (leading to a. o. fytoalexins, flavonoids and anthocyanins) was investigated.

The activity of phenylalanine-ammonia-lyase (PAL), catalysing the conversion of phenylalanine to cinnamate, one of the first steps of the phenyl propanoid pathway, was very low in control batch cultures of *Petunia hybrida* Violet-30. By changing the pH of the medium or by addition of microbial elicitors, a significant increase in PAL activity was observed. Increasing the medium pH from 5.8 (controls) to 6.6, resulted in an eightfold increase in PAL activity, which remained high for at least ten days. Microbial elicitors (e.g. sterilised *Penicillium funiculosum*) led to an tenfold increase in PAL activity within 7 hours, which started to decrease again after about 10 hours. The PAL activity, induced by pH changes and microbial elicitors, was of the same order of magnitude as that, observed in intact *Petunia* flower buds.

The next step will be the study of concomitant changes in chalcon isomerase (an enzyme, specifically involved in flavonoid synthesis) and in various metabolites produced by the phenyl propanoid pathway.

(49) AUXIN AND CYTOKININ CONTENT IN NORMAL AND AUXIN-HABITUATED TOBACCO CALLUS TISSUES

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The auxin-habituated tobacco callus does not require auxin in the medium. Although it has been postulated that habituation is accompanied by an enhanced production of growth promoting substances, Nakajima et al. (1979) could not detect any higher auxin and/or cytokinin concentration in auxin habituated tobacco callus. Also in cytokinin-habituated tobacco callus no enhanced IAA of cytokinin levels were observed (Horseele et al., 1987).

In this report we present kinetics of the endogenous IAA and cytokinin levels during a growth cycle of an auxin-habituated compared with a normal hormone-requiring tobacco callus. Normal hormone-requiring callus of *Nicotiana tabacum* L cv. petit Havana SRI grown on 2 mg/l NAA and 0.3 mg/l BAP contained very low endogenous IAA (5 pmol/g.fr.wt.) and cytokinin (4 pmol/g.fr.wt.) concentrations.

Compared with this hormone-requiring tissue, the auxin-habituated callus showed during the lag phase of its growth cycle an enhanced endogenous IAA concentration (70 pmol/g.fr.wt.). The cytokinin concentration remained very low (4 pmol/g.fr.wt.).

Data presented in this report seem to implicate that an enhanced auxin level (either endogenous in auxin autotrophic tissue or exogenously applied) is a prerequisite for the growth of tobacco callus.

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** Senior Research Associate NFWO.

(50) RECONSTITUTION OF A PHOTOCHEMICALLY ACTIVE PHOTOSYSTEM I COMPLEX OF A THERMOPHILIC CYANOBACTERIUM INTO LIPOSOMES

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The thylakoid membrane is a very complex system of protein-protein and protein-lipid interactions in which light-energy is captured and converted into ATP and reduction equivalents. In order to get more insight in its role in membrane function, the photosystem I complex of the cyanobacterium *Synechococcus* 6716 was isolated by the Fast Protein Liquid Chromatography method. After octyl glucoside extraction of membrane-bound proteins from the thylakoids, the crude extract was loaded on an anion exchange column and separated into four fractions. The photosystem I activities of the column fractions and the crude extract were related to the oxidation rate of reduced cytochrome c under steady-state illumination with oxygen as the electron acceptor. The highest specific photosystem I activity was found in the fourth column fraction. When compared to the crude extract, a sevenfold enrichment could be attained on a protein basis. This photosystem I-enriched fraction was further characterized. It exhibited a 77 K fluorescence emission pattern with a typical far-red emission maximum at 722 nm. Its behaviour on SDS polyacrylamide gels revealed the existence of two main polypeptides of 57 and 69 kDa and some smaller polypeptides in the order of 12 to 14 kDa. A clear double band could be seen at 17 kDa, originating from a contamination of phycocyanin in the photosystem I preparation. The photosystem I complex was reconstituted into liposomes by means of dialysis in the presence of high concentrations of detergents and native lipids. As soon as the detergents were diluted in the dialysis buffer, liposomes were formed which performed cytochrome c oxidation upon illumination in the presence of reduced cytochrome c and oxygen. The rate-limiting step in this reaction was the accessibility of cytochrome c and/or diffusion of oxygen across the liposome membrane, depending on the orientation of the photosystems in the lipid bilayer. By adding 0,02% Triton X-100 to the reaction medium this barrier could be abolished, which was clearly demonstrated by a fourfold increase of the cytochrome c oxidation.

(51) TEMPERATURE-DEPENDENT ALTERATIONS IN CHLOROPHYLL FLUORESCENCE AND LIGHT SCATTERING

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Thermophilic plants like cucumber and tomato show chilling damage at temperatures below 10°C. Lowering the temperature generally decreases photosynthesis and increases the chlorophyll fluorescence (Murata and Fork, 1975).

Chlorophyll fluorescence measurements were used to study the effect of temperature on cucumber and tomato leaf discs. The maximum fluorescence induction value (P) showed abrupt changes, when the temperature was decreased from 30°C to 0°C. P first increased, followed by a slow increase or a slow decrease, and finally increased again. The changes occurred approximately linearly with decreasing temperature and two breakpoints were observed.

For cucumber leaf discs, the breakpoints were found around 9°C and 21°C. The lower temperature breakpoint was hardly affected by the growth temperature but the higher temperature breakpoint shifted from around 21°C, for optimal growth conditions, to around 16°C, for suboptimal growth conditions (Van Hasselt and De Jong, 1984). In the presence of DCMU, only the high temperature breakpoint remained.

Tomato leaf discs of three genotypes differing in growth ability at suboptimal temperatures and light conditions showed breakpoints around 15°C and 24°C when grown under optimal conditions. At present, tomato plants grown at suboptimal temperatures are being studied.

Light scattering (above 700 nm) was studied to investigate whether the breakpoints are related with temperature-dependent conformation changes of the thylakoid membrane (Weis, 1985). A sudden increase around the temperature of the higher temperature breakpoint was evident when the temperature was decreased from 30°C to 0°C.

Possible causes of the temperature-dependent changes in *in vivo* chlorophyll fluorescence will be discussed.

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(52) ETHYLENE FORMATION AND THE EFFECTS OF ETHYLENE AND HIGH TEMPERATURE ON RESPIRATION IN IRIS HOLLANDICA BULBS

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Flowering of small iris bulbs (*Iris hollandica*) can be enhanced by high temperatures (2 weeks 35°C, followed by 3 days at 40°C). At present this high-temperature treatment generally has been replaced by a treatment with ethylene. Both treatments induced alternative, cyanide-insensitive respiration and led to an increase of the mitochondrial cytochrome pathway activity. However, the time course for the development of these respiratory characteristics depended on the treatment: ethylene effects could be measured within 24 h while 40°C must be applied for more than 4 days before the effects were visible.

Mitochondria isolated from Iris bulbs showed oxygen consumption with various respiratory substrates. Pyruvate yielded the highest alternative pathway capacity while this capacity was negligible for NADH as the substrate. Succinate as a substrate gave intermediate results. Therefore, in experiments studying respirational changes succinate and pyruvate were used.

For several plant tissues has been reported that the formation of ethylene from its precursor ACC (1-aminocyclopropane- 1-carboxylic acid) is inhibited at high temperatures (> 35°C, Yu et al, 1980). However, we observed a higher ethylene production for bulbs, stored at 40°C than for control bulbs, stored at 30°C.

The stimulating effect of ethylene on the activity of the cytochrome pathway and on the induction of the alternative pathway appeared to be temperature-dependent: these effects were only observed at 30°C, while a treatment with ethylene at 40°C for 1 day (a period too short to cause the above-mentioned high temperature induction) neither induced the alternative pathway nor increased the cytochrome pathway. The use of the competitive inhibitor NBD (2,5-norbornadiene, 8000 ppm) completely blocked the effects caused by 10 ppm ethylene at 30°C but could not inhibit the alternative pathway induction, caused by a 4 days treatment of 40°C.

This research was carried out in collaboration with the Laboratorium voor Bloembollenonderzoek in Lisse, NL-.

Yu, Y-B., Adams, D.O. and Yang, S.F. (1980), Plant Physiol. 66, 286-290.

(53) BOLTING RESISTANCE AND ACC-METABOLISM IN SELFBLANCHING CELERY CULTIVARS

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Since drought stress can cause an increase in the ACC and N-malonyl-ACC content in celery (Keteleer et al., 1984) and drought stress can suppress vernalization (Benoit et al., 1978), we investigated the relationship between ACC levels and bolting resistance.

Six selfblanching celery cultivars were screened for their bolting resistance. We determined the degree of bolting and the percentage marketable plants at harvest in plants which received a cold treatment of 10 days at 4°C at the age of 60 days. We found substantial differences between the cultivars. The cultivars at both extremes of the bolting scale and an intermediate one (100%, 61% and 18% marketable), were selected for further investigation of the ACC and N-malonyl-ACC content.

On days 60 and 70, before and after the vernalizing treatment, we measured the ACC and N-malonyl-ACC content of the leaves and the ACC-metabolism response to a wilting period (2h at room temperature). The vernalizing treatment gave rise to a higher level of N-malonyl-ACC in all cultivars, the free ACC level was unaffected. The wilting period resulted in an increase in free and N-malonyl-ACC content (relative to the levels in non-wilted plants) but this response to stress was mostly lost or suppressed after the 10 days cold period.

The highest free ACC levels throughout the experiment and the greatest capacity to turn free ACC into N-malonyl-ACC were found in the bolting-resistant cultivar. This cultivar also retained the greatest capacity to respond to drought after a cold period. The cultivar with the highest free ACC levels showed the smallest loss of weight during wilting, at both measuring points.

The connection between bolting resistance, drought resistance and ACC production capacity needs further confirmation in other cultivars.

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(54) EXPERIMENTAL GROWTH ANALYSIS OF CAREX ROSTRATA, CAREX DIANDRA AND CAREX ACUTIFORMIS IN RELATION TO THEIR BIOMASS PRODUCTION IN FENS

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Carex rostrata, Carex diandra and *Carex acutiformis* occur in dense populations in quaking fens in The Netherlands. The first two species have a low annual biomass maximum (250-450 g/m²) and occur in relatively nutrient-poor sites, whereas *C. acutiformis* shows a higher biomass maximum (700 g/m²) in more eutrophic sites.

When the species were grown under controlled conditions in a growth cabinet, their different biomass production was shown not to be the result of inherent differences in relative growth rate (RGR). There were, however, distinct differences in the factors which determine the RGR, i.e. the leaf area ratio (LAR), the leaf weight ratio (LWR), the specific leaf area (SLA) and the net assimilation rate (NAR).

Remarkably, the species of the nutrient-poorest sites, *C. Diandra,* showed the highest NAR and the lowest LAR, whereas the species of the eutrophic sites, *C. acutiformis,* had the lowest NAR and the highest LAR. These values were intermediate for *C. rostrata.*

The higher NAR of *C. diandra* can be explained by a higher chlorophyll content and a higher nitrogen content per m² leaf area, which suggests a higher photosynthetic capacity of this species.

The light climate of *C. acutiformis*, which grows in a higher, more dense vegetation than *C. diandra* and *C. rostrata,* is less favourable than that of the other two species. Several of the characteristics of *C. acutiformis* are similar to those of shade plants.

We think, therefore, that the differences between the three species can be explained by the differences in the vegetational structure of their natural stands.

(55) AMINO ACID UPTAKE BY COTYLEDONS OF EUPHORBIA LATHYRUS SEEDLINGS: REGULATION BY SUCROSE AND SEEDLING AGE

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During germination of *Euphorbia lathyrus* seeds and early growth of the seedling, significant changes take place in the endosperm. Breakdown of reserve proteins and fats is associated with a simultaneous rise in amino acids and sugars, which will be utilized by the growing seedling after uptake by the cotyledons.

Analysis of the soluble products in the endosperm showed that sucrose was the most abundantly occurring sugar. The amino acid composition was rather constant during seedling growth. Arginine, asparagine, glumatine, glutamate and valine accounted for about 50% of the amino acids.

Because of the low uptake capacity of the cotyledons in early stages of growth and a rapid degradation of food reserves, the amino acid and sucrose contents of the endosperm increased progressively and reached their maximum at day 7 and day 10, respectively. Thereafter, the sucrose and amino acid contents declined rapidly. From day 4 to day 10, the sucrose concentration varied between 0.20 and 0.35 M.

The uptake of three different amino acids and sucrose by the cotyledons of intact seedlings was studied in relation to seedling age. The valine and sucrose uptake gradually increased with seedling age and reached their maximum at day 10, just before depletion of the endosperm. In the next few days the uptake capacity for both substrates declined rapidly to zero level. The uptake of glutamate showed essentially the same profile, except that its maximum and subsequent decrease took place at an earlier stage. The uptake of arginine increased slowly in the first week and remained fairly constant until complete depletion of the endosperm. Thereafter, the arginine uptake slowly decreased but still remained substantial.

The uptake of valine was affected by high sucrose concentrations. Different responses were found with excised cotyledons and cotyledons of intact seedlings. Compared with cotyledons of intact seedlings, the uptake by excised cotyledons was reduced. However, the valine uptake by excised cotyledons was partly restored at high sucrose or mannitol concentrations. The valine uptake by cotyledons of intact seedlings was slightly inhibited by low sucrose concentrations.

(56) REGULATION OF CELL RECOGNITION IN CHLAMYDOMONAS EUGAMETOS BY LIGHT

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The heterothallic unicellular green alga *Chlamydomonas eugametos* is a suitable model system to study intercellular communication. As a prelude to sexual fusion, cells of different mating type agglutinate via their flagella. This sex- and species-specific adhesion is mediated by high-molecular-weight glycoproteins ('agglutinins'). In some strains, the agglutinability of the gametes depends on illumination. In the dark, these cells are not agglutinable. Agglutinability is acquired by illumination of 3 W/m^2 within 15 minutes. The flagella of non-illuminated cells contain agglutinin, as shown by gel electrophoresis. When these agglutinin molecules are extracted from the flagella they remain inactive, whereas the agglutinins extracted from adhesive flagella are biologically active. A monoclonal antibody directed against the sexual agglutination site of mating type minus agglutinin is shown to discriminate between active and inactive agglutinins when present in a native state on the flagellar surface. It only binds to the active form. However, the antibody is unable to discriminate between them when they are denaturated in SDS-electrophoresis gels and blotted onto nitrocellulose. Inagglutinable gametes can be partially activated by treatment with low concentrations of glutaraldehyde. These findings suggest that flagellar agglutinability is regulated by an in situ modification of agglutinin on the flagellar surface. The agglutinin-inactivation in the dark is probably a consequence of a change in conformation, whereby the agglutination site becomes inaccessible.

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(57) ISOLATION OF THE QbcCOMPLEX FROM THE THERMOPHILIC CYANOBACTERIUM SYNECHOCOCCUS 6716

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In order to study the Qbc-complex from the thermophilic cyanobacterium *Synechococcus* 6716 in a reconstituted model system, attempts are being made to isolate this complex from the photosynthetic membranes of this cyanobacterium.

The isolation procedure is partially based on the procedure used in our laboratory for the isolation of the ATPase complex from the same organism (Lubberding et al., 1983).

Lysozyme-treated cells (spheroplasts) are osmotically shocked in a buffer of low ionic strength to prepare a suspension of membrane vesicles. The membranes of these vesicles are selectively solubilised with the detergents octylglucoside and sodium cholate.

A partially purified preparation with high duroquinol-cytochrome c oxidoreductase activity but a relatively low sensivity to the inhibitors DNP-INT and DBMIB is then prepared by ammonium sulphate fractionation. The material precipitating at 0-22% ammonium sulphate is resuspended in a minimal volume of buffer supplemented with octylglucoside (30 mM) and sodium cholate (12 mM),resulting in a cyt b-563 concentration of 2-5 μM.

The preparation is further purified by hydrophobic interaction chromatography on a Phenyl-Superose HR 5/5 column (Pharmacia) in a FPLC chromatography system.

The method yields, within 8-10 hours, a functionally active Qbc-complex from the membranes of the thermophilic cyanobacterium *Synechococcus* 6716 with a oxidoreductase activity comparable to that found for chloroplasts (Hurt and Hauska, 1981) and the mesophilic cyanobacterium *Anobeana variabilis* (Krinner et al., 1982).

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Lubberding, H.J., Zimmer, G., Van Walraven, H.S., Schrickx, I. and Kraayenhof, R. (1985) Eur. J. Biochem. 137, 95-99.

(58) APPLICATIONS OF FLUORESCENT PROBES FOR MONITORING ENERGY-LINKED PHENOMENA IN PHOTOSYNTHETIC MEMBRANES

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Our main research is focused on the resolution of bioenergy-transducing mechanisms in photosynthetic and respiratory membranes and simplified reconstituted model membranes derived therefrom. Within this framework we are using several types of commercially available or home-synthesized fluorescent probes for monitoring dynamic structural and functional phenomena related to energy-transducing reactions. We will discuss the following examples of such probes:

(1) Probes for monitoring pH changes inside cells, organelles or proteoliposomes. (a) In reconstituted ATPase proteoliposomes, prepared from a thermophilic, cyanobacterium with extremely good membrane integrity we were also able to quantify established pH gradients and proton translocation kinetics. (b) For measuring the internal pH in intact cells we have synthesized some families of pH-sensitive fluorescent compounds with a dihydropyridine moiety. Being rather lipophilic, these (non-fluorescent) compounds can easily pass a cell membrane after which they can be converted by an intracellular NAD-dependent dehydrogenase or molecular oxygen to a very hydrophylic, impermeant (and fluorescent) pyridinium analogue. (c) For pH measurements inside organelles (chloroplasts, mitochondria) we have developed some lipophilic compounds with pH-dependent fluorescence, which, upon reaction with oxygen, or by slow hydrolysis in water, are converted into very hydrophilic species inside the vesicles.

(2) Probes for monitoring intravesicular oxygen concentrations. The internal light-induced oxygen production could be easily monitored in chloroplasts and intact cyanobacteria with 2-3 times higher sensitivity and much faster response than the oxygen electrode. Moreover, these probes offer interesting tools to study fast kinetics of oxygen evolution in single-turnover flashes.

(3) Probes for estimation of membrane surface charge density and surface pH. There is little information about the size and dynamics of the interfacial pH(and surface potential) profile between membrane surface and bulk solution. In chloroplasts the electrokinetic (zeta) potentials at the plane of shear are definitely different from the 'local' potentials registered by membrane-associated cationic probes and this difference is changed by energization (light or ATP hydrolysis). It appears that the thickness of the layer between bulk solution and membrane surface, in which the pH changes, may not exceed the length of one butylene chain.

(4) Probes for membrane potential changes. In photosynthetic membranes the absorbance of native carotenoids and the extrinsic probe oxonol VI can be used to monitor electric field changes upon (flash) illumination, ATP hydrolysis or relaxation of ion diffusion potentials.

(5) Covalent fluorophore as conformational probe for H^+-ATPase. We have attempted to resolve the kinetics of the conformational change of the membrane-bound ATPase in chloroplasts when these were energized by light. This conformational change is possibly related to the latent active transition, required for ATP synthesis and affinity changes for adenine nucleotides. The fluorogenic label fluorescamine was highly suitable for this purpose. Interestingly, these kinetics are very similar to those of the membrane surface charge rearrangements, as observed with membrane-bound cationic fluorescent probes.

(6) Fluorescence polarization probe for determining lipid phase behaviour. We have used the probe 1,6- diphenyl-1,3,5-hexatriene for determining the phase behaviour of the major lipids of thermophilic cyanobacteria in order to establish the role of non-bilayer-forming lipids (e.g. MGDG) and the effects of protein insertion.

(59) PHYSIOLOGICAL PLASTICITY IN VARIETIES OF WHEAT AND BARLEY, DIFFERING IN SALT RESISTANCE: RESPONSES OF GROWTH AND RESPIRATION AND THEIR POSSIBLE CONNECTION WITH CYTOKININS

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Several varieties of barley and wheat were subjected to growth conditions with and without salt (65 mM NaCl) in their nutrient solution. The growth of hydroponically grown plants was followed on individual plants by measuring root volume and total fresh weight during the experiment.

In general a clear distinction was observed between salt-resistant and salt-sensitive lines concerning their growth responses to salt. Salt-resistant plants demonstrated an immediate lower R(elative) G(rowth) R(ate), especially in shoot tissues upon the addition of salt, which caused a shift in the S(hoot)/R(oot) ratio. In the second period of the experiment we observed a recovery of the RGR, but the altered S/R ratio was maintained. However, plants of salt-sensitive varieties did not show any response after the addition of NaCl for a period of circa 7 days. After that period, the RGR's of shoot and root dramatically decreased, whereas the S/R ratio did not change. The results of root respiration measurements were in close agreement with the growth data.

The following hypothesis will be tested and reported on: salt-resistant plants of barley and wheat possess a cytokinin metabolism, which is in some way sensitive to salt. Salt will lower the production of this plant hormone, leading to growth reduction, which should be interpreted as having regulatory and adaptive value. The insensitivity of the cytokinin production in salt-sensitive plants is considered to be the reason of the unaffected growth after the addition of salt. In these varieties actual salt damage will slow down growth rates.

If this hypothesis is valid, the salt-resistant variety might temporarily alter into a salt-sensitive one by addition of cytokinins to the nutrient solution. This treatments should postpone the growth reduction just as in the salt-sensitive variety. The experiments, as described above, are being currently carried out.

(60) DISTRIBUTION OF THE LATHYROTOXIN ODAP IN MATURE LATHYRUS SATIVUS PLANTS

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Lathyrism is a neurological disease induced by overconsumption of seeds of *Lathyrus sativus* (chickling pea). There is ample proof that the non-protein amino acid ODAP (β-N-oxalyl-L-α, β-diaminopropionic acid) is the cause of this crippling disease (Rutter and Percy, 1984, Spencer et al., 1986). We have previously studied the levels of ODAP during the germination and development of *L. sativus* (Kuo et al., 1987). In this paper we report the distribution of ODAP in mature seed-bearing plants.

Seeds of *L. sativus* cv Dharmapur were grown in the green house (5 seeds per pot with diameter 18 cm, at 20-25°C) and harvested after three months. From a branch on the flowering plant each internode was separated into different parts and 70% ethanol extracts were analyzed for amino acids. The neurotoxin ODAP, homoserine and an unknown compound X (possibly O-acetyl-homoserine) were quantitatively the most important amino acids in all the extracts. Extremely high concentrations of ODAP were detected in the pericarp (120 µmol/g.fr.wt) followed by immature seeds (39 µmol/g. fr.wt). Leaves contained higher ODAP concentrations than the stems and the younger leaves or stems showed higher concentrations than the older ones. The highest concentration of homoserine was found in the fruit: pericarp (50 µmol/g.fr.wt) and immature seeds (50 µmol/g.fr.wt). Unlike ODAP and homoserine the unknown X was concentrated in the stem part of the plant, especially in the new-growing stem. The UV-sensitive isoxazolinone derivatives which were major compounds in seedlings of *L. sativus* were not detected in the mature plants.

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*G. Ongena is recipient of a IWONL fellowship.

(61) THE OCCURRENCE OF ISOXAZOLINONES IN VICIEAE SEEDLINGS

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Non-protein amino acids and some other metabolites containing the isoxazolin-5-one ring are formed in high concentrations during the germination of peas (*Pisum sativum*) and sweet peas (*Lathyrus odoratus*) (Lambein et al., 1976). The isoxazolin-5-one ring is very sensitive to UV-light and to high pH-values, and is probably formed from asparagine. Ten different compounds have been chemically characterised up till now. A great variety of legume seedlings have been screened for the presence of these metabolites, and it was found that only species belonging to the *Vicieae* contained detectable amounts.

We examined 11 species of *Vicia* (7.5% of the genus), half of which contained β-isoxazolin-5-on-L-alanine (I). The genus *Pisum* is very homogenous in this respect: all species and varieties examined contain high concentrations of I (± 2% of the dry weight) and smaller amounts of 2-glucosyl-isoxazolin-5-on-4-yl)- alanine (III) and very small amounts of 2-glucosyl-isoxazolin-5-one (IX). All *Pisum* seedlings also contain the uracilyl-alanines willardiine (IV) and isowillardiine (II) which do not occur in other members of the *Vicieae*. The isoxazolinone compounds from *Pisum* also occur in all *Lens* seedlings in comparable amounts together with the γ-glutamyl peptide of I (XI) and one or more unknown isoxazolinone derivatives.

In 52 species of *Lathyrus* (35% of the genus) we found a variable composition of isoxazolinone derivatives and other non-protein amino acids (some of them toxic). This variability (and toxicity) in the seedling stage is greater than in the dry seeds (Bell, 1962). 80% of the *Lathyrus* contain at least one isoxazolinone, *L. odoratus* contains nine. The distribution of isoxazolinones and other non-protein amino acids in this genus gives mostly unique combinations of these metabolites.

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Lambein, F., Kuo, Y.H. and Van Parijs, R. (1976), Heterocycles 4, 567-593.

(62) DISTRIBUTION OF LECTINS IN SOME DICOTYLEDONOUS PLANTS

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Lectins represent a heteogenous class of proteins sharing a common property: the ability to recognize and bind carbohydrates in a highly specific and selective way. They are not only found in higher plants but also in bacterial, fungi, lichens, invertebrates, and even in mammalian tissue. Although many aspects of their action on foreign, mostly animal cells, is well-documented the function of plant lectins in the plant's physiology is still a matter of debate.

We studied the distribution of lectins in several dicotyledonous plants by using an indirect immunocytochemical labelling technique. This included sequential incubation of cryo-sections of the tissue with polyclonal rabbit anti-lectin antibodies and with goat antibodies directed against rabbit immunoglobulins. The latter were fluorescein-labelled for light microscopy or adsorbed on colloidal gold particles for electron microscopy. For the labelling in the strongly autofluorescent *Datura* seeds we used Texasred in combination with the avidin-biotin staining procedure.

In all cells of root stocks of *Aegopodium podagraria* L. specific fluorescence was associated with the cytoplasm and the nucleus. The cell wall, intercellular spaces and nucleolus were less labelled.

A similar localization of lectins was observed in the cells of root stocks and root tips of *Bryonia dioica* L., the latter, however, showing the highest concentration of lectin in the cell wall.

In root stocks of *Urtica dioica* L. not all cells showed the same distribution. In some, fluorescence was distributed throughout the cytoplasm, in others, it was restricted to small particles in the cytoplasm. Specific labelling was weak in the cell wall and in the intercellular spaces.

In *Datura stramonium* L. seeds the highest concentration of lectin was found in the peripheral layers of the seeds. The seed coat and the epidermis surrounding the endosperm were intensely stained, whereas the endosperm itself was completely devoid of lectin. Electron microscopic localization in the seed confirmed these results. In the embryonic tissues (cotyledons and primary axis) a low level of specific fluorescence was found in small inclusions in the cytoplasm.

Localization studies as described here indicate where the lectins are present in the cell and in the plant at the moment of sampling. Any proposed physiological function of lectins based on biochemical research has to be consistent with the localization of the proteins in the cell.

(63) CHANGES IN VALINE UPTAKE DURING PEA SEED DEVELOPMENT

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During the development of the pea seed (*Pisum sativum* L. cv Marzia) the growth rate of the cotyledons, measured as an increase in dry weight, changes permanently. It is not known whether this change is accompanied by changes in the uptake characteristics of the assimilates. The aim of the present study was to determine the kinetics of L-valine uptake by isolated cotyledons.

The results show that the best way to describe the uptake of valine is to postulate three uptake components: two saturable components, one with a high affinity (Km_1) and one with a low affinity (Km_2) for the substrate, and a linear component, giving the following equation: $v=Vmax_1*S/(Km_1+S)+Vmax_2*S/(Km_2+S)+K_{lin}*S$. The parameters of the uptake isotherm change during development. For young cotyledons (19 days after flowering) the following values were calculated: $Vmax_1$=not detectable, Km_1=n.d., $Vmax_2$=19.5, Km_2=14.0, K_{lin}=2.5. The values for older cotyledons (31 d.a.f.) were: $Vmax_1$=0.6, Km_1 = 0.09, $Vmax_2$=169, Km_2=6.3, K_{lin}=1.6. (Vmax:nmol/(gFW*min), Km:mM, K_{lin}:nmol/(mM*gFW*min)).

The uptake of valine by young cotyledons can be described by the equation but at concentrations below 250 µM the uptake isotherm is sigmoid. This phenomenon can be explained by the fact that isolated cotyledons leak amino acids from their internal pool into the bathing medium. The uptake of labelled valine is inhibited competitively by those amino acids, leaking in great quantities from the young cotyledons during the uptake period.

These results may imply that at an early developmental stage valine is taken up predominantly by the linear component ($v=K_{lin}*S$), whereas at later stages uptake occurs predominantly by the saturable components.

(64) PARAMETRIZATION OF THE CO_2-EXCHANGE CYCLE OF CAM-PLANTS

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Crassulacean acid metabolism (CAM)-plants show a daily CO_2-exchange cycle composed of four distinct phases (numbered I-IV): (I) the nocturnal CO_2-uptake, (II) the burst of CO_2-fixation after the start of the light period, (III) the decarboxylation phase and (IV) a final increase in CO_2-fixation at the end of the day (Kluge et al., 1982). Therefore, integration of CO_2-exchange data over a whole day is necessary to estimate the plant's rate of CO_2-assimilation in response to environmental and genetic influences.

Net CO_2-exchange rates were measured in an open gas exchange system with a diffefential infrared gas analyser (ADC, type 225-MK3). Continuous registration using a multipoint datarecorder (Philips, type MP 8237A) provided a set of points which, after digitizing (Calcomp 9000) and calibration for 289 fixed time intervals, yielded series of standardized curves. From these, several parameters can be calculated, e.g., net CO_2-assimilation over 24 hours, during the night, during light, during the burst of CO_2-fixation and during phase IV; duration of phase II and phase IV; the maximal nocturnal net CO_2-uptake rate and the time at which this occurs.

This methodology was applied on CO_2-exchange data of four *Aechmea*-cultivars, grown under different conditions. Significant differences were found for all parameters mentioned above. The proposed methodology seems to offer applications for studying genetic and environmental influences on CO_2-exchange. The parameters could also be used in descriptive regression models for studying relations between the distinct phases of the CO_2-exchange cycle such as a possible relation between net CO_2-uptake during phase IV and the time of maximal nocturnal CO_2-uptake rate.

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(65) EFFECTS OF SHORT-TERM H_2S AND SO_2 FUMIGATION ON THE SULFUR METABOLISM OF SPINACH PLANTS

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Fumigation of spinach with H_2S or SO_2 for one to six days resulted in accumulation of sulfhydryl (SH) compounds in the shoots of H_2S- and SO_2-exposed plants. The sulfate concentration in the shoots of the SO_2-exposed plants increased linearly with time. SH-accumulation showed saturation kinetics as a function of time as well as H_2S-concentration, which were ascribed to the internal H_2S-concentration in the plant and the availability of substrates for GSH synthesis, respectively. SH-compounds accumulated more at lower exposure temperatures, whereas sulfate accumulation was more pronounced at higher temperatures. These results are discussed in relation to the possible foliar uptake of H_2S and SO_2, the temperature-dependence of uptake and the water-solubility of these gases. An exposure to 0.25 $\mu l\ l^{-1}$ SO_2 or H_2S for 24 h did not affect the transpiration of spinach. The possibility of SO_2- induced H_2S emission rather than sulfate accumulation as a source for SH-accumulation is also discussed. Cessation of the fumigation resulted in a decrease in SH-compounds and sulfate content, which could be accounted for by S-metabolism and growth, respectively.

(66) THE ACC METABOLISM IN ISOLATED BEAN SEEDLING SEGMENTS

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S-adenosylmethionine (SAM) and 1-aminocyclopropane carboxylic acid (ACC) are intermediates in the biosynthetic pathway of ethylene (Adams and Yang, 1977, 1979). We have studied the influence of exogenously applied ACC or SAM on the ACC metabolism of isolated segments of 8 days old etiolated bean seedlings (cotyledons, primary leaves, epicotyls with apex, hook regions, lower part of the hypocotyls and roots). After 24 h of incubation with or without ACC or SAM, the ethylene production was measured and the ACC and MACC content analysed. Isolated epicotyls in a control series produced during this period the highest level of ethylene (105 $nl.24h^{-1}.g.fr.wt^{-1}$) in comparison to the other tissues studied. Due to this incubation period the ACC content of the leaves, roots and cotyledons increased. The MACC concentration increased especially in the epicotyl. Exogenous added ACC can be converted both to ethylene and MACC in each isolated segment. At the saturated ACC concentration for maximal ethylene release (10^{-3}-5.10^{-3} M ACC) we were able to measure the ethylene forming enzyme (EFE) activity of the tissues by recording the ethylene evolution. After ACC application leaf tissue produced high amounts of ethylene (6240 $nl.24h^{-1}.g.fr.wt^{-1}$), while cotyledons and hooks formed high levels of MACC. After SAM addition the ethylene production increased in the epicotyl, hook and root tissue. The ACC content in the leaves and the hook region was stimulated by the SAM concentration which gave maximal ethylene release (10^{-4}-10^{-3} M SAM), while the MACC level also augmented in the epicotyl and the roots under these conditions.

These results show that ACC synthase is most active in isolated leaves, epicotyls and hook regions. Leaves and epicotyls have a great EFE activity, while the hook and the cotyledons better convert ACC to MACC.

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(67) DEVELOPMENT OF CALLUSES AND SHOOTS FROM POTATO PROTOPLASTS: INFLUENCE OF SUCROSE AND POTASSIUM DISULFITE

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Great variation in the regeneration capacity exists between different plant clones of potato. This is often correlated with differences in tissue browning *in vitro*, probably caused by derivatives of (poly) phenolic compounds (quinones). The influence of high sucrose concentrations in the medium on the production of these compounds has been described in literature.

In our experiments it appeared that the addition of potassium disulfite (an inhibitor of the conversion of phenolic compounds into quinones) caused a decreased browning of calluses at an early stage. This indicates that turnover of phenolic compounds plays a part in tissue browning in potato. (Determination of the (poly)-phenolic compounds in calluses is in progress).

Lowering the sucrose level resulted in decreased tissue browning and increased shoot regeneration from protoplast-derived calluses of di(ha)ploid potato plant clones.

(68) CLONING AND CHARACTERIZATION OF MRNA'S DIFFERENTIALLY EXPRESSED IN T-DNA (T-CYT GENE) TRANSFORMED VERSUS NORMAL TOBACCO PLANTS

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Plant cells transformed by the T-DNA from the Ti plasmid of the soil bacterium *Agrobacterium tumefaciens* provide an interesting model system for studies on the molecular basis of plant morphogenesis. The morphogenetic properties of such plant cells are determined by two loci on the transforming T-DNA, the auxin locus and the cytokinin locus, which code for the biosynthesis of the plant hormones auxin and cytokinin, respectively. When both loci are active, the transformed cells are forced to develop into autonomously proliferating callus tissue which is not capable of differentiation. When, however, only the auxin locus or only the cytokinin locus is active, the cells develop into tissues which spontaneously differentiate abnormal roots or shoots.

In vitro cultured tobacco shoots resulting from transformation of tobacco cells by T-DNA with an active cytokinin locus (T-cyt gene) and an inactive auxin locus lack apical dominance (strongly reduced length, numerous adventitious sprouts) and are unable to form roots. This aberrant phenotype is caused by the cytokinin stress imposed on the shoots by the T-cyt gene. In search for plant genes which are regulated by the high endogenous cytokinin level and involved in the establishment or maintenance of the aberrant phenotype, we screened cDNA libraries of *in vitro* cultured transgenic T-cyt shoots and normal tobacco plants by differential hybridization. Thus far we have isolated five cDNA clones (pCNT 1 to 5) of mRNAs that have a much higher steady state level in T-cyt transformed shoots than in the shoot part of normal plants. Surprisingly, the pCNT1, 4 and 5 mRNA levels are also particularly high in the root part of normal plants. The pCNT1, 2 and 3 mRNAs can be induced in normal shoots to levels comparable to those found in T-cyt transformed shoots by culturing the shoots on cytokinin-containing medium. Clones pCNT3, 4 and 5 were found to correspond to mRNAs which are induced in tobacco leaves upon infection with tobacco mosaic virus (Hooft van Huijsduijnen et al, 1986): pCNT 3 mRNA codes for a protein belonging to the PR-1 group of pathogenesis-related proteins, pCNT 4 mRNA for chitinase and pCNT 5 mRNA for a yet unknown protein. In contrast, the pCNT 1 and 2 mRNAs are not induced in leaves after virus infection. Their possible involvement in plant growth and differentiation processes is currently under investigation.

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(69) DE NOVO GA SYNTHESIS IN DE-ETIOLATING PHASEOLUS VULGARIS L. SEEDLINGS

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Many enzymes in etiolated plants have been reported to increase in activity in reaction to illumination. This is the case, for instance, for an (the) enzyme(s) essential for kaurene synthesis in pea seedlings (Gomez-Navarette and Moore, 1978). We have studied the involvement of ent-kaurene and thus GA-synthesis in the leaf expansion of *Phaseolus vulgaris* seedlings induced by white light.

First, the effects of exogenously applied GA_3 and/or AMO 1618 - an inhibitor of ent-kaurene synthesis - on the light-induced leaf expansion were studied. In plantlets having their hook region in the epicotyls, AMO, when applied from the onset of de-etiolation, significantly inhibited this process. GA_3 counteracted the inhibitory effect, but did not enhance leaf expansion when applied alone. There was no effect of AMO when given several hours after de-etiolation. Next, we determined the ent-kaurene synthesizing capacity in cell-free extracts of leaves and epicotyls. Although the rate of synthesis of the GA-precursor was low, a peak was found at about 4 hours after the onset of illumination in the epicotyls, and after 9 or more hours in the leaves. Our results suggest that GA synthesis is one of the prerequisites for light-controlled leaf expansion.

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(70) IS DE NOVO GA SYNTHESIS INVOLVED IN CEREAL LEAF UNROLLING?
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Gibberellins are involved in several photomorphogenetic phenomena. For instance, cereal leaf unrolling is a phytochrome-mediated process accompanied by a rapid increase of the acidic ethyl acetate soluble GAs (for a review see Smith, 1980). Klein et al. (1963) reported the unrolling process to be accompanied by alterations in starch metabolism.

We studied the unrolling of *Zea mays* leaves. Attention was focused on the possible involvement in the expansion process of GAs synthesized *de novo*, and of starch breakdown.

The effects of AMO 1618, tetcyclacis (BASF) (both inhibitors of GA synthesis) and of norflurazon (a bleaching herbicide from Sandoz) appeared to inhibit the unrolling of leaf sections.

The time-dependent ent-kaurene synthesizing capacity in cell-free extracts derived from leaves during the de-etiolation process was also determined. An initial phase of rapid decrease was followed by a gradual increase leading to a maximum about 7 hours after the onset of light. Ent-kaurene synthesis was not markedly reduced by norflurazon. Starch breakdown was demonstrated in de-etiolating leaves and leaf sections by electron microscopic, enzymatic and chemical methods. Whether GA synthesis, starch breakdown and leaf unrolling are causally related processes remains to be determined. Our results suggest that the unrolling of maize leaves depends on *de novo* GA synthesis and/or on process(es) occurring in chloroplasts.

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Smith, H. (1980), British Plant Growth Regulator Group, Monograph 5, 95-109.

(71) GA SYNTHESIS IN CROWN GALL OF NICOTIANA TABACUM L.
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The presence of GAs (or GA-like substances) has been demonstrated in several kinds of tobacco calluses as well as in crown galls (Park et al., 1984). However, their function in regulating growth and differentiation of these tissues is still unclear.

We were interested in the *de novo* GA-synthesis of tobacco crown galls. First, we tried to determine the capacity of cell-free systems to synthesize the GA-precursor ent-kaurene from mevalonate. Separation of the reaction products could not be achieved by conventional methods, so that a sample clean-up procedure followed by HPLC was developed. By this method, ent-kaurene synthesis could be demonstrated in shoot forming crown galls. A maximum was reached about 3 days after subculture.

To get an insight in the role of GAs in this material exogenously applied GA_3 and two growth retardants interfering with GA synthesis, tetcyclacis and LAB 150 978 (both from BASF), were studied with regard to their effects on growth, morphology and chlorophyll content. Neither GA_3, nor the inhibitors significantly affected the increase in fresh weight of the crown galls. As for morphology, GA_3 had a remarkable influence upon the shape of the 'leaves'. The effect could partially be overcome by both of the retardants. Chlorophyll content was increased as a result from tetcyclacis treatment, the effect being counteracted by GA_3. Our data suggest an influence of tetcyclacis and LAB 150 978 upon GA action, and a possible involvement of GA in chlorophyll synthesis and/or breakdown. Alternatively, GA_3 and the retardants might compete for being taken up.

Park, K.H., Fujisawa, S., Sakurai, A., Yamaguchi, I. and Takahashi, N. (1984), Plant Cell Physiol. 25, 1303-1306.

(72) MICROTUBULE ORGANIZATION DURING INTERPHASE AND MITOSIS IN CULTURED MESOPHYLL PROTOPLASTS OF HIGHER PLANTS. AN IMMUNOFLUORESCENCE MICROSCOPIC STUDY
Eltjo G.M. Meijer* and Daina H. Simmonds, Plant Research Centre, Agriculture Canada, Ottawa, Canada.

Microtubule organization in mesophyll protoplast cultures of *Medicago sativa* L. (alfalfa, lucerne) and *Nicotiana tabacum* L. (tobacco) was investigated by means of indirect immunofluorescence in combination with simultaneous staining of DNA with Hoechst 33258 and cell walls with Calcofluor White. Prior to mitosis a very dense network of rather fine cortical microtubules developed. In cultures of *M. sativa* this dense array of microtubules was not formed in the absence of auxin. Protoplasts isolated from recalcitrant *Medicago* genotypes which did not divide in culture failed to produce the dense network. A prophase band of microtubules appeared at the interphase-prophase transition and was present until late prophase when the spindle was formed. Telophase was characterized by the presence of a phragmoplast and, during the later stages, by the beginning of the re-establishment of interphase microtubule networks. Aberrant microtubule organization during karyokinesis and cytokinesis and the resulting mitotic abnormalities were frequently observed in cultures of both species.

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(73) SOME ASPECTS OF THE DEVELOPMENTAL PHYSIOLOGY OF SOMATIC EMBRYOGENESIS IN MEDICAGO SATIVA L.- ETHYLENE AND POLYAMINE SYNTHESIS DURING THE DIFFERENTIATION PROCESS

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In vitro regeneration in *Medicago sativa* is strongly genotype-dependent. Extensive genotype screening and medium manipulations allowed the development of a novel rapid high frequency somatic embryogenesis system in *M. sativa* in which the first embryos can be discerned 15 days after the explant (leaf, periole) is put into culture (Meijer and Brown, 1985, 1987). Explants were cultured on a medium with 2,4-D for 10 days for embryo induction followed by 20 days on a basal medium for embryo formation. Ethylene and polyamine synthesis were monitored during the first 16-20 days in culture by means of gas and high pressure liquid chromatography, respectively. During embryo induction there were rapid increases in the rates of ethylene and putrescine biosynthesis. These rates of biosynthesis began to fall after transfer of the cultures to basal medium. The rate of growth, however, increased following transfer to 2,4-D free medium. Spermidine and spermine levels were relatively low and there were only minor fluctuations during the culture period under investigation. Exposure of the cultures to inhibitors of ethylene and putrescine biosynthesis during the first 5-6 days on basal medium resulted in decreased levels of these substances as well as inhibition of somatic embryo formation (but not of tissue proliferation).

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(74) SHOOT AND ROOT GROWTH IN ZEA MAYS L. EFFECT OF SOIL COMPACTION

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The growth of aerial parts of maize appears to be very sentitive to conditions affecting root growth and development. Soil compaction due to repeated passages of heavy machinery is suspected to affect growth and dry matter yield. To obtain quantitative assessments of these effects soil was systematically compacted with a tractor in small plots layed out in a complete randomized blocks design with 4 replications. Treatments took place after ploughing and before seedbed preparation. In 1985, the factors studied were the intensity of compaction (weight of tractor; the total surface area of treated plots was compacted) and the soil humidity at the time of treatment (21% and 26% soil water content, on a fresh weight basis). Soil type was AbaO. In 1986, two soil types (Aba (b), and Gcp according to the Belgian classification) were investigated, and compaction was applied to various proportions of the total plot surface area. Compaction was monitored using a penetrometer (pezograph) equipped with a device permitting graphical recording. Excavations at different depths were made, roots were washed from soil monoliths (Böhm, 1979) and dry weight distribution with depth and position between rows was determined. In 1986, root length was also estimated according to Newman (1965).

In 1985, seedling emergence was delayed by three days (relative to controls) in plots compacted under more humid conditions. Final population density was 20% lower, silking occurred with a 7 days delay, plants were 22 to 43% smaller (according to time) and total leaf area (at final harvest in October) was decreased by 23%. Final dry matter production was reduced by 41% (aerial parts) or 46% (ears only). All these results were highly significant ($p \leq 0.01$). Measurements obtained in 1986 indicated a decrease of 41% in final total dry matter (aerial parts) or 21% in ear dry weight (per ha), when the whole surface area of the plot was compacted (Aba (b) soil). This corresponded to a more superficial root system: 82% of total root length was in the upper 10 cm (below soil surface), against 39% in the controls. A higher lateral development was also observed in compacted plots (in middle of the row between 5 and 10 cm depth we observed 5,4 cm root length/cm^3 soil volume in treated plots and 0.9 controls). Similar observations were made in the experiments conducted in 1985. They also corresponded to the conclusions reached by Grimes et al. (1975). The results were, however, influenced by the type of soil: in the Gcp soil, the decrease of total dry weight (aerial parts) was much lesser (13%, significant $p \leq 0.05$), and although initially root growth was slower, the differences had disappeared at anthesis.

Thus roots appear to be sensitive to soil compaction, but the effects depend on factors such as soil type or soil humidity at treatment time. An important part of the shoot dry weight (yield) variation from field to field might be attributable to effects at the root level. For this reason, more attention should be paid to the physiology of roots.

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(75) CELL RECOGNITION IN THE GREEN ALGA CHLAMYDOMONAS EUGAMETOS

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One of the best systems for studying cell recognition in plants is the mutual recognition between compatible *Chlamydomonas* gametes, that precedes sexual reproduction. This single-celled alga can be mass-cultivated as vegetative cells on an agar containing mineral medium and then, when flooded with water, all the cells generate two flagella and swim free from the agar as sexually competent gametes.

The isogamous sexes, referred to as mt^+ and mt^-, when mixed, immediately agglutinate together via components exposed on their flagellar membranes. These so called agglutinins have been identified and characterised as huge (1.3×10^3 kDa) linear glycoproteins (50% sugar) that are extrinsically attached to the membrane. Agglutinins involved in sexual adhesion induce the formation of an intracellular signal (cAMP) which in turn, triggers the outgrowth of a mating structure through the cell wall, between the two flagellar bases. Gametes eventually fuse in pairs by these mating structures, but first they have to be positioned exactly opposite each other. This positioning is accomplished by two flagellar properties as will be illustrated: (a) agglutination sites first involved in agglutination are transported to the flagellar tips; (b) the flagella, which now can adhere over their entire length, assume a specific orientation, positioned around the mt^- cell body. This brings the cells into a vis-à-vis position with the mating structures in direct apposition. Cell fusion is initially limited to the formation of a plasma bridge. The cell-tandems deagglutinate and resume swimming, using only their mt^+ flagella. Within 48 h, the pair has settled on the substrate and the two cell bodies have fused completely to form a zygote.

(76) MEASUREMENTS OF KINETIC PARAMETERS OF HERBICIDE BINDING

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Many widely used herbicides act by blocking the photosynthetic electron transport between photosystem II and the PQ-pool. They displace the secondary quinone acceptor Q_B from its binding site, and thus prevent the reoxidation of the primary acceptor Q_A. The binding site is located on the 32-kD protein of PSII. Compounds of different chemical nature, e.g. DCMU, atrazine and dinoseb, all display similar modes of action.

When a herbicide is bound to the Q_B-site, the reaction center can make one more turnover under the formation of Q_A^-, and then is blocked. Most herbicides are exchanged with Q_B on a seconds time-scale (Vermaas, 1984). In this study the exchange parameters of herbicides of different classes are calculated. These parameters can give more insight in the working mechanism of PSII-herbicides.

The kinetic parameters are calculated using the flash-induced oxygen evolution patterns, measured in isolated broken chloroplasts of *Chenopodium album*. As predicted by the Kok S-state model (Kok et al., 1970) these patterns show a periodicity of 4 in the amplitude of oxygen production, caused by the stepwise oxidation of water to oxygen. This oscillation is damped as a result of the misses and double hits of the RC by the excitation light. In thoroughly dark-adapted thylakoids almost all of the RC's are in the stable S_1-state, and must make 3 turnovers before oxygen is released. When a slowly exchanging herbicide is present, with a residence time on the binding site in the same order of magnitude as the duration of the flash train, the probability of exchange with Q_B during a train of 10 consecutive flashes is low. This means that some RC's will remain locked during the experiment. The net oxygen production is lowered, but the pattern stays identical. When a more rapidly exchanged herbicide is added, the displacement probability during the flash train is higher and RC's can switch between the blocked and free state. This means that they will get out of phase with each other, and an increased damping of the oscillation of oxygen production is seen. The kinetic parameters of the exchange can be calculated by comparing patterns obtained for different flash frequencies and herbicide concentrations. For a description of the model, and the results obtained with this method, see Naber and Van Rensen, (1987).

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(77) TRITERPENOL BIOSYNTHESIS AND ACCUMULATION IN EPIDERMIS AND MESOPHYLL OF ILEX AQUIFOLIUM LEAVES

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Leaves of *Ilex aquifolium* L. accumulate the triterpenols α- and β-amyrin as native compounds and as esterified to long-chain fatty acids. The esters are to a high degree concentrated in cytoplasmatic lipid globules found in epidermis and mesophyll cells. Free triterpenols are found in all tissues, but accumulate on the leaf surface in the cuticle and epicuticular wax.

Selectivity in the utilization of different labeled precursors indicated the existance of separate sites for triterpenol and triterpene ester synthesis (Niemann, 1985). Different rates of synthesis were found for both processes (Niemann and Koerselman-Kooy, 1987).

The synthesis of triterpenols is mainly located in the upper epidermis. Accumulation of triterpenols starts with that of β-amyrin at a leaf age of around 3 weeks and is largely completed, with α-amyrin as major constituent, at the age of 15 weeks.

A very active ester biosynthesis was found in both epidermis and mesophyll of 3 weeks-old leaves. In these leaves a large accumulation of esters of both α- and β-amyrin (ratio 2.8) had already occurred, indicating a very early start of ester biosynthesis. Triterpene ester synthesis is still present in leaves of 1 3/4 years old.

In spite of the triterpenol synthesis in the upper epidermis, the triterpene ester pool seems to be a major source of the free triterpenols accumulating in the (chloroform-soluble) epicuticular wax.

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(78) PHENOL METABOLISM IN HIGHER PLANTS: RELATION WITH RESISTANCE AGAINST VASCULAR WILT IN CARNATION

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In many plants, environmental signals as light, wounding and fungal attack control the levels of enzymes involved in secondary phenolic biosynthesis. These signals lead to variation in the diversion of aromatic amino acids into soluble phenolic glycosides and esters, and into phenolic polymers in lignin and suberin. Enhanced production of phenolic derivatives is a nearly universal plant response to infection by pathogens, and as such, phenolics are believed to play a role in many resistance mechanisms. Phenol-derived compounds are considered to be directly toxic to the pathogen or are incorporated into structural barriers as, for instance, phenol-conjugated, lignified or suberized cell walls.

In vascular wilt of carnation, caused by the fungus *Fusarium oxysporum* f.sp. *dianthi*, the physiological response of the host (*Dianthus caryophyllus* L.) varied with the degree of susceptibility (Baayen and Elgersma, 1985). In resistant cultivars, vascular infection induced gel formation in colonized and surrounding vessels.

The infusion of phenolics, followed by oxidation, is held responsible for the orange to dark brown colour change of the gels, which were otherwise composed of various polysaccharides. Adjacent primary cell walls appeared to become slightly lignified. Later on, the infected area also became surrounded by periderm tissue, and vascular regeneration took place. In susceptible cultivars vessels were rarely occluded with gel. Vascular browning was observed which appeared to be caused by discolouration of primary walls at the edges of colonized tissue. In addition, infection with *F. oxysporum* can induce the carnation to produce a number of new, fungitoxic compounds (phytoalexins) of phenolic nature (Ponchet et al., 1982). This phenomenon may be more pronounced in resistant varieties. Preliminary investigations indicated that resistant and susceptible cultivars also may differ in cell-wall(?)-bound phenolics found after infection.

It is hypothesized that phenol metabolism forms an integral part of the complex polygenic resistance mechanism of carnation against *F. oxysporum* f.sp. *dianthi*.

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(79) MODIFICATION OF THE PHOTOSYNTHETIC MEMBRANE

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Thylakoid membranes isolated from spinach and pea leaves were fragmented by a Yeda-press treatment of 10 MPa. According to Åkerlund and Andersson (1983), the disruption of thylakoid membranes leads to the formation of right side-out photosystem I and inside-out photosystem II enriched thylakoid vesicles.

This vesicle population was separated in an aqueous two phase polymer system. Separation occurs according to different surface properties (Albertsson et al., 1982; Andersson et al., 1985).

The orientation of the membranes was tested by measuring the light-induced pH-changes in the medium. The oxygen evolving complex is normally located at the side facing the thylakoid-lumen. The proton liberation accompanying the water splitting can thus be measured as a pH-decrease in a suspension containing inside-out orientated photosystem II vesicles.

The two types of vesicles were further characterized by: (i) measuring their electron transport capacity using different combinations of electron donors and acceptors; (ii) measuring the electrochromic P515 signal which gives information about the membrane energization and the integrity of the vesicles.

Studies with inside-out vesicles will lead to more detailed information about kinetic aspects of the proton release inside the thylakoid lumen, because the protons can now be measured directly in the surrounding medium.

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(80) PLANT REGENERATION THROUGH SOMATIC EMBRYOGENESIS FROM NORMAL AND AGROBACTERIUM RHIZOGENES-TRANSFORMED ROOTS IN THE TETRAPLOID POTATO CULTIVAR BINTJE

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A procedure resulting in genetically stable plant regeneration is highly desirable for successful application of genetic manipulation methods in plant breeding. In the tetraploid potato cv. Bintje plant the regeneration process from shoot culture-derived protoplasts was highly unstable: 60% of the regenerated plants were aneuploid or octoploid (Bokelmann and Roest, 1983; Sree Ramulu et al., 1986). By contrast, the long-term cultures of root lines transformed by *Agrobacterium rhizogenes* and plants regenerated from them were found to be genetically stable (Hänisch ten Cate et al., 1987). It has been suggested that the Ri-plasmid can be used as a virulent DNA vector in the binary genetic transformation system (Hoekema et al., 1985). In this context we developed an efficient regeneration procedure for *A. rhizogenes*-transformed root lines of cv. Bintje.

A regeneration procedure consisting of three distinct steps each lasting three weeks was developed with root segments of 0.5 to 1.0 cm long after certain modifications of the procedure described by Ooms et al., (1985). a). The callus phase required at least a minimum of three weeks. Sufficient callus formation and subsequent shoot development were obtained on MS medium containing 2% sucrose and supplemented with 2,4 D and zeatin. The presence of both 2,4 D and zeatine during this phase was essential for shoot induction. b). The shoot induction phase required three weeks in addition. In the shoot induction medium the sucrose concentration was 3% and 2,4 D was absent. In addition, zeatin was replaced by BAP and GA_3 was added. Several shoots developed on the green compact calli. c). During the shoot development phase, it was observed that optimal growth of shoots occurred on liquid MS medium without hormones. The shoot development was poor on solid medium.

2,4 D-induced green compact calli on both sides of root explants, whereas NAA induced light brown calli throughout the surface of the roots. In the shoot induction phase somatic embryoid structures were observed on the green calli of roots, whereas calli from leaves and shoot protoplasts form shoots from adventitious buds. These results suggest that the genetic stability of the regeneration procedure from *A. rhizogenes*-transformed root lines is caused by the regeneration via somatic embryogenesis.

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(81) INTERACTION OF HORMONES WITH REGARD TO IN VITRO FLOWER BUD FORMATION IN TOBACCO

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Both cytokinins and auxins are required for flower bud formation in tissue explants of tobacco. Earlier investigations have indicated that the cytokinin concentration, in particular, determines whether flower buds are formed (Van den Ende et al., 1984[a,b]). The auxin concentration mainly determines the position of the buds on the explants, e.g. polar versus non-polar bud formation, as well as callus growth.

This study deals with the interaction between these classes of hormones with respect to flower bud initiation and development. The *in vitro* flower bud formation was studied on tissue explants consisting of epidermis and subepidermal cortex from the pedicel of *Nicotiana tabacum* cv. Samsun. The tissue explants were cultured on Murashige-Skoog medium containing glucose and different hormone concentrations as described earlier (Van den Ende et al., 1984[a,b]).

The cytokinin benzylaminopurine (BAP) was given at 4 different concentrations in combination with 7 concentrations of 3 different auxins, i.e. indolylacetic acid (IAA), naphthaleneacetic acid (NAA) and indolylbutyric acid (IBA). It was previously shown that at relatively high concentrations (10^{-6}M) of both BAP and NAA, flower bud formation takes place within 3 days of culture, while the first visible flower buds appear after 8-10 days (Van den Ende et al., 1984[a,b]). Explants cultured at relatively low auxin concentrations (e.g. 10^{-8}M), in combination with a high cytokinin concentration (e.g. 10^{-6}M), had delayed flower bud formation, i.e. the first visible flower buds appeared 19 days after the onset of culture. Furthermore, auxin at low concentrations may become the limiting factor in flower bud initiation at all the cytokinin concentrations employed, while the cytokinin concentration may become limiting only at low auxin concentrations. These results indicate that auxins and cytokinins do interact with regard to flower bud formation. There are no significant differences in the effects of IAA, NAA and IBA on flower bud formation.

Auxins as well as cytokinins are rapidly metabolised by the explants. The major metabolites of NAA and BAP were shown to be conjugates (Barendse et al., 1986) which are believed to have little biological activity. At present the metabolism of IAA, an endogenous hormone, is under investigation in order to elucidate the effect of metabolism on the concentration of free hormones in the explants and, consequently, the effect on flower bud formation.

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(82) FLOWER INDUCING TREATMENTS FOR FLOWERING MATURE AND FLOWERING IMMATURE VRIESEA OSPINAE PLANTS

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Vriesea ospinae plants become flowering mature, if they have reached a fresh weight of 25 g. The flowering mature plants can be induced to flower by an ACC (1-amino-cyclopropane-1-carboxyl acid) application, while flowering immature plants remain vegetative after this treatment.

Flower induction by ACC of flowering mature plants can be detected by an increased ethylene production (40-50 nl/h.plant) as early as 1-3 days after the ACC treatment. Flowering immature plants do not have an ACC-stimulated ethylene production (Philippe et al., 1984).

A pretreatment with the auxin (2)-NAA (α-naphtyl acetic acid) caused an increase of the ACC-induced ethylene production of flowering immature plants. The ethylene evolution reached the highest levels after a time lapse of 7 days between the (2)-NAA and the ACC application. The combined treatment also resulted in a flower induction of flowering immature plants. Still, the plants had to reach a fresh weight of 7,5 g before the (2)-NAA/ACC flower induction occurred.

(2)-NAA application also influenced the ACC metabolism of flowering immature plants. Flowering immature plants treated with ACC or (2)-NAA showed an increased free ACC content (1,5 nmol/g fr wt) in the first days after the treatment and the malonyl-ACC content rose to 4 nmol/g fr wt.

(2)-NAA pretreatment followed by ACC application resulted in a free ACC content of 2,5-8 nmol/g fr wt. The highest free ACC levels occurred, if there was a time lapse of 7 days between the 2 treatments. The amount of M-ACC was not influenced by the (2)-NAA pretreatment (4 nmol/g fr wt).

We can conclude that a (2)-NAA pretreatment causes an increased uptake of exogenously applied ACC by immature plants and this results in a stimulated ethylene production.

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(83) THE EFFECTS OF IRRADIANCE ON THE GROWTH OF LEAVES, INTERNODES AND FLOWERHEAD OF SUNFLOWER

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Seedlings of sunflower were grown at 15, 30 and 60 W m^{-2}, 16 h per day, 22°C and 70% RH in a subirrigated gravel culture. Higher irradiance level (IL) accelerates development in a constant way from germination to anthesis. This acceleration is the consequence of a light-dependent increase of relative growth rate (RGR) in the primordial phase of growth together with a proportional decrease of the duration of growth. In the linear phase of growth RGR decreases with leaf number independent of IL; this patternised retardation of growth may be related to flower induction. The volume of the growing shoot (GS) may be defined as the summation of volumes of successive internodes growing in length. The increase of this volume plays an important role in the adaption of absolute growth rate of the plant to IL, because this volume is an important determinant of leaf initiation rate and of the size of the initiated primordia. At constant conditions the initiated primordia grow out according to a growth pattern depending on leaf number and IL. The size of GS reflects the size, architecture and phyllotactic order of the vascular system (Larson, 1975): the larger the number and diameter of the individual vascular sympodia, the larger the diameter of GS, and the larger the length of the orthostichy, the longer GS. The rate of increase of GS depends on IL. One orthostichy below the leaf primordium, neighbouring leaf vascular bundles (direct bundles) merge and form together the so-called synthetic bundles (Thoday, 1922). This special cambial system expands tangentially in the also expanding medullary rays with a rate proportional to IL and this development is related to the diameter the ripe flowerhead will reach (Pieters, 1986). The unequally distributed tangential growth in the vascular cylinder causes the stem to be ultimately hollow.

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(84) THE ROLE OF THE GROWING SHOOT IN DIMINISHING STRESS IN NO_3^--DEFICIENT PLANTS

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In previous experiments, in which plants were grown at various irradiances, it was shown that the growth patterns of leaves were unaffected by irradiance. Adaptation of absolute growth rate of a branch to irradiance is the result of a changing size of the growing shoot (GS). The growing shoot can be defined in several ways, a: GS is that part of the shoot, growing in length (thus including all lengthening internodes) or b: GS is that part of the shoot that bears growing leaves. It was also shown that GS reflects the size of the vascular system. The development of the vascular system of poplar was elegantly analysed by Larson (1979). The first definition of GS bears upon the vertical extension of the primary vascular system (growth in height); the second definition upon the formation of the subsidiary vascular system, ultimately leading to cambial growth (growth in stem thickness). It was also shown that plants react on NO_3^--deficiency in the same way as on low irradiance (Pieters and van den Noort, 1985). Therefore it was hypothesized that NO_3^- and light both affect the size of GS similarly and that the growth patterns of leaves and internodes are relatively unaffected. An experiment was designed to analyse accurately growth of individual organs and several other physiological parameters in reaction to light and NO_3^- dosage. Cuttings were taken from weakly growing plants, thus assuring a low level of assimilates and nitrates in the experimental material. On each cutting one shoot was allowed to grow. The plants were cultivated at 20°C, 60% RH, 16 h day-length on a subirrigated gravel culture. There were two irradiation levels and 3 NO_3^--levels. At 7.5 W m^{-2} (LI), NO_3^- dosage was 0.0, 0.25, and 1 and, at 30 W m^{-2} (HI), 0.0, 1.0, and 4.0 meq.$plant^{-1}$.-day^{-1}. The highest NO_3^--dosage was nearly saturating. Growth patterns of leaves and internodes were slightly affected only at the lowest NO_3^--level. As expected, adaptation of absolute growth rate proceeded mainly via the size of GS. Nitrate reductase activity (N-act) depended strongly on NO_3^-- and light-dosage (especially the NO_3^-/light ratio) and plant age. No N-act was found in the roots. Only at LI the number of plants entering dormancy increased with increasing NO_3^--deficiency. No dormant plants were observed at HI. Chlorophyll content per unit area decreased with increasing NO_3^--deficiency. Especially at HI anthocyanin content increased in petioles and stipulae of NO_3^--deficient plants.

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(85) RATE OF DISAPPEARANCE OF THE STIMULATING EFFECT OF LIGHT ON GERMINATION
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Red light normally stimulated the germination of *Plantago major* and *Origanum seeds*. When the seeds were temporarily incubated in polyethylene glycol (PEG-6000; -1.2 MPa) at 22°C, after illumination by red light, this stimulation disappeared. Germination of *P. major* dropped to 20% after 13 hours incubation, but *O. vulgare* required 48 hours for such a decrease. It was apparently not the rate of dark reversion of phytochrome in the far-red absorbing form (Pfr) that was primarily responsible for this difference. A much lower level of Pfr was sufficient for the stimulation of germination of *O. vulgare* compared to *P. major*. This allowed a longer period for the action of Pfr in *O. vulgare*. The germination of *O. vulgare* is stimulated by a short exposure to red light. *P. major* required an exposure of longer duration or several intermittent short exposures. Presumably, the level of Pfr dropped below the critical threshold level before its action was completed; prolonged or repeated exposures maintain a sufficiently high level. This difference in light requirement is also not primarily based on a difference in the rate of dark reversion, nor on a difference in escape time. The most important reason is again the difference in the level of Pfr required for germination.

(86) INTERSPECIFIC DIFFERENCES IN RELATIVE GROWTH RATE: A FIRST EXPERIMENT
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Why do slow-growing plants grow slower than fast-growing plants? Slow-growing species often occur in dry or nutrient-poor habitats. But even when grown with ample supply of water and nutrients, these species cannot grow with a high relative growth rate (RGR). A preliminary experiment was carried out to obtain insight in this problem. Plants of nine species were grown in growth cabinets in nutrient solution. A growth analysis was carried out, and the rate of photosynthesis measured halfway during the experiment. The mean RGR ranged from 150 to 270 $mg.g^{-1}.d^{-1}$. Surprisingly, no correlation was found between RGR and either net assimilation rate (NAR) or photosynthesis (expressed per unit leaf area): fast-growing species do not have a higher rate of photosynthesis than slow-growing species. A marked difference was found in biomass allocation. Fast-growing species invest relatively more material in their leaves and have a slightly higher specific leaf area. Moreover, the RGR was negatively correlated with the dry matter percentage, suggesting a difference in chemical composition between fast- and slow-growing species. From this first experiment, the conclusion might be drawn that biomass allocation is a more important factor in explaining interspecific differences in RGR than the rate of photosynthesis.

(87) EFFECTS OF COOL STORAGE ON THE PHYSIOLOGY OF TOMATO PLANTS
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Cultivating young tomato plants preceding the growth for production in the spring is expensive (high costs of heating and artificial light). Application of cool storage may enable the cheaper cultivation of plants grown in the summer for prolonged growth in the spring. An important obstacle for cool storage of tomato plants is their chilling sensitivity. The aim of this study is to investigate the processes underlying the chilling sensitivity, in particular cell membrane functioning at lower temperatures in order to develop a temperature treatment for the storage of tomato plants. Four week old plants grown under greenhouse conditions are placed in a cool room at 10°C or 4°C at low light intensity (12h, 3000 lux).

Growth of intact plants and temperature-dependent conductivity, chlorophyll fluorescence and photosynthesis of leaf samples were measured weekly. Preliminary results showed that 1) plant growth at 10°C is reduced to 20% of the control, 2) chlorophyll degradation occurs in older, not yet fully expanded leaves, 3) flower initiation continues, 4) chlorophyll fluorescence and photosynthesis are unchanged, 5) conductivity first increases, followed by a fast decrease. At 4°C, 1) growth is inhibited completely, 2) the leaf turgor decreases, 3) there is no change of chlorophyll content, 4) photosynthesis and chlorophyll fluorescence are inhibited.

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(88) ENDOGENOUS IAA PRODUCTION IN PISUM SATIVUM UPON INFECTION BY AGROBACTERIUM RHIZOGENES

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Although the T-DNA genes of *Agrobacterium tumefaciens* are genetically as well as functionally well-defined, little is known about the *Agrobacterium rhizogenes* system. In this report we present data on the endogenous IAA and IAM (indole-3-acetamide) levels related to the initial stages of an *A. rhizogenes* 1855 infection on *Pisum sativum*.

At the infection site and accompanying the gall formation the endogenous IAA levels rose during the first seven days from 250 to 750 pmol/g.fr.wt. Even in the region directly underneath the infection site a similar time course was found ranging from 250 to 400 pmol/g.fr.wt. However, in the latter case it was impossible to distinguish clearly these regions after five days due to an overgrowing gall formation. In an uninfected wounded control tissue, an average IAA level of 250 pmol/g.fr.wt. was observed. The rise of the endogenous IAA upon infection with *A. rhizogenes* was accompanied by a drastic increase of the endogenous IAM levels. These observations indicate that the incorporation of the *A. rhizogenes* Ti- and TR- regions induces an IAA biosynthetic pathways (tryptophan → IAM → IAA) which was also found in plant tumor tissue transformed with *A. tumefaciens* strains (Schröder et al., 1984, Van Onckelen et al., 1986).

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(89) PLASMA MEMBRANE FUNCTIONS IN HIGHER PLANTS: PHOTOTROPISM AND IAA

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Phototropism has been explained on the basis of the induction by unilateral blue light (about 450 nm) of an asymmetrical IAA-distribution mainly in coleoptiles. Extensive studies support this so-called Cholodny-Went hypothesis (Went and Thimann, 1937). Nevertheless this model has been challenged recently (Firn and Digby, 1980). Since one of the criticisms concerns the methodology for determination of the hormonal concentration, an accurate method was adopted for measuring IAA levels (Horemans et al., 1984). Mung bean hypocotyls were used. Previous to phototropic curvature, increased IAA concentrations in the shaded hypocotyl part were measured. Also the differential growth of the cells in reaction to irradiation was investigated by cell measurements on microscopic sections of bending hypocotyls.

Dose-response curves of etiolated and green seedlings differ considerably, an indication for differential changes in sensitivity between the two systems. The influence of phytochrome upon phototropic behaviour is under current investigation. The eventual change in responsiveness may be explained by alterations at the plasma membrane level, since it has been proposed that responsiveness is correlated with changes in properties of hormone-receptor sites (Trewavas, 1984). The characteristics of plasma membrane IAA receptors (or carriers) need to be investigated.

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*H. Asard is a NFWO research assistant.

(90) SENSITIZATION OF KALANCHOE SEEDS BY GIBBERELLINS: FACTORS AFFECTING THE VERY LOW AND LOW FLUENCE RESPONSES.

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Fluence-response curves for the effect of two R irradiations, each consisting of half of the total fluence and given on days 7 and 8 after sowing to *Kalanchoë* seeds, incubated on suboptimal GA_3 concentrations, are clearly biphasic (Rethy et al., 1987). GA_3 adds a very low fluence response (VLFR), requiring more than 10,000 times less R fluence to the low fluence response (LFR). When only one R irradiation is given in the presence of GA_3, a VLFR is observed, which is lower than with two R pulses, but no LFR occurs (De Petter et al., 1985). The LFR requires 3 to 4 daily short R irradiations, when seeds are incubated on water or KNO_3-solution and at least 2 R pulses, separated by 24 h, in the presence of GA_3. Both pulses do not need to be LF: a VLFR saturating first pulse, in combination with a second LF pulse, already induces the LFR.

The VLFR is promoted by GA_3 concentrations up to ± $5x10^{-5}$M, whereas maximum LFR, induced by 2 equal R pulses, is already obtained with $3x10^{-5}$M GA_3. In contrast with other systems, in *Kalanchoë* seeds, GA_3, the factor sensitizing the seeds, also affects the LFR, although the different GA_3 concentration ranges promoting LFR and VLFR separate both responses physiologically. A factor as KNO_3, increasing the LFR in the absence of GA_3, also clearly increases the VLFR, when added to GA_3 concentrations, suboptimal for the VLFR.

Presence of GA_{4+7} also results in biphasic fluence-response curves. In comparison with the effect of GA_3, sensitization takes place at ± 10-fold lower concentrations.

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(91) COMPARISON OF ENDOGENOUS PHYTOHORMONE LEVELS IN CLONED AND NON-CLONED WILD TYPE TOBACCO CROWN GALL TISSUES

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Non-cloned crown gall tissues are clusters of transformed and untransformed cells. Until now most of the investigations on the endogenous phytohormone levels of crown gall have been established using non- cloned tissues.

The recent availability of cloned lines not only offers the opportunity to define more specifically the influence of the T-DNA genes on the endogenous phytohormone levels but also to detect possible interactions between transformed and non-transformed cells.

In this respect we determined the evolution of the endogenous levels of IAA and cytokinins in different types of cloned an non-cloned wild type crown gall tissues.

A non-cloned octopine type crown gall (induced by pTiB6S3 on *Nicotiana tabacum* L. cv. Wisconsin 38) was compared with three different cloned lines. The endogenous IAA level of the non-cloned line (ranging from 5 to 450 pmol/g.fr.wt.) was higher (up to 7 times) than the level found in cloned lines. The endogenous cytokinin level of one green-coloured cloned line was comparable to the level of non- cloned tissue (respectively ranging from 500 to 5000 and from 100 to 6000 pmol ZR equivalents-/g.fr.wt.), whereas the level of two pale cloned lines only ranged from 10 to 200 pmol ZR equivalents/g.fr.wt.

Nopaline type crown galls (pTiC58) were induced on *Nicotiana tabacum* L. cv. petit Havana SR1. Analysis of a non-cloned nopaline tissue revealed IAA levels ranging from 10 to 50 pmol/g.fr.wt. and cytokinin levels ranging from 100 to 1300 pmol ZR equivalents/g.fr.wt. In contrast two pale cloned lines showed higher levels of IAA (50-300 pmol/g.fr.wt.) and lower levels of cytokinins (<5-100 pmol ZR equivalents/g.fr.wt.).

Although one would expect the endogenous phytohormone levels to be more elevated in cloned tissue, due to the lack of possible 'dilution' by non-transformed cells, our data pointed out that this was not the case. Complex interactions between transformed and non-transformed cells on one hand and the effect of selection during the cloning procedure on the other hand have to be envisaged.

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(92) SURVIVAL REACTIONS OF GERMINATED POLLEN FROM LILIUM LONGIFLORUM AFTER HEAT STRESS WITHOUT HSP-FORMATION

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Several kinds of tissue subjected to heat stress react uniformly by the formation of a set of heat shock proteins (HSPs). In contrast, mature pollen incubated at high temperatures in germination medium do not form HSPs.

Pollen incubated in germination medium have a high metabolic activity that results in a physiological response characterized by pollen tube formation and -growth. During the incubation period proteins are synthesized at a high rate, while transcription is neither necessary nor could be demonstrated during germination and pollen tube growth. Formation and growth of pollen tubes can be prevented by inhibition of protein synthesis with cycloheximide, but also by external conditions such as high temperatures. This suggests that the physiological response to heat treatment and concomitant qualitative changes in protein synthesis can be used as parameters for monitoring the reaction of germination pollen, in this case from *Lilium longiflorum*.

We present the reactions of lily pollen after incubation at 39°C during two periods. Morphological changes were checked microscopically. Proteins were labeled with [35-S] methionine for one hour periods and analyzed on fluorograms after 2-D electrophoresis.

Control experiments demonstrated that the qualitative protein composition in lily pollen is constant during development and growth of the pollen tube in germination medium. In response to heat treatment, pollen tubes of *Lilium longiflorum* formed a protrusion at the tip. At the same time plasma streaming in the tip cells stopped temporarily and their synthesis rate of several proteins changed. However, none of these affected proteins have common characteristics with the HSPs. The physiological and morphological changes were influenced by the moment as well as by the duration of heat treatment. Protusion formation, plasma streaming and protein synthesis in germinated pollen were affected in a different way for pollen which were heat-treated twice, i.e. after imbibition (3', 39°C) and after pollen tube formation (30', 39°C), in comparison to pollen heat-treated only once, after pollen tube formation (30', 39°C).

Keeping in mind that pollen of lily have a time lag of two hours between imbibition and pollen tube growth, these results suggest that lily pollen seem to have a 'translation memory' even though transcription is absent.

(93) NAPHTHALENEACETIC ACID UPTAKE, TRANSPORT AND METABOLISM DURING IN VITRO FLOWER BUD DEVELOPMENT IN TOBACCO

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The *in vitro* flower bud development on tissue explants from the floral stalks of *Nicotiana tabacum* L. cv. Samsun is particularly suited to study auxin transport and metabolism, since the tissue strips consist of only epidermis and a few layers of cortex cells, and many of the cells are directly exposed to the medium.

The tissues were cultured on Murashige-Skoog medium containing glucose, benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) (Van den Ende et al., 1984). At the optimal cytokinin concentration (10^{-6}M BAP) and low concentrations of NAA, polar buds are formed at the basal edge (maximum at 4.5×10^{-7}M NAA). At higher concentrations bud formation takes place on the remaining surface (maximum at 2.2×10^{-6}M) and the polar buds disappear. The shape of the concentration vs. response curve is the same for the two groups of buds. With the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) (10^{-6}M) in the medium, the buds develop evenly spread over the whole explant at all NAA concentrations. This is regarded as a strong indication that, in the absence of NPA, longitudinal auxin transport through parenchymatous cells is taking place.

In a first approach to establish the relation between external and internal concentrations, we have studied NAA uptake. The uptake appears to be strictly proportional to the concentration in the medium, and to be linear in time during the first four days of culture - during which the majority of buds is initiated. From the amount of NAA taken up, the internal concentration cannot be directly calculated, since exogenously supplied NAA is conjugated at a high rate (Cohen and Bandurski, 1982). When extracts from explants incubated on medium with ^{14}C - NAA were analysed on HPLC, the counts were recovered in NAA and a number of conjugates. The main conjugate accounted for approximately 45 per cent of total radioactivity, and is presumably naphthaleneacetylaspartic acid (Vijaraghavan and Pengelly, 1986) accounted for 15 and 5 per cent. Only 5 per cent of the radioactivity was in free NAA. These proportions were the same during the first two days of culture, for all NAA concentrations tested.

Since NAA uptake is linear in time, we conclude that free NAA is accumulating in the explants at a constant rate. At a medium concentration of 10^{-6}M NAA, this rate is 14 pmol free NAA.explant^{-1}.day^{-1}, which results in an internal concentration of 9.4 nmol.gram^{-1} or 9.4×10^{-6}M after one day.

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(94) REGENERATION IN PLANTAGO-SPECIES

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There is a difference in regenerative potential between the species of the genus *Plantago* occurring in grasslands in The Netherlands, affecting their ability to maintain themselves under mechanical stress.

It was found that *P. lanceolata*, which is the species that is most sensitive to damage by treading and grazing (Blom, 1979), is in fact the species which is able to regenerate profusely, not only by the growth of axial buds, but also by neo-formation of root-borne shoots (Soekarjo, 1979, 1980).

The vulnerability of this species turned out to be due to the position of the thickened stem base (caudex) relative to the ground level. In the other species investigated (*P. coronopus, major* and *media*), the stem basis is retracted below ground level, due to a much stronger root contraction (Rimbach, 1898, 1899, 1929).

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(95) APICAL DOMINANCE IN PLANTAGO SPECIES

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When no nutrients are added and the plants are not subjected to mechanical injury, only in *Plantago lanceolata* and *Plantago maritima* axillary daughter rosettes can be observed within the first growing season. In older plants they are also found in *Plantago media and Plantago major* and occasionally in *Plantago coronopus*. Removal of the apical meristem and of all the leaves, induced development of daughter rosettes in all of the five species investigated. When the apical meristem and all leaves were left intact, and no mineral nutrition was supplied, kinetin (10^{-4}) administered to the roots induced development of all axillary buds in *P. lanceolata*. Availability of water and nutrients increased the development of daughter rosettes in all species (Soekarjo, 1981). In the presence of the apical meristem, removal of all leaves only promoted the development of daughter rosettes in *P. maritima, media and lanceolata*, but had no effect in *P. coronopus* and *P. major*. Removal of part of the leaves in *P. lanceolata* increased the rate of leaf appearance as well as the development of daughter rosettes (Soekarjo, 1985).

The observations and results given, indicate that apical dominance in the true rosette *Plantago* species is regulated in a way similar to that of the apical dominance in branched plants. Under low nutrient conditions the development of axillary buds is impeded (Gregory and Veale, 1957; McIntyre, 1977). The regulation can be seen as an internal competition between the different parts of the plant. The sink strength of both the apical meristem and the developing leaves does not allow supply to the axillary buds. They are 'ignored', both in the distribution of upward nutrient and downward carbohydrate. Only when the nutrient supply is greater than the demand of these growing regions, the surplus may become available to the axillary buds. This may also explain the common phenomena of 'critical size' in flowering and 'compensatory growth'.

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(96) A SIMULATION MODEL OF PLANT GROWTH UNDER DIFFERENT N AVAILABILITY

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This model has been constructed to test some assumptions about nitrogen- and carbon metabolism and nitrogen (re)distribution, pertaining to the growth of vegetative plants.

Three different plant parts have been distinguished: roots, mature leaf parts and growing leaf parts. Uptake of nitrate by the roots, formation of amino acids, proteins, sugars and structural carbon in the different plant parts, and transport of nitrogen and carbon compounds through the xylem and the phloem, have been included. These biochemical and transport processes and the corresponding growth, were simulated for several days, and under different levels of nitrogen availability.

The model was adapted to maize plants. With ample supply of nitrate, the simulated growth of shoot and roots was similar to that in experimental plants, as was the simulated growth of roots, when the whole root system was deprived of exogenous nitrate. The simulated shoot growth in this situation was reduced and stopped within a few days, while in plants, shoot growth was only reduced (50%). When part of the root system received nitrate, the simulated shoot growth was reduced and the growth of the nitrate receiving roots was increased, as was found in experimental plants. The simulated growth of the part of the root system, which did not receive nitrate, behaved like the growth of a whole root system deprived of nitrate, whereas in the experimental plants root growth was reduced.

(97) BICARBONATE UTILIZATION BY LEAF PROTOPLASTS FROM POTAMOGETON

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Leaves from the submerged angiosperm *Potamogeton lucens* are able to assimilate bicarbonate. These leaves behave polarly: during bicarbonate utilization protons (H^+) are excreted by the cells of the lower epidermis, while hydroxyl (OH^-) ions are excreted by the upper epidermal cells. It has been proposed that acidification of the apoplast is a prerequisite for bicarbonate utilization. To test this hypothesis ^{14}C-fixation by leaf protoplasts was determined at different pH values. Also experiments, using the isotopic disequilibrium technique, were performed. They showed that at pH values > 8, bicarbonate is a major carbon source for photosynthesis in protoplasts, despite the absence of cell walls and polarity. At pH values around 6, the rate of ^{14}C-fixation in protoplasts equals that of intact leaves. At pH values > 8, however, intact leaves showed a higher rate of ^{14}C-fixation. This indicates that, though bicarbonate utilization occurs in protoplasts and in intact leaves, it is more efficient in the latter. From this, and other experiments, we conclude that at least two processes contribute to bicarbonate utilization in *P. lucens* leaves: active transport (H^+-HCO_3^- symport?) and acidification of the apoplast resulting in the conversion of bicarbonate into CO_2. Polarity may increase the efficiency of both.

(98) THE EFFECT OF CALCIUM ON THE SODIUM AND CHLORIDE TRANSPORT IN SALT-STRESSED BARLEY ROOTS

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Hordeum vulgare (C.V. Menuet) seedlings were grown for six days on a 0.25 mM $CaSO_4$ culture solution, to obtain Low-Salt-Roots (LSR). On the seventh day, roots were excised and arranged in a plexi-glass transportbox, which was divided into three compartments. First a large (30 ml) compartment wherein the radio-active tracer solution was brought and where the apical part of the roots were immersed. A second compartment (3 ml) was used as buffer zone, to prevent eventual leakage between the two main compartments. As last, the recipient-compartment (8 ml), where the basal cut end of the roots emerged. The latter two compartments are filled with the uptake solution in the unlabelled form. The solutions used were 100 mM NaCl and 100 mM NaCl+ 10 mM $CaCl_2$, labelled for ^{22}Na, $^{36}Cl^-$ and ^{45}Ca. A first set of measurements was done with LSR and a second set with High-Salt-Roots (HSR), used after a 24 hour uptake period. Each experiment included 8 to 16 replicates and was done at 0°C and 20°C.

Results show us three main parts of the ionic distribution. Radio-activity found in the apical root segment, after rinsing, is due to local accumulation. In the basal root segment, which was not in direct contact with the tracer solution, the presence of radio-activity can be attributed to re-distribution within the root. The radio-activity found in the recipient solution, where the cut ends of the roots were able to exudate, can be shown to originate from xylem transport.

In the absence of calcium, very high percentages of sodium and chloride accumulation were found. While in LSR only 10% of the radio-activity was transported through the xylem, the xylem transport became more important (to about 40% of the total influx) in HSR.

In the presence of calcium, a general decrease of influx was observed for both monovalent ions. Especially the accumulation in LSR was decreased (60%); the transport was decreased by about 40%. Im HSR the decrease was smaller with regard to the accumulation (30%), but very strong as for the xylem transport (up to 90%).

So the action of calcium on both ions has different aspects. First, it decreases the general influx of these ions. Then, due to a better segregation (vacuolar accumulation), calcium prevents large amounts of sodium or chloride to reach the central cylinder and to be loaded into the xylem. The loss of inside mobility of monovalent ions is clearly seen in the practical stop of ion redistribution within the root when calcium is present.

Calcium itself was a rather low influx, most of which can be described as a passive flux. No differences were found between experiments done at 20°C or 0°C.

(99) INITIAL PROCESSES OF FROST INJURY IN SPINACH LEAVES

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During the last 15 years the role of membrane lipids as a factor in frost hardening and chilling has been extensively studied. Besides lipids also proteins are affected by freezing stress. Levitt (1962) suggested that freezing injury results in, or is even caused by, denaturation of proteins due to formation of inter- and intramolecular disulfide bonds during freezing-induced dehydration of the cell. In order to obtain insight into the mechanism of freezing injury and into the relevance of Levitt's SH-hypothesis (1962) we investigated the effect of freezing and thawing on the sulfhydryl content of water-soluble proteins and water-soluble non-protein compounds in spinach leaves.

In our experiments leaf discs were gradually frozen from +2°C to -12°C, or from +2°C to -7°C, the latter in experiments in which freezing was started by seeding ice crystals at -2°C. Freezing injury was estimated by electrolyte leakage from frozen and subsequently thawed leaf discs and measured as electrical conductivity. Frozen leaf discs were extracted instantly, or, after a 20 h thawing period, in a nitrogen-bubbled sodium ascorbate solution. The content of water-soluble protein-SH-groups and non-protein-SH-compounds was determined with the DTNB colour reaction (Ellman's reagent; De Kok et al., 1985). A direct relation between freezing injury and oxidation of water-soluble protein-SH-groups, measured during the 7 h-freezing process, was demonstrated. In experiments in which freezing was started by seeding ice crystals at -2°C, in frozen leaf discs oxidation of protein-SH-groups was observed immediately after frost initiation. Using the latter method, protein-SH-oxidation appeared to be reversible; protein-SH-oxidation preceded frost injury. In both types of experiments the content of water-soluble non-protein-SH-compounds, mainly glutathione, was hardly affected during freezing, indicating that glutathione does not play a direct role as an antioxidant, as suggested by Levitt (1962). Our results stress the importance of protein-SH-groups in freezing injury of plants.

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(100) NITRATE ACCUMULATION IN SPINACH LEAVES: A STUDY WITH ISOLATED VACUOLES

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Spinach plants can accumulate nitrate to concentrations as high as 7000 mg. kg^{-1}. The concentration reached is influenced by various environmental factors, as nutrient level, light intensity, temperature and relative humidity. When vegetables as spinach and lettuce are commercially grown in greenhouses under winter conditions at low light intensity and low temperature, the plants accumulate nitrate to concentrations, potentially toxic for human health.

An investigation into the physiological mechanism of nitrate accumulation showed that 1) nitrate uptake by the root is regulated by the demand for osmotic solutes in the shoot and 2) nitrate accumulates in the shoot, when the demand for osmotic solutes can not be met by organic solutes (Steingröver 1986).

Compartmentation experiments showed that nitrate is accumulated in the vacuoles. Dependent on the experimental and environmental conditions vacuolar nitrate can be replaced by sugars, organic acids and/or other anions as chloride. The partitioning of nitrate between cytoplasm, where it can be reduced and finally incorporated into amino acids and proteins, and the vacuoles, where it serves as an osmotic solute will be investigated. Exchange processes at the vacuolar membrane level will be studied with vacuoles, isolated from spinach leaves. A method for the isolation of protoplasts and vacuoles of spinach is presented.

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(101) ACTIVATION OF BINDING CAPACITY DURING SEXUAL INTERACTION IN CHLAMYDOMONAS EUGAMETOS

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Chlamydomonas eugametos displays an interesting contact-activating mechanism. When gametes of the two mating types (mt^+ and mt^-) of this unicellular green alga are mixed, they agglutinate sexually via their flagella. On the flagella sex-specific adhesion factors, agglutinins, are located. These are large, linear glycoproteins that are extrinsically bound to the flagellar membrane. We present evidence that during agglutination, these agglutinins are activated which results in the increase of agglutinability. Before contact, a minor part of the agglutinins present on the flagella is operational and only functions as recognition molecules. After contact between complementary agglutinins, more agglutinins become activated and adhesion is intensified. Therefore, the agglutinin molecule is not only both receptor and ligand, but acts also as erogenic agonist. We have indications that two major changes occur: first, a conformational change that exposes the binding site; second, a change in the anchoring of the agglutinin. During agglutination, the activated molecule is transported to the flagellar tip. This 'tipping' is an essential step in de lining up of the flagella that is required to position two complementary gametes so that they can fuse via very short plasma papillae. CSLM (Confocal Scanning Laser Microscope) images of immunogold-labeled gametes revealed that the agglutinins are not randomly distributed over the flagellar membrane but are arranged in a row. We have indications that the row is 2-3 times twisted around the flagellum, thus forming a spiral domain. Other glycoproteins are also grouped in spiral domains, but since these components do not play a role in the sexual process and are not redistributed during agglutination, we propose that there is a functional distinction between these domains and the agglutinin domain. The finding that the agglutinins are placed in (helicoidal) avenues may be helpful to understand the transport of these molecules.

(102) THE SOLUBILISATION OF THYLAKOID MEMBRANES WITH NON-IONIC DETERGENTS

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Purification of chlorophyll protein complexes involves solubilisation of thylakoid membranes with non-ionic detergents. In this work, the effects of two closely related non-ionic detergents (det)(NP-40 and TX-100) were compared with regard to electron transport activity, protein composition and some fluorescence properties of spinach thylakoids.

Thylakoid membranes were isolated according to Dunahay et al. (1984). Membranes were resuspended to 1 mg chlorophyll (chl/ml) and were incubated on ice during 30 min with increasing concentrations of NP-40 or TX-100 (expressed as det/chl from 1 to 160). Chlorophyll was determined according to Arnon (1949). Photosystem I (PSI) and II (PSII) activities were measured as described by Böger and Kunert (1978). An aliquot of the samples was centrifuged during 10 min at 48000g. The polypeptide composition of the pellet was analysed on SDS-PAGE according to Delepelaire and Chua (1982).

A rapid decrease of PSII activity was observed with increasing detergent concentration. The effect was independent of pH and type detergent used. PSI activity also declined with increasing detergent concentration. However, this activity showed a minimum around the CMC but increased with higher concentrations. With very high det/chl ratios (80-160) the activity was even higher than in the control.

The intensity of the polypeptide bands corresponding to the PSI complex, the CF_1-factor and some components of the PSII complex decreased with increasing det/chl ratios, as appeared from SDS-PAGE of the pelleted material. Moreover, a blue shift of the red absorption maximum and a dramatic decrease of the low temperature 740/685 nm fluorescence ratio was progressively induced with increasing detergent concentrations.

In conclusion, membrane solubilisation is independent of the type of detergent used and of the pH of the incubation medium. The sensitivity to the action of the detergents is different for both photosystems. Increase of the PSI activity and the corresponding decline of the low temperature 740/685 fluorescence ratio with rising detergent concentrations will be discussed.

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(103) ON THE PHYSIOLOGICAL ROLE AND LOCALISATION OF ZINC-INDUCED ISOPEROXIDASES IN PHASEOLUS VULGARIS

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In primary leaves of *Phaseolus vulgaris*, treated with toxic concentrations of zinc in the nutrient solution, the guiacol-peroxidase (POD) capacity strongly increased as compared to control plants, receiving optimal zinc nutrition. Electrophoresis of a crude leaf extract revealed the appearance of a group of relatively fast migrating anionic isoPODs. Toxic zinc treatment had no significant effect neither on POD capacity, nor on isoPOD pattern in the roots. Using syringaldazine as substrate, a similar increase in leaf POD capacity was observed. Zinc, however, did not affect the ascorbic acid POD capacity. A fraction, enriched in extracellular proteins was prepared from the leaves of plants, treated with excess zinc (Hendriks et al., 1985). This fraction contained almost exlusively the zinc-induceable group of anionic isoPODs. After removal of the extracellular proteins the activity of these isozymes was strongly reduced in the extract of the remaining leaf tissue.

In *Populus* a fast migrating group of anionic isoPODs specifically oxidizes syringaldazine, a substrate with strong affinity for lignifying cell wall POD. Therefore this group was considered to be involved in lignification (Imberty et al. 1985). In *Petunia* (Hendriks et al. 1985) and tobacco (Mäder et al., 1980), extracellular fast migrating anionic isoPODs were also associated with lignification. Our results therefore suggest that toxic zinc treatment induces lignifying isoPODs in the primary leaf of *Phaseolus vulgaris*. Suberisation in *Phaseolus* roots is also controlled by fast migrating anionic isoPODs with similar electrophoretic characteristics (Sijmons et al., 1985). Both isoPOD functions will be considered in further research.

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(104) A SPECIFIC PROTEIN IS INDUCED IN RICE CELLS SUBMITTED TO NaCl

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Accumulation of salt and especially NaCl in soil is a worldwide problem. The effects on the cells are both osmotic imbalance and ion toxicity. The complexity of the response of the whole plant to a saline environment makes it difficult to study the physiological mechanisms of tolerance in organized tissues. So *in vitro* cell suspension cultures can contribute to a better understanding of the molecular mechanisms implied in salt agression and to improve selection of salt-tolerant plants. Moreover, salt-tolerant plants have been regenerated from cells adapted to NaCl stress in tissue culture. Still the metabolic changes identified in cells have to be correlated to those in the tissues of the stressed plant. So far, a 26000 D protein has been reported to be associated with adaptation to NaCl (Singh et al., 1985).

In order to investigate the synthesis of proteins during salt stress in rice (*Oryza sativa* L. cv. Rosa Marchetti), callus cultures were initiated from seed embryos on solid medium R2 and after two months transferred into liquid medium. Exponental-phase cultures of rice cells were first exposed to different concentrations of NaCl during 16h and then labeled with [^{3}H]-leucine for two hours. Proteins were extracted and analysed by 10-20% gradient-SDS-PAGE. It appeared that protein synthesis was severely repressed at 15 g/l of NaCl. Nevertheless, a 32000 D protein was produced in large amounts. It is known that abscisic acid (ABA) accelerates adaptation of cultured cells to salt (La Rosa et al., 1985). So we added ABA (0,5 and 1 mM) in order to see if induction of the salt protein was stimulated. The addition of ABA in the medium did not induce this protein. However, the synthesis of a set of proteins of lower molecular weight was induced, especially a 26000 D protein. The treatment of the cells with both ABA and NaCl resulted in the synthesis of both polypeptides. Thus ABA causes neither stimulation nor repression on the synthesis of the 32000 D protein.

In order to investigate if this salt protein is involved in the adaptation to salt, we have isolated, after mutagenesis by MNNG treatment, several NaCl tolerant cell lines and this protein will be sought.

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(105) BENZYLAMINOPURINE METABOLISM DURING IN VITRO FLOWER BUD FORMATION IN TOBACCO

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In tissue explants of epidermis plus subepidermal cortex from the flower stalks of *Nicotiana tabacum* flower buds are formed (Tran Thanh Van, 1973; Van den Ende et al., 1984) at appropriate concentrations of benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). The number of flower buds formed depends mainly on the BAP concentration if the NAA concentration $\geq$ 0.22 µM.

In order to get some insight in the relationship between the number of flower bunds formed and the internal BAP concentration, it is necessary to study the metabolism of BAP in the explants. Exogenously supplied cytokinins can be metabolized to their respective ribonucleosides and ribonucleoside- 5'phosphates and to various conjugates (Letham and Palni, 1983). We have studied the more polar and non-polar metabolites formed after incubation of tissue on medium supplemented with ^{3}H-BAP.

Two non-polar metabolites, one of which is BAP, were detected by reverse-phase HPLC. The other metabolite co-chromatographed with standard 7-G BAP. The spectra of the metabolite and that of authentic 7-G BAP were identical. On two-dimensional TLC the metabolite and 7-G BAP banded at the same position.

Three polar metabolites of BAP were detected after a 1-h pulse and 3-h chase incubation by ion-exchange HPLC. The retention times of the three metabolites were close to the retention times of AMP, ADP and ATP. Enzymatic hydrolysis resulted in the disappearance of the metabolites from their respective positions. This indicates that possibly the metabolites are 9-R BAP 5'mono-, di- and triphosphate. Laloue and Pethe (1982) found evidence for the existence of these metabolites in a cell culture of tobacco.

Further studies will investigate which metabolic forms are responsible for flower bud formation and whether the metabolism of other cytokinins is comparable with that of BAP.

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(106) INTERACTION OF HYDROXAMATE-ACTIVATED PEROXIDASES WITH THE DETERMINATION OF THE CYTOCHROME AND THE ALTERNATIVE PATHWAYS

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Hydroxamates generally are considered as specific inhibitors of the oxygen uptake by the mitochondrial, alternative pathway. However, side effects on the cytochrome pathway and on various enzyme systems also are possible. In potato tuber callus tissue a very active hydroxamate-stimulated NADH-dependent O_2- uptake develops during the growth of the callus, which is caused by a peroxidase. The total activity may be as high as 1000 times the respiratory activity of the callus tissue. The chief properties of this perixidase are:

a. Maximal stimulation is observed with 1-3 mM benzhydroxamate (BHAM) and 1-15 mM salicylhydroxamate (SHAM). Higher concentrations, especially of BHAM, give less or no stimulation.
b. Hydroxamates are not consumed during the reaction.
c. Both NADH and NADPH can serve as the elctron donor for the reaction. The affinity for NAD(P)H is very low (Km near 10 mM). In the absence of hydroxamates NAD(P)H is only slowly oxidized, with an even lower affinity.
d. The peroxidase can carry out two reactions: an O_2-consuming and an H_2O_2-consuming reaction. In both reactions one NAD(P)H is consumed. In the first reaction H_2O_2 is formed which can be consumed in the second reaction, resulting in an overall stoichiometry of 2 NADH consumed for each O_2 molecule and in the production of H_2O.
e. The reaction of completely blocked by cyanide, superoxide dismutase and (excess) catalase, but not by antimycin A or azide.

This peroxidase-mediated O_2-uptake might interfere with respiratory measurements. In experiments with isolated mitochondria this interference can be prevented by the addition of catalase to the reaction mixture. The use of high concentrations of hydroxamate is not allowed because of inhibitory effects on the cytochrome pathway. In intact callus tissue hydroxamates only stimulate O_2-uptake in the presence of exogenous NADH. In vivo the peroxidase does not appear to function in O_2-uptake, probably because of its localization (at least partly in the cell wall) and/or its low affinity for NADH.

The conditions for the use of hydroxamates in the determination of cytochrome and alternative pathway activity will be discussed.

(107) XYLEM-TO-PHLOEM TRANSFER OF AMINO ACIDS IN THE PETIOLE OF THE THIRD LEAF OF TOMATO

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The xylem vessels in tomato (*Lycopersicon esculentum*) provide the main translocation path of amino acids produced in the roots (Van Die, 1963). Along the upward route, the amino acids partly escape from the transpiration stream and traverse to the phloem. Subsequent transport in the sieve tubes favours the N-supply of the apical plant parts (Van Bel, 1984), which illustrates the importance of the xylem-to-phloem transfer for the nitrogen economy of the plant (Pate, 1986; Simpson, 1986).

A substantial part of the amino acid escape takes place in the petioles. Excised leaves were therefore adopted as model systems to study the determinants of amino acid distribution. ^{3}H-amino acids fed to the petiole base of excised leaves were similarly distributed along the petiole as in whole plants. Chasing the vessels with water did not alter the distribution which suggested uptake by metabolic compartments. Amino acid withdrawal from the vessel by surrounding tissues was active, as simultaneously administered ^{14}C-carboxyl inulin (a marker of xylem transport) was hardly retained. This active uptake by the petiole tissues amounted up to 70% of 1 mM amino acids applied to the system.

Xylem-to-phloem transfer in the petiole was monitored by exudation experiments. First, the optimal conditions for phloem bleeding were established by phloem exudation (7 hours) in a range of EDTA-concentrations after $^{14}CO_2$-application (1 hour) to excised leaves. In the presence of the optimal EDTA-solution (10 mM in 5 mM MES-buffer, pH 6), exudation of xylem-fed ^{14}C-amino acids was recorded. Amino acid circulation via the lamina was prevented by heat girdling of the petiole just below the basal leaflets. Xylem-fed ^{14}C-labelled amino acids showed up in considerable amounts in the phloem exudate. The xylem-to-phloem transfer probably includes for stages: (1) diffusional escape of amino acids from the vessels, (2) active uptake by the tissues around the vessels, (3) release from the symplastic compartments into the sieve tubes, and (4) phloem transport to the base of the petiole and subsequent exudation.

The mechanism of xylem-to-phloem transfer and its function in whole-plant physiology are under current investigation.

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(108) A METHOD FOR THE DETERMINATION OF THE RESPIRATORY COSTS FOR THE MAINTENANCE OF ROOT BIOMASS, FOR ROOT GROWTH AND FOR ION UPTAKE

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The respiratory costs for the maintenance of root biomass, for root growth and for ion uptake in *Carex diandra* and *Carex acutiformis* were calculated, in order to explain the higher rate of root respiration in *Carex diandra* plants. The three respiration components were derived with a multiple regression analysis, using the relative growth rate and the net rate of nitrate uptake as independent variables and the rate of root respiration as a dependent variable. There was no significant difference in the rate of maintenance respiration, in growth respiration or in respiration per unit of ions absorbed, between the two species. It is concluded, that the higher rate of root respiration of *Carex diandra* is caused by a higher rate of nitrate uptake. At relatively high rates of growth and nitrate uptake, the contribution of the rate of respiration for ion uptake to the total rate of respiration amounted to 38% and 25% for *Carex diandra* and *Carex acutiformis*, respectively. The results suggest that ion uptake is a major sink for respiratory energy in the roots.

(109) ACID RAIN EFFECTS ON NET PHOTOSYNTHESIS, LEAF CONDUCTANCE AND BIOMASS DISTRIBUTION OF PICEA ABIES (L.) KARST.

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Three year old spruce plants (*Picea abies* (L.) Karst.) were grown in a greenhouse. The plants were exposed three times a week to simulated acid wet precipitation (solution of pH 4.3 or 3.5) or to a control precipitation (pH 5.6). Net photosynthetic rate increased gradually with decreasing pH, with a significant difference between pH 3.5 and 5.6. Leaf conductance for CO_2 and H_2O of both acid rain groups was also greater than that of the control group. The increase of net photosynthesis was only small and is possibly due to the increased leaf conductance. The water use efficiency of the treated plants was 20 to 25% lower than that of the control group, indicating an adverse effect of acid precipitation on the water balance of spruce which may limit the productivity in periods of water stress. Neither total chlorophyll content nor the ratio chlorophyll a/b showed statistically significant differences.

There were no significant effects on dry weight of different plant parts or on root/shoot ratio, but the ratio wood/needle dry weight increased with decreasing pH. This is likely to be due to a significantly increased proportion of previously formed stems and twigs and possibly also to a decreased proportion of current year needle biomass (although not significant).

(110) AIR HUMIDITY AS REGULATING FACTOR IN LIPID DIFFUSION THROUGH CUTICLES. A COMPUTER-AIDED SIMULATION OF LIPID TRANSPORT

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Cuticles contain substances with extreme differences in polarity. Such substances occur usually not as stable mixtures but in separate polar and a-polar complexes. Histochemical analyses of *Ilex aquifolium* indeed demonstrated an inhomogeneous, more or less granular lipid distribution pattern in their thick cuticles.

Lipid diffusion is only possible within the lipid particles. The cuticular membrane will loose water in dry air. The water-containing parts will shrink, but the lipid particles cannot shrink. As a result more and more particles will make contact with each other, which facilitates lipid diffusion. Together with the increased permeability to lipids, the resistance to water transfer through the cuticle increases.

A BASIC-program was developed for computer-aided simulation of the inverse relation of water and lipid permeability in cuticles. The program visualizes the transformation of isolated lipid globules into paths of lipid transfer across the cuticle.

(111) HISTOCHEMICAL LIPID DISTRIBUTION PATTERNS IN ILEX AQUIFOLIUM L. (AQUIFOLIACEAE), HOYA AUSTRALIS R. BR. EX TRAILL. AND HOYA CARNOSA H. BR. (ASCLEPIDIACEAE)

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Like many other *Hoya* species, *Hoya australis* has a white latex with a high triterpenol ester content. The latex in the small laticifers absorbs lipid stains like the Sudan stains, in contrast to *Hoya carnosa* which has a clear yellow water-soluble latex. Triterpenolesters were not detected in the latex of *Hoya carnosa*. Small lipoid droplets occur in most mesophyll cells (sclereids included) of both *Hoya* species. Some epidermis cells have a latex-like granular content. Thin-layer chromatography of *Hoya carnosa* extracts indicate that the lipoid droplets in the mesophyll mainly contain triterpenol fatty acid esters, whereas the epidermis also contains triterpenol cinnamates. *Ilex aquifolium* has no laticifers, but lipoid droplets with triterpenol fatty acid esters occur in most living leaf cells.

Both *Ilex* and *Hoya* have triterpenol-rich cuticular membranes which can be easily coloured with Sudan stains. But they differ in oxidative products of the triterpenols. It seems probable that the type of lipoid accumulation in the epidermis cells correlates with the oxidation pattern of the cuticular membrane triterpenols.

(112) TRITERPENE OXIDATION IN CUTICULAR MEMBRANES OF ILEX AQUIFOLIUM L. (AQUIFOLIACEAE)

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Holly plants contain lipid droplets with fatty acid esters of the pentacyclic triterpenols alpha-amyrin and beta-amyrin in most living cells. The leaf wax contains the pentacyclic triterpenes ursolic acid, oleanolic acid, alpha-amyrin and beta-amyrin. In both triterpenol acids the methyl group at carbon atom 28 is replaced by a carboxyl group. The triterpenol acids may be produced by oxidation of triterpenols in either the epidermis cells or in the cuticular membrane. Analysis of trace compounds in the leaf wax demonstrated the presence of alpha-amyron, beta-amyron, uvaol, erytrodiol, ursolic aldehyde, and oleanolic aldehyde. The presence of these compounds indicates the activity of two alternative oxidation routes for the triterpenols in leaf wax. The oxidation of triterpenols to triterpenons (oxidation at carbon atom 3) in holly leaf wax is quantitatively insignificant.

(113) PHYTOCHROME EFFECT ON ETHYLENE PRODUCTION BY INTACT ETIOLATED BEAN SEEDLINGS: THE ESCAPE FROM REVERSIBILITY

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Intact etiolated bean seedlings (*Phaseolus vulgaris* L. cv. Limburgse vroege) were illuminated with red and far-red light for 10 min. After different time intervals, ethylene production (using a continuous flow system), ACC and M-ACC contents were measured.

A 10 min red light treatment induces a fast, strong and long-lasting decrease of ethylene production in 8 days old seedlings. The inhibition of ethylene production by red light is fully reversed a subsequent far-red irradiation, as measured 6 h after the light treatment. This suggests the involvement of the phytochrome system. Reversibility is still observed when far-red is given with dark intervals of 15 min up to 3 h after red light irradiation. Three hours after the red light treatment, ethylene production has already reached its minimum level. After the far-red treatment, given at that time, it takes 2 h before the initial dark ethylene production is re-established. A subsequent second red light treatment induces a decrease in ethylene production similar to the first one. Reversibility is lost between 3 and 6 h after the first red light treatment. Six hours after red irrediation, ethylene production has almost completely escaped from far-red reversibility.

The inhibitory effect of the light treatment on ethylene production results from reduction in the availability of free-ACC, through increased ACC malonylation and suppression of ACC formation. Red light temporarily promotes the malonylation of ACC, reducing the level of ACC and hence the ethylene production rate. Far-red irradiation, even when given without any interval after red light treatment, fails to induce a reconversion of M-ACC to ACC. This temporary increase of malonylation after a red/far-red treatment is reflected in a corresponding, also temporary, decrease of ethylene production (minimum after about 1 h). The reversible inhibition of free-ACC formation shows an escape from reversibility very similar to that of ethylene production.

In conclusion, it seems that the initial, non-reversible malonylation of ACC plays an important role in the first, quick part of the light response, while the reversible inhibition of ACC-formation is responsible for keeping both the free-ACC pool and the ethylene production at the reduced level for a prolonged period of time.

(114) THE UPTAKE OF ATMOSPHERIC AMMONIA BY LEAVES

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There is increasing evidence that the high NH_3 emission in areas with intensive livestock breeding in The Netherlands contributes significantly to the serious dieback of forests and to the strong decline of plant species in these areas (Roelofs et al, 1984). About two third of the total deposition of NH_3 is via dry deposition (Van Aalst, 1984). Many aspects of the dry deposition of NH_3 on vegetation are not well understood. In this context the process of NH_3 uptake from the atmosphere by leaves is studied. The uptake is measured with a leaf chamber, in which a leaf attached to the plant is enclosed. A continuous flow of purified air, containing a known amount of NH_3 is led through this chamber. At the inlet and outlet of the chamber the NH_3 concentration is measured. The NH_3 flux into the leaf is calculated from the difference between inlet and outlet concentration. Simultaneously, transpiration and photosynthesis of the leaf are measured. In this way the NH_3 flux can be directly related to stomatal behaviour and photosynthesis of the leaves. In a first series of experiments, leaves of *Phaseolus vulgaris* L. were exposed for 9 hours to different NH_3 concentrations, at different light intensities. The leaves appeared to have a high affinity for NH_3. Even at high NH_3 concentrations (up to 400 $\mu g\ m^{-3}$) the NH_3 flux into the leaf increased linearly with the NH_3 concentration in the leaf chamber. Resistance analysis indicated that NH_3 transport into the leaf is via the stomata: transport via the cuticle is negligible under the experimental conditions. There is no internal resistance against NH_3 transport. So NH_3 flux into the leaf can be predicted by data on the boundary layer resistance, stomatal conductance for H_2O and ambient NH_3 concentration. The NH_3 flux was found not to influence the photosynthesis. (Van Hove et al., 1987) Also the adsorption of NH_3 on the leaf surface is studied. The measurements were carried out in the dark avoiding stomatal uptake. Preliminary measurements showed that the adsorbed quantity of NH_3 is strongly dependent on relative humidity of the air. In addition, the adsorbed quantity appeared to be proportional to NH_3 concentration. This indicates that an equilibrium between the adsorbed quantity of NH_3 and its concentration in the atmosphere exists. An explanation for this equilibrium situation is the presence of a waterfilm coating the cuticle of the leaf, in which NH_3 dissolves. This waterfilm may play an important role in the deposition of other air pollutant compounds like SO_2 on vegetation.

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(115) CELLS OF COMMELINA BENGHALENSIS MINOR VEINS: MORPHOLOGY, ONTOGENY, PLASMODESMATAL FREQUENCIES AND ATPase-ACTIVITY

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Commelina benghalensis leaves, in different stages of development, were prepared for transmission electron microscopy. The ontogeny of the minor veins was determined and the respective cell types were defined. The distribution of 1200 plasmodesmata between the cells was counted and depicted in a so-called plasmodesmogram. ATPase staining was performed with mature and immature leaves.

These are two types of sieve tubes. A companion cell/protophloem sieve tube complex originated from a common initial cell at an early stage of vein development, whereas a metaphloem sieve tube developed from the same precursor cell as the phloem parenchyma cells at the stage of vein maturation. This ontogeny might reflect the development of two physiologically separate units in the minor vein.

In mature leaves, the minor vein comprises a one-layer mestome sheath surrounding one xylem vessel, 2-5 vascular parenchyma cells, one thin-walled protophloem sieve tube + companion cell, and one thick-walled metaphloem sieve tube. Both sieve tubes show an open lumen. The protophloem sieve tube (diameter 4-5 μm) has an intense symplastic contact with the companion cell (3,700 plasmodesmata mm^{-1} vein), constituting an isolated symplast unit, The metaphloem sieve tube (diameter 3 μm) is tightly connected with the vascular parenchyma (13.700 plasmodesmata mm^{-1} vein) and this symplastic contact continues from the metaphloem sieve tube via the vascular parenchyma and the mestome sheath to the mesophyll.

In growing leaves, ATPase staining was most intense at the inner side of the plasmamembrane of the protophloem sieve tube. In full-grown leaves, however, the staining in both sieve tubes and the companion cell was negligible, while the mestome sheath cells displayed high ATPase-activity at the plasmamembrane site. The parenchyma cells showed relatively high ATPase activities during all investigated stages of development. The absence of ATPase activity in the metaphloem sieve tube suggests that metaphloem sieve tube loading does not occur at the plasmamembrane.

The results might indicate different ways of phloem loading (symplastic to the metaphloem sieve tube and non-symplastic to the protophloem sieve tube) in importance varying with the stage of development and/ or the supply of assimilates (cf. Fisher, 1986; Van Bel, 1987).

Van Bel, A.J.E. (1987), Plant Physiol. Biochem., in press.
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(116) FLUORESCENCE AS AN INDICATOR OF AIR POLLUTANT EFFECTS ON PLANTS

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Cuttings of poplar (*Populus euramericana* L. cv. 'Flevo') and bean plants (*Phaseolus vulgaris* L.) were grown in chambers with different NH_3 concentrations (0, 50 and 100 $\mu g/m^3$). Temperature was 20°C, relative humidity 70% and a light period of 16 hours at a light intensity of 60-70 W/m^2(PAR). Air within each chamber was changed 6 times per minute, avoiding CO_2 depletion and water vapour condensation on the internal walls. The poplar cuttings were placed in the exposure chamber, when their shoots appeared. The exposure of the bean plants was started, when the first ternary leaf became visible. The fluorescence measurements were started after a NH_3 exposure of two weeks for beans and four weeks for poplars. The method used was the fluorescence-light-doubling technique (Schreiber et al, 1986). Before a measurement the plant was dark-adapted in a normal environment for 20 minutes. The effect of the relatively low doses of NH_3 on the plants was small. Bean leaves showed no significant change in the minimum and maximum fluorescence levels (F_0 and F_{max} respectively). This implies no change at the light harvesting and membrane structure level (Krause and Weis, 1984). Both the energy-dependent quenching (q_E) and the photochemical-dependent quenching (q_Q) changed slowly in the course of time (Schreiber et al., 1986). Energy-dependent quenching under continuous illumination rises after 3 weeks of NH_3 exposure, while the photochemical-quenching diminishes. This rise in q_E and fall in q_Q can be attributed to a lower rate of photosynthesis in concurrence with aging of the leaf. This aging seems to be accelerated by the presence of low concentrations of NH_3. The effect of NH_3 on poplar leaves is even smaller. However, when exposed to a high concentration of NO (> 2ppm) for a short time, plants exposed for 5 weeks to NH_3 reacted strongly by a sharp rise in q_E and diminishing q_Q (NO_x is NO or NO_2). The short NO-exposure had, on the contrary, hardly any effect on the control plants: q_E raised slightly, while q_Q did not change at all. The effect of the short exposure to NO_x seemed to wear off in 10 days. Our preliminary conclusions are the following:

a. prolonged exposure to low doses of NH_3 enhances the aging process of bean leaves;
b. there are no structural changes due to prolonged exposures to relatively low doses of NH_3;
c. there is a synergistic effect between the stress induction of NH_3 exposures and other stress-inducing factors such as a short exposure to a high concentration of NO_x.

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Schreiber, U., Schliwa, U. and Bilger, W. (1986), Photosynth. Res. 10, 51-62.

(117) FLUORESCENCE AS A STRESS INDICATOR IN PLANT BREEDING

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When a plant is grown under optimal conditions, it will utilize the light captured by its photosynthetic pigments as efficiently as possible, i.e. the majority of the trapped photons will be used for photosynthesis. However, there is always a small loss due to thermal deactivation and to the re-emittance of photons from the pigments, which can be detected as red light (wavelength=685nm). This light is called fluorescence and can be used as a means to get information on the functioning of the chloroplast (Krause and Weis, 1984). Recent advances in fluorescence research have led to a new measurement technique called the fluorescence-light-doubling method (Schreiber et al., 1986). This has turned fluorescence measurements into a powerful tool for fast stress detection in plants. We have applied this technique to young plants of two different maize varieties (Flint and Dent), which have been bred for resistance(+) or susceptibility(-) to cloroses as a consequence of a mild cold treatment (Dolstra and Miedema, 1986). The maize plants were grown at 70% rh, 16 h light($60 W/m^2$)/18 h dark and 20°C. After 2 weeks 4 plants of each population were moved to the greenhouse as a control. Six plants of each population were kept under the same conditions except that the temperature was changed to 15°C at day and 10°C at night. The fluorescence curves of young greening plant leaves were measured daily starting 24 hours after the change of the temperature regime. The plants were kept in the dark and at room temperature for at least 30 minutes before each measurement. After statistical analysis of the results the following conclusions could be drawn: a). The cold treatment inhibits photosynthesis at the Calvin cycle level; b). Reversible photo-inhibition is enhanced in the cold-treated populations; c). Structural changes, causing changes in light scattering, occur in the cold-treated plants; d). Only the negative (susceptible) populations have a significant rise in the minimal fluorescence (Fo) after 24 hours of cold treatment of the Dent- and after 3 days of the Flint-. The positive (resistant) has a much lower rise in Fo during or after the cold treatment.

Laboratory experiments with young maize plants have revealed an improper assembly of the light-harvesting pigment protein complex (LHCP IIa) within the thylakoid membrane upon 5 hours of 5°C treatment (Hayden et al., 1987). A malfunctioning LHCP IIa would immediately lead to an enhancement of Fo. We tentatively conclude that our positive populations ($Dent^+$ and $Flint^+$) assemble their LHCP IIa properly at 15°C/10°C, while the susceptible populations ($Dent^-$ and $Flint^-$) incorporate an improper protein during the assembly of the LHCP IIa.

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(118) REGULATION OF PHOTOSYNTHETIC ELECTRON TRANSPORT BY BICARBONATE, FORMATE AND HERBICIDES

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Bicarbonate, formate, and many herbicides act between the primary quinone electron acceptor Q_A and the plastoquinone pool. Herbicides like the ureas, triazines and the phenol-type herbicides probably act, by the displacement of the secondary quinone electron acceptor Q_B from its binding site on a Q_B-binding protein located at the acceptor side of photosystem II. Formate appears to be an inhibitor of electron transport; this inhibition can be removed by the addition of bicarbonate. There appears to be an interaction of the herbicides with bicarbonate and/or formate (Van Rensen and Snel, 1985). It has been suggested that both the binding of a herbicide and the absence of bicarbonate may cause a conformational alteration of the environment of the Q_B-binding site. The alteration brought about by a herbicide decreases the affinity for another herbicide or for bicarbonate. The change caused by the absence of bicarbonate decreases the affinity for herbicides. Moreover, this change in conformation causes an inhibition of electron transport (Jansen et al., 1986).

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(119) THE STATOLITH APPARATUS IN HOOKS OF ETIOLATED PHASEOLUS SEEDLINGS: AN ELECTRON MICROSCOPIC STUDY

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During their development the majority of seed plants exhibit typical gravitropic responses: roots have a positive growth reaction, stems show a negative tropism. Roots and stems have a uniform perceptive system: the statocytes. It is generally accepted that gravity is perceived in these cells primarily by sedimenting amyloplasts on membranes of the endoplasmic reticulum.

In former light microscopy work, we demonstrated that the hook plays a predominant role in the perception and reaction to gravity in the stem of etiolated seedlings of *Phaseolus vulgaris* L.. We also investigated the distribution of the amyloplasts within the cells of the perceptive system. This report describes the ultrastructure of these cells with special attention to location of the endoplasmic reticulum (E.R.) and the distribution of amyloplasts.

We used 7 days old dark-grown bean seedlings (21°C, 70% rh). Tissue was collected at different positions in the hook and along the stem, and fixed and embedded using standard techniques.

In all samples, well-developed amyloplasts were found, which were always aggregated at the topological lower side of the statocytes, confirming our former observations. As seen in longitudinal sections, the E.R. was always present in the periphery of the cells. We quantified the E.R.-distribution in samples of different stem positions. We divided the statocytes in quadrants and expressed for each quadrant the amount of E.R. as the percentage of cell wall covered by E.R. The results of this analysis demonstrated a clear polarity in the distribution of E.R.: the highest concentration was always found in the topological lower part of the cell. Depending on the position in the stem this lower part coincides with the morphological lower side of the cell (stem and part of the hook facing the stem) or with the morphological top side (part of the hook facing the stem meristem). The polarization is most pronounced in the upright part of the stem. As the stem grows, cells migrate continuously through the hook. In this stem area, the distribution of the E.R. thus has to be 'repolarized' in a period of one day in each statocyte. This behaviour is very different from the 'undirectional' polarization of statocytes, known in roots and green stems. Whether the repolarization involves a redistribution of persisting membranes or localized breakdown and synthesis cannot be concluded from our results.

(120) VITAL STAINING OF MITOCHONDRIA FOR PROTOPLAST MANIPULATION

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One approach in our current research project is the transfer of organelles by microinjection. For this technique, a culture system and a microinjection system have been developed (De Laat and Blaas, 1987). For the visual control of the injection procedure, it was neccessary to use fluorochromes, which stain specifically and vitally certain organelles, such as the nucleus and the mitochondia.

Suspension cultures from *Nicotiana plumbaginifolia* (Doba cell line, kindly provided by Dr. Shields, Sittingbourne, U.K.) were used for protoplast isolation and staining experiments. Concentrations up to 10 microgram/ ml DiOC7(3) (3,3'-diheptyloxocarbocyanine, purchased from Serva) showed no toxic effects on the growth of cultured protoplasts. Both the specificity of the stain and the retention inside the cell were good. The last was determined by cocultivation of stained and washed protoplasts with unstained protoplasts in the same Petri dish. Exchange of stain was observed only in case the protoplasts were damaged, of after a few days of culture. The fluorochrome was extremely resistant to fading, even after 30 minutes of epi-illumination with blue light.

DiOC7(3) can be used to stain mitochondria vitally during a few days, and the fluorochrome is very resistant to fading, thus allowing long observation periods.

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De Laat, A.M.M. and Blaas, J. (1987), Plant Sci., in press.

(121) INDUCTION, ISOLATION AND FLOW CYTOMETRIC SORTING OF MICRONUCLEI FROM CELL SUSPENSION CULTURES OF NICOTIANA PLUMBAGINIFOLIA

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One of the objectives of this project is to establish a method for limited gene transfer through chromosome transplantation. During the previous period of the BEP programme, the isolation and sorting of metaphase chromosomes on the basis of their DNA content was achieved. Also transfer of chromosomes by liposomes was investigated but this technique was found to be unsatisfactory. This report focuses on the use of micronuclei.

Micronuclei were induced by prolonged incubation of a cell suspension culture of *Nicotiana plumbaginifolia* (Doba cell line, provided by Dr. Shields, Sittingbourne, U.K.) with APM(18 µM amiprophosmethyl). Samples were taken at regular intervals and stained with orceine or Feulgen. Protoplasts were isolated, lysed in a neutral pH buffer and stained with ethidium bromide for flow cytometry.

In addition to high metaphase arrest by APM, micronuclei were induced after prolonged exposure. The results indicated that a high cell division activity is a prerequisite for an efficient induction of micronuclei. Up to 30% of the treated cells showed multinucleation. The number of micronuclei per cell was found to be dependent upon the duration of the treatment, showing a maximum between 28 and 32 h after addition of APM. Micronuclei formed from one or more chromosomes can be sorted by FCM on the basis of DNA content. Feulgen microdensitometric measurements of DNA content of micronuclei sorted by FCM, showed the presence of a number of populations within a sorted sample.

Transfer of micronuclei may offer a good alternative for the transfer of metaphase chromosomes. As micronuclei contain interphase chromosomes, their integration into a recipient genome can be more stable than that of the isolated metaphase chromosomes.

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(122) EFFECTS OF ETHYLENE ON THE TUBERIZATION OF POTATOES

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The literature data on the effects of ethylene on tuberization in the potato plant are contradictory. Both inhibition and stimulation of tuber formation have been described (Mingo-Castel et al., 1976; Garcia-Torres and Gomez-Campo, 1973). The reason for this discrepancy might be that tuber formation is a complex phenomenon, consisting of a sequence of physiological processes, each regulated by different factors. In the tuberization of potato plants at least two important processes can be discriminated: 1. the inhibition of the elongation of the stolon, and 2. the subsequent induction of radial growth. We have tried to analyze the possible effects of ethylene on these processes separately.

Using *in vitro* culture of stem segments with an axillary bud, it was shown that ethylene, applied as ethephon, inhibited the elongation of axial shoots. Ethylene significantly suppressed tuber formation, when applied under conditions favourable for tuberization, i.e. a high sucrose concentration of the medium.

The same effects were observed after the addition of ACC (1-aminocyclopropane-1-carboxylic acid), the precursor of ethylene. Both effects could be antagonized, at least partly, by silver ions. These obsevations indicate a dual effect of ethylene on tuberization of potatoes: it has a positive effect by blocking the elongation of the stolon, but it suppresses tuber formation per se.

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(123) INTERACTIONS BETWEEN NADH- AND SUCCINATE OXIDATION IN POTATO TUBER CALLUS MITOCHONDRIA

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In plant cells glucose is degraded via the glycolysis and the citric acid cycle. Plant mitochondria oxidize (endogenous) NADH derived from the citric acid cycle and succinate via dehydrogenases located on the inner surface of the mitochondrial inner membrane. Glycolysis-derived (exogenous) NADH can be oxidized via an extra dehydrogenase located on the outer surface of the mitochondrial inner membrane. The question arises whether this endogenous NADH-oxidation plays an important role *in vivo*. Therefore, the effect of a combination of exogenous NADH and endogenous succinate on the electron transport chains was studied in mitochondria isolated from callus-forming potato tuber discs (*Solanum tuberosum* L. cv. Bintje).

With respect to the cytochrome pathway, a combination of exogenous NADH and succinate resulted in respiratory rates which were higher than the rates with the substrates given separately, but less than the sum of the individual rates. In the combination both substrates contributed to oxygen uptake, but NADH oxidation was inhibited to a greater extent than succinate oxidation.

As far as the alternative pathway is concerned, a combination of succinate and NADH resulted in respiratory rates which were about the sum of the separate rates. A stimulation of NADH oxidation mediated by the alternative pathway was observed in the presence of succinate.

In conclusion, it can be said that there is a difference in the interaction between succinate and exogenous NADH with respect to the cytochrome pathway of the alternative pathway. In the cytochrome pathway NADH oxidation is partly inhibited by succinate, but NADH oxidation still contributes to a considerable extent to oxygen uptake in the presence of this substrate derived from the citric acid cycle.

In the alternative pathway NADH oxidation is even stimulated in the presence of succinate. Possible mechanisms for this effect will be discussed.

(124) CORTISOL ENHANCES POLYAMINE CONTENT IN ETIOLATED MUNG BEAN SEEDLINGS

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Application of corticosteroids to intact mung bean seedlings results in very pronounced physiological processes. Root elongation growth and lateral root formation are doubled by a number of glucocorticoids and their 5ß-reduction products. The 5α-reduced glucocorticoids and mineralocorticoids are inactive or inhibiting. Also the hypocotyl growth is stimulated (20-50%) by various glucocorticoids (Geuns, 1983[a]). Cortisol, 5ß-DHF and 5α-DHFstimulate the adventitious root formation on the hypocotyls of intact mung bean seedlings (Loeys and Geuns, 1978; Geuns 1983[b]). As polyamines are involved in a large number of physiological processes and were reported to be related to adventitious root formation, we followed the free polyamine content in different sections of etiolated seedlings, growing in water or in cortisol solution, during 5 subsequent days. Methods were described elsewhere (Walter and Geuns, 1987). Cortisol enhanced the putrescine content of roots from day 2 on, but did not influence the amounts of spermidine or spermine. In the cotyledons only a small decrease in the content of putrescine on day 3 was observed after a cortisol treatment. In leaves and epicotyls the only influence of cortisol was a 30% lowering of the spermine content on day 3. Very interesting results of cortisol application were found in the lower hypocotyl region. From day 3 on cortisol significantly enhanced the putrescine content in the lower 2 cm sections and from day 4 on the contents of putrescine, spermidine and spermine were significantly stimulated by a cortisol treatment in the lower hypocotyl sections. In these lower sections a very pronounced adventitious root formation occurred after a cortisol treatment (Loeys and Geuns, 1978). The exact correlation between polyamines and adventitious root formation is under investigation.

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(125) THE EFFECT OF PARAQUAT ON THE ETHYLENE PRODUCTION OF INTACT GREEN PHASEOLUS VULGARIS L. SEEDLINGS

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Irradiation of 10 days old green seedlings of *Phaseolus vulgaris* L. with white light (150 $\mu mol.m^{-2}.s^{-1}$) results in a 31% enhancement of the ethylene production. Previous results suggest that active photosynthesis is involved in the light-stimulated production of ethylene, more specifically through the generation of free radicals in the water-splitting reaction of photosystem 2.

Paraquat (= methyl viologen) is a potent herbicide known to stimulate oxygen radical production through the reaction of molecular oxygen with reduced paraquat radicals (Halliwell, 1984). Paraquat applied to green bean seedlings is useful in our study of the light-stimulated ethylene production in two respects. First, its action is specific for the cloroplast site and secondly, it is taken up rapidly by the intact roots (Halliwell, 1984).

The seedlings were placed with their roots in 50 ml paraquat (10^{-4}M and 10^{-3}M) or water (=control). CO_2 and ethylene were determined in white light or darkness after 6 h stabilisation of the intact seedlings in a continuous flow system. After 6h of darkness, the plants did not show any difference of ethylene or CO_2 production neither in water nor in paraquat (10^{-4}M and 10^{-3}M). However, in light the paraquat-treated plants showed a significant stimulation of their ethylene production with respect to the controls (10^{-4}M : 17%; 10^{-3}M : 52%). Net photosynthesis was only inhibited by the highest paraquat concentration.

Time-course experiments were performed during 9h light or darkness. Paraquat (10^{-3}M) was applied to the plants at the beginning of the experiment. During the 9h dark period, ethylene and CO_2 production remained fairly constant. Moreover, no difference was observed between controls and paraquat-treated seedlings. In contrast, there was an increase of the ethylene production of paraquat-treated seedlings in light. Compared to the controls, their ethylene production was stimulated after 5 to 6h exposure to light. With time, paraquat treatment also increasingly inhibited net assimilation of CO_2. Over the whole experimental period no visible symptoms of damage by the paraquat solution were noticed.

These data suggest a close relationship between the action of paraquat (i.e. production of free radicals), light and ethylene production. Free radicals generated by an interaction between the herbicide and light seem to be involved in the light-stimulated ethylene production of bean seedlings.

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(126) SHORT- AND LONG-TERM GROWTH RESPONSES AFTER FAR RED END-OF-DAY IRRADIATION OF HELIANTHUS

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Helianthus plants (4-6 weeks old) were irradiated with broad band red (R) or far red (FR) light obtained by use of Cinemoid filters. Leaf fresh weight decreased and stem fresh weight increased after FR-irradiations. Although total stem dry weight was higher in the FR-irradiated plants than in the controls, the concomitant increase in stem elongation indicates increased water uptake. Water stress was induced by adding concentrated NaCl to the potting compost of the plants. Growth reduction, as a function of osmotic stress was similar in the presence or absence of FR end-of-day irradiation. This suggests that phytochrome action and water transport are not directly coupled. The effects of one FR-irradiation on elongation could be observed over a 36 h period. The FR-effect increased, when the light treatment was repeated at the end of 2 or 3 consecutive days. Growth was measured utilizing transducers at 10 s intervals. Means of 30 measurements, i.e. 5 min were computed. The running means of growth over 4 periods of 5 min were computed on an hourly basis (apparent growth rate) or as a percentage of the maximum value during the dark period. Apparent growth rates during the dark period showed an undulating pattern in FR-treated plants, osmotically stressed plants and controls. Apparent growth rates were higher in FR-treated plants, but the positions of the peaks remained the same as in the controls. Differences occurred in the second half of the dark period, where apparent growth rates increased in FR-treated plants and decreased in the controls. The FR-action could be partially reversed by R during the first 6 h of the dark period. The phenomenon of undulating growth also occurred in FR+R-treated plants.

We conclude that these undulations are not related to phytochrome action. Our results indicate that the low P_{fr} levels induced by FR during the dark period cause a more or less permanent change in the characteristics of the stem tissue or stem metabolism.

(127) SUGAR AND AMINO ACID TRANSPORT INTO DEVELOPING SEEDS OF PEA (PISUM SATIVUM L.). CONTROL MECHANISMS OPERATING IN THE SEED COAT

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During accumulation of assimilates by developing seeds of legumes, solutes are transferred from tissue of maternal origin (seed coat) to tissues of the developing embryo. After the removal of the embryo from a developing seed, the 'empty' ovule can be filled with a substitute medium to trap the solutes released from the seed coat (Wolswinkel, 1985). Several lines of evidence support the view that sugar and amino acid transport into 'empty' seeds is not reduced, in comparison with transport into intact seeds within the same fruit (provided that the solution filling the seed coat cavity has an optimal osmolality (Wolswinkel, 1985)).

The rate of phloem transport into developing seeds of legumes is reduced by a low osmolarity of the substitute medium (e.g. 50 mM mannitol, in comparison with a 400 mM mannitol solution). Possibly, in developing seeds of legumes and many other taxonomical groups, a high solute concentration in the sink apoplast is required to maintain the rate of assimilate transport from source regions into the developing sink. In experiments on the efflux of sucrose and amino acids from excised seed-coat halves and cotyledons of developing seeds, it could be demonstrated that the cell membranes of these tissues have a relatively high permeability (there is a high rate of efflux). The rate of uptake of sucrose and amino acids from the seed coat or cotyledonary apoplast is controlled by cell turgor (influenced by the osmolality of the apoplast solution). Uptake from a bathing medium is reduced, when a low solute concentration is present in the solution (e.g. 50 mM mannitol, in comparison with a 400 mM mannitol solution (Wolswinkel et al., 1986)). When a relatively low osmolality of the solution in the seed apoplast is reducing carrier-mediated uptake of sucrise and amino acids from the apoplast (and possibly also of other solutes, such as K^+ ions), this phenomenon may assist in maintaining a relatively high solute concentration in the seed-coat apoplast. A relatively low solute concentration in the seed-coat apoplast would reduce the sink strength of a developing seed, since it can lead to a relavitely high cell turgor at the sink end of the phloem pathway (Wolswinkel, 1985; Wolswinkel et al., 1986). This may be prevented, when uptake of solutes from the apoplast is controlled by cell turgor.

Wolswinkel, P. (1985), Physiol. Plant. 65, 331-339.
Wolswinkel, P., Kraus, E. and Ammerlaan, A. (1986), J. Exp. Bot. 37, 1462-1471.

(128) THE ROLE OF CHELATION IN THE UPTAKE AND FATE OF CADMIUM IN TOMATO PLANTS

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Chelation of metal ions in the vicinity of plant roots plays an important role in the supply of nutrients to plants. Were it not for this important phenomenon, most soils would be devoid of plant growth because iron and in some cases zinc, copper and manganese are too insoluble in soils to maintain adequate levels of soluble nutrient. Chelating agents are regarded to combine with micronutrient cations and increase the total level of these nutrients in solution. Chelation increases nutrient availability by increasing both mass flow and diffusion of nutrients to roots.

In soils contaminated with heavy metals, the phenomenon of chelation not only increases available levels of necessary cations, but also those of toxic metals. The mobility of metal ions in the plant may depend on whether metal complexes enter the root intact or only after dissociation at the root surface. Although both mechanisms may be operative, chelates are also indicated as being excluded from plants.

In the present study the uptake and distribution of cadmium in tomato plants (*Lycopersicon esculentum*, Mill, cv Tiny Tim) was examined, with and without the presence of EDTA as chelating agent. Eight weeks old intact and de-rooted tomato seedlings were used in hydroculture experiments with cadmium applied as $^{115}Cd(NO_3)_2$ in a range of concentrations, with or without EDTA and/or DNP (dinitrophenol). Measurements of the ^{115}Cd content of roots, stems and leaves were carried out by γ-ray spectroscopy. The data showed that applications of both EDTA and DNP result in reduced total Cd accumulation in the plants, but enhanced Cd transport into the above-ground plant parts. The Cd mobility in the transport channels in the shoots was increased by EDTA in both intact and de-rooted plants. Application of DNP increased Cd import in leaves in de-rooted plants, but resulted in reduced import in leaves of intact plants. These results suggest the possible formation of Cd-complexes in root cells before root-to-shoot transport takes place. Furthermore, initial Cd uptake may be associated with adsorption on the negative charges of the cell walls of the root-system. The high Cd-mobility in shoots, in experiments with intact plants and Cd-EDTA application indicates the possibility of Cd-uptake as a Cd-EDTA complex.

(129) PLASMALEMMA-LOCATED ADENOSINE TRIPHOSPHATASE ACTIVITY IN EPIDERMAL TRANSFER CELLS OF DEVELOPING PEA COTYLEDONS

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During pea seed development the embryo takes up assimilates released by the seed coat. The positioning of the embryo within the seed suggests that this uptake mainly occurs via the abaxial epidermis of the cotyledons. These epidermal cells develop wall ingrowths which are entirely restricted to the outer tangential walls. Later in development wall accretions are formed, obliterating the original investments of the ingrowths. The cotyledons are able to take up assimilates actively. A proton-pumping ATPase activity is involved in such a process. In this paper we report on the localisation of an ATPase activity in the epidermal transfer cells.

Pea plants (*Pisum sativum* L. cv. Marzia) were grown in a controlled environment. Seeds were harvested about 3 weeks postanthesis. ATPase activity has visualised by standard histochemical methods employing lead and cerium phosphate precipitation. The (pre-)incubation medium contained 80 mM Tris-malate buffer (pH 7.2), 240 mM sucrose, 5 mM $Mg(NO_3)_2$ and 2 mM $Pb(NO_3)_2$ or 1 mM $CeCl_3$. The incubation medium contained besides 2.3 mM ATP. After washing, the slices were postfixed in a mixture of OsO_4 and $K_4Fe(CN)_6$ and then processed for electron microscopy. Suitable control treatments were carried out.

In the epidermal transfer cells, a heavy ATPase reaction product was present on the plasmalemma adjoining the ingrowths of the outer tangential cell wall. Minor amounts were present on the plasmalemma adjacent to the smooth inner tangential walls and the radial walls. ATPase reaction product was nearly absent on the plasmalemma of the parenchyma cells and on that of the adaxial epidermis.

Control incubations without ATP showed little activity. Deposits were absent after heating the slices or after incubating sections in a medium with ATP and NaF. Adenylate cyclase activity could not be demonstrated under the present assay conditions. Substitution of β-glycerophosphate for ATP gave slight deposits.

The amount of the lead and cerium phosphate precipitate on the plasmalemma of the epidermal transfer cells obtained with ATP as substrate is much greater than that obtained in control media. This indicates the presence of a plasmalemma-located Mg^{2+} activated ATPase activity associated with the outer tangential wall ingrowths. The abaxial epidermal cells are thus polarised with respect to their structure and the localisation of a plasmalemma-located Mg^{2+} ATPase activity. This supports the suggestion that they are involved in the active uptake of assimilates by the developing embryo.