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Initiation of Starch Biosynthesis in Photosynthetic Tissues

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Since the first publications reporting the characterization of starch synthase and starch phosphorylase, it was found that they both required exogenous glucan primer to form new α-glucosidic bonds, i.e. they could only transfer glucose from ADPglucose or glucose-1 phosphate, respectively, to a pre-existing glucan primer. Later, however, a number of reports showed that starch synthases from potato tuber and spinach leaf and the phosphorylase of potato tuber can catalyse the synthesis of α-glucan in the absence of added primer, i.e. they have 'unprimed activity'. Several studies on potato tuber were the basis for the hypothesis that a phosphorylase isoform localized on amyloplasts can synthesize α-1,4-glucan chains on a protein acceptor; the protein-glucan so formed would act as a primer for the starch synthase. If initiation of starch biosynthesis *in vivo* followed the pathway proposed, unprimed phosphorylase activity would be expected to be present in all plant tissues that accumulate starch. The phosphorylase from spinach chloroplasts was chosen for this study because it is homologous to the potato-tuber amyloplast enzyme and because in the leaf, the chloroplast is the site of starch accumulation. The questions to be answered were the following. Does the chloroplastic phosphorylase have activity in the absence of added primer? What is the nature of the product of the unprimed activity? Is the endogenous primer a protein? Could the unprimed reaction occur in physiological conditions? Indeed, the chloroplast phosphorylase from spinach leaves was found to be capable of synthesizing α-1,4-glucan in the absence of added glucan primer but, apparently, no protein primer is required. The 'unprimed' activity seems to consist of the elongation of pre-existing, endogenous primer associated with the enzyme in a non-covalent fashion. The unprimed activity of the choloroplastic phosphorylase resembles those of the starch synthases and glycogen synthases of several sources as far as the stimulation by citrate, presence of non-covalently attached polyglucose in the enzyme preparation, properties of the product, and in the interactions with branching enzyme.

It is concluded that the criteria used in the past for the measurement and characterization of putative proteoglucan are inadequate and that a new approach is required in the investigation of the initiation of starch synthesis.

Root Respiration of Fast-Growing and Slow-Growing Species in Relation to N Supply

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Inherently fast-growing grass species lose approximately 30 and 14% of the carbon assimilated per day in total plant and root respiration, respectively, when plants are grown at an optimum nitrate supply. For inherently slow-growing grasses, these fractions are higher: approximately 41 and 16%, respectively. Despite their vastly lower rates of growth and nitrate uptake, roots of slow-growing species respire only at a marginally lower rate than those from fast-growing ones. This discrepancy is not due to an intrinsic inefficiency of the respiratory apparatus of the slow-growing species. Comparison of calculated and measured rates of root respiration suggests that slow-growing species have constitutively lower respiratory energy costs for ion uptake than fast-growing ones.

When grown at a growth-limiting supply of nitrate, inherently fast-growing species lose approximately 71 and 52% of the carbon assimilated per day in total and in root respiration, respectively. For inherently slow-growing species, grown at the same nitrogen supply, these fractions are approximately 58 and 38%. At this growth-limiting supply of nitrate, the rate of root respiration is very similar for fast- and slow-growing species. Despite the vastly lower rates of growth and nitrate uptake at the growth-limiting nitrate supply, in comparison with that under optimum conditions, the rate of root respiration is only marginally lower. Comparison of calculated and measured rates of APT-production indicates that the total calculated respiratory energy consumption is far less than the actual one. We suggest that this discrepancy is at least partly due to an increased specific respiratory energy demand for nitrate uptake.

Phloem Loading: a Climate-Associated Machinery?

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Plasmodesmograms (pictograms of symplastic continuity in the phloem-loading zone of the minor vein) predict the existence of multiprogrammed phloem-loading (Van Bel & Gamalei 1991). Recent experiments evidenced indeed a strong correlation between the mode of phloem loading and the plasmodesmatal connectivity between sieve element/companion cell complex (SE/CC-complex) and the adjacent cells. Abundant plasmodesmatal connectivity seems to coincide with symplastic phloem-loading, symplastic isolation of the SE/CC-complex with apoplastic phloem-loading. Also, on the basis of plasmodesmograms, several multiple mechanisms with combined symplastic/apoplastic phloem-loading are to be expected. The plasmodesmatal minor vein configuration is a family feature. Distribution of the plant families over the global surface reveals a potential correlation between minor vein configuration and the terrestrial ecosystem. The distribution suggests that mainly drought and temperature stress have provoked the evolution of the ancient symplastic phloem-loading into the more advanced apoplastic mode. The resultant ecophysiological concept of phloem loading contends that the mode of phloem loading is grossly correlated with the climate.

Van Bel, A.J.E. & Gamalai, Y.V. (1991): Multiprogrammed phloem loading. In: Bonnemain, J.L., Delrot, S., Lucas, W.J. and Dainty, J. (eds): *Recent advances in phloem transport and assimilate compartmentation*. 128–139, Ouest Editions, Nantes.

Hormonal Regulation of Assimilate Partitioning: a Micro- and Macro-Economic Exploration

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The involvement of plant growth regulators in the transport of assimilates towards developing seeds has been the subject of study for a number of years. Of all classes of hormones, abscisic acid (ABA) seemed to be the most suitable candidate for a role in assimilate partitioning. We studied this role with the use of ABA-deficient mutants of *Arabidopsis* and pea. Both long-distance transport of assimilates and partitioning of the assimilates among the different types of storage materials in the seed were investigated.

The fresh and dry weights of the seeds of ABA-deficient mutants did not differ significantly from the corresponding wildtypes.

Next, we measured growth rates of seeds with different ABA-content on the same (ABA-deficient) motherplant. Growth rates of pea seeds were determined by measuring the seed diameter with a pair of callipers during development; *Arabidopsis* plants were labelled with $^{14}CO_2$ to study the growth rate of the seeds. In both cases no influence of ABA was detectable.

Unloading of sugars from the seed coats was studied with the empty seed coat technique. The concentration of ABA in the buffer did not significantly influence the import rate of sugars.

Finally, we investigated the influence of ABA on the fate of the imported photosynthates. Both lipid and carbohydrate content of mutant and wildtype *Arabidopsis* seeds were compared. Presence of ABA in the seeds significantly increased the content of long-chain fatty acids, and simultaneously decreased the content of both soluble sugars and poly-saccharides.

We concluded that ABA exerts no effect on the long-distance transport of assimilates, but seems to play a role in the distribution of these assimilates among the different types of storage materials, at least in cruciferous seeds.

Control of Thiophene Biosynthesis in *Tagetes patula*

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Tagetes species (marigolds) accumulate in their roots sulphurous compounds known as thiophenes. Little is known yet about the factors controlling the synthesis of these compounds. We are investigating the regulation of thiophene metabolism in hairy root cultures of *T. patula*.

One possible way to influence thiophene production is by changing the sulphate concentration in the liquid growth medium of the hairy root cultures. Lowering the sulphate concentration from 2 mM to 0·1 mM had no significant effect on morphological development of the roots. The ability of the roots to take up [^{35}S]-labelled sulphate also did not change. In contrast, both thiophene content and the capacity to synthesize thiophenes decreased dramatically. This indicates that culturing at low sulphate concentration, not only limits the availability of an essential precursor but also leads to a modification of the sulphate-channelling in the cell. The latter might be achieved by a decrease in the activity of enzymes involved in thiophene synthesis.

An assay has been developed to quantify butenenyl-bithienylacetate (BBTOAc) esterase, one of the enzymes controlling thiophene metabolism. We are trying to identify more enzymatic steps in thiophene metabolism. As a first approach we study more thiophene bioconversions using polyacetylenes or thiophenes as substrate *in vivo* in root cultures and *in vitro* in partially purified enzyme extracts from *Tagetes* species.

Reflected Additional Lighting can Affect the Crops of Neighbours

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Nowadays, additional lighting of greenhouse crops is widely applied in The Netherlands, especially for roses, but also for lilies, chrysanthemums, freesias and pot plants. Due to the often dense concentration of greenhouses, a neighbouring greenhouse is generally very close, and leakage of light may affect the crops therein. Leakage of light through the side walls of the greenhouses is restricted by screens, but some of the added light leaves the greenhouses through the roofs. The light may then be reflected by clouds, especially when the clouds are low, and be diffused over the surroundings. Light levels of up to 20 lux may be expected.

Therefore, the effect of 4 and 20 lux of HPS-light, either in the first or in the second part of the night, was studied on the growth and development of some greenhouse crops. Sunlight-daytime was 10 h, the night, either with or without 'neighbours' light was 14 h. In the light treatments, 6 h of darkness was maintained.

Elongation growth of Cucumber 'Farbio' was reduced by light compared to dark controls, and fruits developed later and on a higher node. Flowering of the short-day plant *Chrysanthemum morifolium* 'Daymark Cream' was delayed (4 lux first as well as second part of the night, 20 lux first part), or fully inhibited (20 lux second part of the night). In the short-day plant *Euphorbia pulcherrima* (Angelica) (Poinsettia) all light treatments prevented flowering. The long-day plant *Fuchsia* 'Beacon' started to flower with 20 lux, but not with 4 lux. *Callistephus chinensis* (China Aster) 'Milady', 'Kometa' and 'Starlight' responded to all light treatments: 20 lux induced flowering and caused a strong leaf expansion and elongation of stems and flower stalks. Four lux induced flowering without extra elongation and leaf expansion. Generally light in the second part of the night was more effective than light in the first part of the night.

These results indicate that growers should take care with the use of additional lighting in areas with many greenhouses, and they may have significant implications for future use of additional lighting in The Netherlands.

Relationship of Nitrate and Tonoplast ATPase Activity in Lettuce

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Nitrate uptake in lettuce vacuoles is stimulated by ATP. Intriguing questions were whether this nitrate transport is related to ATPase activity and how this ATP-stimulation can be reconciled with the fact that nitrate inhibits APTase activity, at least at high concentrations. We evaluated the effect of nitrate on the two properties of ATPase: proton pumping and ATP hydrolysis. In the presence of MgATP, addition of nitrate (at concentrations up to 50 mM) to tonoplast vesicles resulted in generation of a proton gradient, comparable to the effect of chloride. The effect of nitrate on the rate of quenching depended on the concentration. When the vesicles were incubated with bafilomycin, a very specific inhibitor of the tonoplast ATPase, no quenching occurred after addition of nitrate. Apparently, the effect of nitrate on generation of the proton gradient in the presence of MgATP is exclusively due to an ATPase effect. Nitrate inhibited proton pumping when the addition sequence was changed and the anion was added to the assay before addition of MgATP. The stimulating or inhibiting effect of nitrate on proton pumping may depend on the status of the ATPase. When ATP is added first, it may bind to the bindings site of the enzyme, preventing nitrate from binding. Then nitrate acts as a permeant anion. When nitrate is added first, proton pumping is prevented.

ATP hydrolysis was inhibited by nitrate. So, the effect of nitrate on ATP hydrolysis differed from the effect on proton pumping.

The oxonol V fluorescence quenching showed that nitrate dissipates the membrane potential in the presence of MgATP, suggesting that nitrate will be transferred into the vesicles driven by the membrane potential and stimulating ATPase to pump H^+. Quinacrine fluorescence quenching with vacuoles showed that they already contain a large stable proton gradient after isolation, notwithstanding the presence of MgATP. Oxonol V fluorescence quenching with vacuoles showed that they do not have a membrane potential after isolation. Only after addition of MgATP a membrane potential is generated.

We conclude that nitrate uptake by vacuoles is only possible after ATP stimulated generation of a membrane potential. Moreover, nitrate will not inhibit the proton pumping property of ATPase under all circumstances.

Identification of the Quantitative Most Important Energy-Requiring Maintenance Processes in Leaves; The Costs of Assimilate Transport and Protein Turnover

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The nature of processes influencing the dark respiration of non-growing leaves was studied. Sugar export and protein turnover were considered to be most important. To test this hypothesis, respiration measurements were performed by differential infrared gas analysis. Sugar and protein content were determined. For the experiments, the comparable primary leaves of bean (*Phaseolus vulgaris* L.) were used. The correlation between leaf protein content and dark respiration rate was significant. This could not be explained by sugar transport. Transport rates of assimilates were low, and, therefore, the resulting respiration requirements appeared to be negligible. The dark respiration rate of non-growing leaves was not completely explained by the combination of these processes.

Limitation of Essential-Oil Production in Caraway by Assimilate Availability

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In a Dutch National Research Program, the potential of caraway (*Carum carvi* L., Umbelliferae) as an industrial crop is being investigated. So far, caraway seed (or actually fruit) is mainly being used as a spice. Several authors report useful biololgical activities of the two main monoterpenoid compounds of the essential oil contained in the fruits: carvone and limonene, which may lead to new uses. For new industrial applications, a stable production of caraway essential-oil will be a prerequisite. Currently, up to six-fold differences in essential-oil yield between years and individual farmers occur. Weather conditions seem to have a strong influence on seed yield and essential oil content. Analysis of data from Dutch farmers suggests that there is a positive relationship between solar radiation and essential-oil content. Data from a field experiment in which plant source-sink-ratio's were altered by removal of leaves or flowers, suggested that seed set and filling and essential-oil synthesis were limited by the availability of carbohydrate. In a greenhouse experiment, a positive relationship between assimilate availability and essential-oil synthesis was found. Plants with a high sugar content showed a higher rate of essential-oil synthesis than plants with a low sugar content. This was confirmed in a semi in-vitro experiment: After pollination on the plant, umbels were cultured for 1 week on a liquid MS-medium containing a range of sucrose concentrations. There was a positive relationship between sucrose concentration and both fruit growth and essential oil synthesis. Essential-oil synthesis rate was of the same order of magnitude as in the greenhouse experiment.

Assimilate availability during seed set and filling seems to be crucial for a high and stable yield of caraway essential-oil. Further studies are aimed at handing tools to breeders and agronomists to improve varieties and crop management which may increase and stabilize caraway essential-oil production.

A Comparison of Some Methods to Determine the Degree of Frost Hardiness in Winter Wheat

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Frost hardiness of winter wheat plants was determined by the amount of ion leakage, the amount of triphenyl tetrazolium chloride (TTC)-reduction and chlorophyll fluorescence of leaf tissue.

Two-week-old winter wheat (cv. Urban) plants were hardened in a growth cabinet at low temperature and low light conditions (4°C; 60 $\mu E\ m^{-2}\ s^{-1}$, 10 h) for several weeks. At different times frost hardiness was determined. For both the ion leakage and the TTC test, leaf parts of 3 mm were frozen in glass tubes at a rate of 4°C/h. At different temperatures tubes were removed from the cooling bath, stored at 4°C in the dark overnight, after which ion leakage and TTC reduction were measured (Steponkus, P.L. & Lanphear, F.O. 1967, *Plant Physiol.* **42:** 1423–1426). Chlorophyll fluorescence was measured on leaf parts of 1 cm placed on moist filter paper on an aluminium cuvette of 4°C. The temperature of this cuvette could be lowered by cooling it at one side at a rate of 4°C/h with a cooling bath so that a temperature gradient from +4°C to −18°C could be established. When the final temperature was reached, the cuvette was rewarmed again quickly to 4°C. Initial fluorescence and maximal fluorescence were measured before and after the freezing treatment.

Winter wheat showed a hardening (LT_{50}) from −8·5°C to −12°C in 8 weeks with both ion leakage and TTC-reduction. Similar results were found when hardiness was determined with chlorophyll fluorescence starting from the second week of hardening. However, unhardened leaves had a killing temperature of −4°C when measured with cholorophyll fluorescence. This difference seemed to be caused by the possibility of the leaf parts, which were frozen in test tubes, to supercool, as when these leaf parts were

nucleated with ice crystals at −1°C, the LT_{50} was raised to −4°C as measured with ion leakage. Leaf parts frozen in glass tubes also showed a survival until −8°C when measured with chlorophyll fluorescence.

From these experiments it can be concluded that besides ion leakage and TTC-reduction, chlorophyll fluorescence can be a useful tool to determine the degree of hardiness in winter wheat leaves.

Physiological Effects of Enhanced UV-B on *Phaseolus vulgaris*. Simulation of 10% Ozone Reduction Under Diffuse Belgian Climatic Conditions

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Ozone depletions of 10–20% can be expected in the near future, resulting in increased levels of UV-B radiation (280–320 nm) reaching the earth.

The purpose of this study was to evaluate the sensitivity of young bean plants (*Phaseolus vulgaris* L.) cv. Label under simulated light conditions of overcast weather at normal and reduced (10%) ozone concentrations (probable Belgian situation). UV-B effects being directly dependent on the amount of visible light (Cen, Y.P. 1990, *J. Exp. Bot.* **41**: 1489–1495), it seemed possible that diffuse light conditions would increase damage. A second purpose was to study the possibilities of image analysis for fast and non-destructive pigment measurements.

After 30 days of irradiation, the dry weight of the plants was reduced by 22% (comparable to results at higher light intensities). The diminished growth was explained by the significant photosynthesis reduction at all leaf ages (average: 12·6%). The high sensitivity was due to the low visible light levels which inhibited flavonoid synthesis (the main protective pigment against UV, Robberecht, R. 1978, *Oecologia* **32**: 277–287) and resulted in plants with relatively thin leaves. Significant colour differences beteween test and control group were detected and correlated with the changes in chlorophyll-content (chlorophyll synthesis was stimulated by the higher UV-B intensities, probably a defense mechanism). Further study is necessary to find the exact relationship between colour-change and pigment-concentration.

The Development of Shoots and Axillary Buds of Tulip Bulbs Grown *in vitro*

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Attempts to develop in-vitro adventitious bulbs of tulip have not been very successful so far.

In the present study shoots of dry stored bulbs were isolated, together with a small piece of the basal plate, carrying the innermost daughter bulb. The explants were cultured in a liquid medium (modified Murashige and Skoog medium), supplied with 120 g/l sucrose and various amounts of GA_{4+7}. The dry stored bulbs had been exposed to a 5 or 17°C temperature treatment for 0, 6 or 12 weeks.

The results show that a previous cold treatment of the mother bulb enhances the growth of the daughter bulbs *in vitro*. Adding GA_{4+7} had no effect on buds from the 0 or 12 week 17°C bulbs. It stimulated growth of daughter bulbs from mother bulbs exposed to 12 week 5°C, the optimum being 0·1 mg GA_{4+7} per explant. As soon as the daughter bulbs started growing, the growth of the attached shoot stopped.

The growth rate of the shoot was significantly higher when they were excised from 12 week 5°C bulbs as compared with bulbs that received other treatments (0 and 6 week 5°C, 12 week 17°C). The highest concentration GA_{4+7} (1 mg per explant) inhibited shoot growth of 12 week 5°C bulbs.

It is suggested that the sensitivity of shoots and/or axillary buds to GA_{4+7} increased during the cold treatment.

Cryopreservation of In-Vitro Shoot Tips of Chicory (*Cichorium intybus* L.)

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Cryopreservation may become an important tool for the long-term conservation of valuable germplasm of crops under genetically stable conditions. Shoot tips are most appropriate for cryopreservation because of their genetic stability upon regrowth.

Shoot tips of about 2 mm, isolated from *in-vitro* cultured root fragments of chicory (*Cichorium intybus* L.) variety Flash, were subjected to several cryopreservation protocols. Cooling rates between 0·1°C/min and 0·7°C/min to a transfer temperature of −30°C, −40°C or −50°C were investigated. The influence of the DMSO content and the duration of preculture and cryoprotection treatments on the survival were examined. The best result, regrowth of 83%, was obtained after 2 days preculture on medium without DMSO, cryoprotection with 15% DMSO for 1 h and freezing to −40°C with a cooling rate of 0·5°C/min prior to immersion in liquid nitrogen.

During regrowth of the cryopreserved shoot tips, callus formation was observed. A callus phase prior to shoot initiation is undesirable as callus formation potentially increases the frequency of somaclonal variants.

Further investigations on the reaction of different varieties, the survival after prolonged storage periods and the regrowth of cryopreserved shoot tips into entire plants are in progress.

To have more shoot tips, we inoculated leaves *in vitro* on a new medium. The shoots formed on these leaves were isolated and induced for further proliferation. In this way, we can obtain a lot of shoot tips starting from a small root segment. Shoot tips isolated from root or leaf explants do not show a different behaviour after cryopreservation.

GA-deficient Seeds of *Arabidopsis thaliana* (L.) Heynh. show Fluctuations in GA Sensitivity During Seasonal Changes in Dormancy

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Buried seeds of a variety of species show seasonal fluctuations in dormancy. These patterns are often regulated by temperature. Previous studies showed correlations between dormancy and sensitivities to light and nitrate.

Germination of the GA-deficient (*ga-1*) mutant of *Arabidopsis thaliana* absolutely depends on the application of gibberellins (GAs) to the incubation medium. In light, seeds require less GA than in darkness. The sensitivity to GAs is also increased by a chilling pretreatment of 7 days 2°C. Wildtype seeds depend in darkness also on GA application, but become independent of exogenous GA when a chilling pretreatment and light are combined. Therefore it is concluded that light and chilling stimulate both GA-biosynthesis and sensitivity to GA.

Seeds of wildtype and *ga-1* were exposed to field conditions in water in darkness during 18 months. Germination capacity was tested after several intervals of incubation outside, either in a GA_{4+7}-range in darkness or in water after a 15-minute red-light irradiation. Germination temperature was 24°C. Wildtype seeds showed changes in the response to red light, while both genotypes showed *identical* fluctuations in GA requirement during the seasons.

Therefore, fluctuations in GA sensitivity are part of the seasonal pattern of dormancy. For germination, seeds require the light-induced GA-biosynthesis during a GA-sensitive phase in the cycle.

Determination of Free IAA and IAM in *Cucumis sativus* Roots Transformed with *Agrobacterium rhizogenes*

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Roots were obtained after inoculation of the basal part of cucumber stems with different strains of *Agrobacterium rhizogenes* of the agropine-type and grown *in vitro*. They are able to grow on a hormone-free medium. The T-DNA of this type consists of two different fragments, TL (left) and TR (right) (White *et al.* 1985, *J. of Bacteriol.* **164:** 33–34). Homology was detected between the TR aux-loci and the *A. tumefaciens* tms-genes responsible for indole acetic acid (IAA) synthesis from tryptophan over indole acetamide (IAM) (Levesque *et al.* 1988, *Plant Mol. Biol.* **11:** 731–744). It has been shown that open reading frames 10, 11 and 12 (rol A, B and C), which are located on the TL, can induce roots on wounded tissue (Carderelli *et al.* 1987, *Mol. Gen. Genet.* **209:** 475–480). Using solid-phase purification and HPLC (Van Onckelen *et al.* 1988, *Meded. Fac. Landbouwwet. Rijksuniv. Gent* **53:** 1685–1693) we determined IAA and IAM concentrations in transformed roots. High amounts of IAA and IAM found in roots transformed with the TR alone and which showed a spongy phenotype confirm the time-persisting expression of the aux-genes on the TR-fragment. Preliminary data on the increased IAA content of young proliferating roots transformed with only the rol-genes seem to confirm the recently proposed function of rol-B. Incorporation of rol-genes and aux-genes together resulted in an intermediate phenotype and IAA concentration.

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The Use of Triple-Split Rooted Stem Cuttings in Studying Phloem Transport to Tomato Root Systems Infected with *Meloidogyne incognita*

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Stems of young tomato plants (5–6 full-grown leaves) contain three main and three smaller alternating bicollateral vascular bundles. It was possible to isolate the main bundles by making three longitudinal incisions of about 15 cm through the stem. During incubation on nutrient solutions, adventitious roots developed at the three stem parts. The three root systems, thus formed, were placed separately in pots filled with silver sand. One root system of each plant was inoculated with about 1500 second-stage juveniles of *M. incognita*. Five weeks after inoculation the triple-split, singly infected shoot-root systems were ready for use in the experiments.

Solutions containing Rb^+ or 6(5)carboxyfluorescein (CF, a fluorescent phloem-sap tracer) were applied to the transpiration stream of one of the uninoculated stem

parts for various periods. The other root systems remained in their pots or were placed in aerated tap water. Rb-concentrations and dye movement were determined using atomic absorption spectrophotometry or epifluorescence microscopy, respectively. The results show that infected root systems were stronger sinks for Rb^+ (15×) than corresponding uninfected root systems. The Rb^+-loss from infected root systems was also enhanced (70×) compared to that of uninfected corresponding ones. Hand-cut sections of CF-treated plants revealed a potential transport pathway from the nematode-induced giant cells, via the body of the nematode, out of the root. This pathway may contribute to the higher leakage of Rb^+ from infected roots. Like aphids, excreting a lot of sugars and retaining nitrogen containing compounds, plant parasitic nematodes might also excrete their surplus of compounds, which might hence exert an extra drain on the host.

Factors Influencing Epinastic Responses in Tomato Plants

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Increasing occurrence of ethylene pollution in greenhouses, brought about the onset of a renewed study about epinastic responses of *Solanum lycoperisicon* plants. Epinastic responses were examined in order to evaluate sensitivity of tomato plants for low-level ethylene pollution ranging from 0–1 ppm.

In control conditions (only background ethylene present), the angle between the stem and the leaf petiole (petiole curvature) depends on the position of the leaf on the plant. The leaf closest to the top with an angle of at least 20° was numbered 1. The petiole curvature increases for the successive leaves from the top downwards to become maximal at the lowest leaf on the stem (up to 150°). Due to new formation of leaves at the apex (one leaf every 3 days), ageing of the observed leaves results in increases of its leaf number, which reflects an increase of the petiole curvature.

The epinastic response was described as the difference in petiole curvature between control and exposed plants. After application of exogenous ethylene, a decreasing epinastic response of the leaf was observed from top to bottom. Optimal and repeatable epinastic responses were obtained for leaves 2 and 3. Dose-response curves were obtained for the top leaves numbers 3, 4, 5 and 6 using different ethylene concentrations and a fixed exposure time of 48 hours. The third leaf gave the best linear fit for the dose-response curve.

Tomato plants placed under a 12 hour photoperiod were exposed to different concentrations of ethylene during different periods of time. Petiole and mid-vein curvatures were followed kinetically during and after ethylene treatment by means of a video camera and image analysis software. Even during ethylene exposure, drastic changes of more than 100% in the epinastic curvatures could be observed depending on the time of the day at which measurements were taken.

Epinastic curvatures are the result of complex interactions between environmental and endogenous factors which were over-simplified in the past.

Plant Density and the Effect of CO_2 Enrichment on Yield and Yield Components of Spring Wheat

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The effect of CO_2 enrichment on spring wheat grown at two plant densities has been studied. A lower plant density allows for a prolonged period during which CO_2 can most effectively stimulate growth. This period ends when canopy closure reduces light penetration and diminishes the stimulative effect of CO_2. Therefore, we expect a greater stimulation by CO_2 during the vegetative period under low plant density, which could result in a greater stimulation of yield and biomass at final harvest.

Plants of spring wheat cv. 'Minaret' were grown under crop-like conditions in two naturally-lit greenhouse compartments from April to July 1991. Temperatures were 20/15°C day/night until 15 May, and 22/18°C thereafter. CO_2 was controlled at levels of 350 and 700 ppm. Plants were grown in 2231 containers, filled with a heavy clay soil. Nutrients and water were added in excess in similar amounts per plant. The two plant densities were: high density (333 plants per m^2) and low density (111 plants per m^2); row spacing was 10 cm in both treatments.

During the early vegetative period, tillering showed a significant interaction between CO_2 level and plant density: stimulation of tillering by CO_2 was greater at low plant density. Later, this difference disappeared. A higher CO_2 level increased, but lower plant density decreased final yield and biomass per m^2. At final harvest, no interaction remained between CO_2 and plant density per unit area. These results agree with literature data and suggest that CO_2 effects during the (early) vegetative stage are relatively unimportant for the effect of CO_2 on yield.

Preparation of Monoclonal Antibodies, an ELISA and Determinations of Abscisic Acid in Sprouts of Tulip

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Monoclonal antibodies against abscisic acid (ABA) were prepared by coupling this hormone to porcine

thyroglobulin through its carboxylic group. Selection occurred on plates coated with ABA-keyhole limpet haemocyanin, and hybridomas solely reacting with (+)ABA were isolated. Cross-reactivities were determined. A competitive ELISA was developed with a monoclonal antibody of which the affinity was about 50 times higher to (+)cis,trans ABA than to (+)trans, trans ABA. Methylation of the hormone was not necessary. The sensitivity of this assay ranged from 1–15 pmol. With this ELISA, the ABA content in sprouts of tulip cv. Apeldoorn was determined. Several extraction procedures were followed, varying mostly in the number of steps. Unfortunately for the tulip material, an extensive extraction procedure was necessary. The ABA content in sprouts of tulip bulbs which were completely, partly or not at all prepared to produce a quality flower (treatment at 5°C) and their respective controls (treatment at 17°C) was determined. Part of this research was carried out in collaboration with The Centre for Agrobiological Research in Wageningen.

Cadmium Effects in Mung Bean Seedlings

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Results are obtained with 3-day-old etiolated Mung bean seedlings that received Cd-sulphate (1–100 μM) for an additional 2 days under TL-tubes (16 h, 25°C).

Mung bean growth was inhibited by 10 μM Cd^{2+} in the liquid culture medium. Cd was transported and accumulated in the different plant parts even after 1 h exposure of the roots.

Polyamines were studied in the different plant parts. Even at 1 μM concentration, the concentration of putrescine increased considerably in roots, the lower and upper hypocotyl segments and also in the epicotyls. The leaves and cotyledons were less affected. Spermidine levels increased in the hypocotyls, decreased in the leaves and were not influenced in the roots, epicotyls and cotyledons. Spermine levels decreased in all plant parts.

Tissue permeability as measured by ion- and ^3H_2O-efflux studies decreased by Cd-treatment.

Isolated plasma membranes of treated roots and hypoctyls showed a much higher polyamine content as well as an increased stigmasterol to sitosterol ratio as compared to untreated seedlings. Studies on the phospholipids and fatty acids are in progress.

The Role of Light Quality in the Propagation of Hyacinth Bulbs

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Hyacinth bulbs are usually propagated by the so-called scooping technique: after excision of the basal plate the bulbs are incubated at 25°C in the dark. Dependent on the cultivar, 30–50 young bulbs are formed at the wound surface of each bulb. The main problem with this propagation technique is not the number of newly formed bulbs but their tendency to remain dormant after planting (thus reducing the yield at harvesting). In the present study, scooped bulbs of the cultivar Viking (well-known for its dormant daughter-bulbs) were incubated under different light conditions: darkness, red, white and blue fluorescent light. Light treatment had no effect on the number of newly formed bulbs. The appearance of these bulblets, however, was markedly affected by the light treatment. Under blue light the bulblets were smaller but more differentiated than in darkness. Pigmentation (chlorophyll and anthocyanin) was also enhanced by blue light. Under red light some differentiation occurred, but without pigmentation. The most striking effects of the light treatment were observed after planting. Leaves emerged sooner and a larger amount of leaves per mother bulb was formed when a light treatment was given. With respect to the latter process, light intensity seemed to play a more important role than light quality. A larger amount of leaves results in an enhanced yield of new bulbs. These results demonstrate that a light treatment during the formation of the young bulbs (partially) prevents the bulbs from becoming dormant. Although we are still far from understanding the mechanism behind this light effect the practical use of these findings is evident.

Aluminium Avoidance in Split-Root Experiments with the Velvet Bean *Mucuna pruriens* var. Utilis

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Velvet beans are fast-growing leguminous plants used as cover crops in the humid tropics. Field experiments showed that shallow root development on an acid soil was not due to an absolute toxicity of the subsoil, but to a relative preference for topsoil conditions (Hairiah *et al.* 1991 *Plant and Soil* **134:** 95–105). Split-root experiments on a recirculating nutrient solution were carried out to test the hypothesis that this subsoil avoidance is based on an Al avoidance mechanism acting at the level of the intact root system rather than on that of individual roots. A solution containing 0·185 mM Al was found to stimulate root dry weight and to reduce shoot/root ratio when applied to both sides of a split-root system. Application of such an

Al-containing solution to half of the roots led to a significant shift in root growth to the control side. In further experiments the hypothesis was tested that the Al-avoidance reaction is related to the local response of plants under P stress leading to increased root branching close to a P source. Increasing the P supply to the plant results in the disappearance of the Al-avoidance reaction.

Frost Damage of Leaves Monitored by Chlorophyll Fluorescence During Freezing

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Changes of chlorophyll fluorescence parameters during a freeze–thaw cycle which caused irreversible damage were studied in holly and spinach leaf discs.

When stationary fluorescence after induction at +6°C was recorded during freezing, only a small increase was measured below zero degrees until freezing of the leaf tissue occurred around −6°C. Quenching of fluorescence remained present below 0°C, provided that ice formation had not yet occurred.

At the moment of freezing, a fast increase in stationary chlorophyll fluorescence was evident. It decreased when the temperature was increased again. After thawing, at a constant temperature of +6°C, stationary fluorescence continued to decrease slowly. Induction of the thawed leaf disc showed a complete absence of quenching capability indicating a freezing-induced block of the electron transport beyond Q_a. The loss of quenching ability occurred when the leaf froze. A frost-induced decrease of maximal, inducible, variable fluorescence occurred below zero degrees during thawing. Analysis of the fast changes in chlorophyll fluorescence induction at different temperatures during a freeze–thaw cycle, showed a decrease in the slope of fluorescence increase beyond the first dip (D) below 0°C in unfrozen discs. This transition disappeared upon freezing and remained absent after thawing. Osmotic stress induced a comparable disappearance of the dip, indicating that osmotic stress induced by ice formation causes damage to the electron transport.

Ca^{2+} Antagonizes Gibberellin-Induced Endo-β-Mannanase Activity, Endosperm Weakening and Germination of Tomato Seeds

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Gibberellin (GAs)-induced endo-β-mannanase activity and subsequent weakening of the endosperm layer around the radicle tip are crucial steps in the germination of tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) seeds (Groot, S.P.C. & Karssen, C.M. 1987, *Planta* **171:** 525–531; Groot *et al.* 1988, *Planta* **174:** 500–504). In order to further unravel the signal transduction pathway between perception of the GA signal and ultimate visible germination, the role of Ca^{2+} has been studied. With the aid of the Ca^{2+} chelator EGTA, the Ca^{2+} ionophore A23187, and the Ca^{2+} channel blockers $LaCl_3$ and verapamil, free cytosolic Ca^{2+} levels were manipulated in cells of isolated endosperms from the GA-deficient *gibl* mutant of tomato.

Ca^{2+} and A23187 antagonized GA_{4+7}-induced mannanase activity and endosperm weakening, whereas La^{3+} and verapamil could induce mannanase activity and endosperm weakening in the absence of GAs. EGTA also strongly enhanced mannanase activity without GAs but endosperm weakening was less than expected and germination capacity was lowered. It was found that, contrary to GA_{4+7}, EGTA failed to induce mannohydrolase activity which then became the limiting factor in endosperm weakening and germination.

The calmodulin antagonists W7 and chlorpromazin were ineffective, suggesting that calmodulin is not involved in pre-germinative processes in tomato. It is proposed that lowering of free cytosolic CA^{2+} may be part of a GA-induced signal transduction pathway.

Effects of Abscisic Acid on Somatic Embryos of *Daucus carota*

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Somatic embryos of carrot are not desiccation tolerant when grown in simple Gamborg's B5 medium, containing 2% sucrose. Application of 50 μM ABA 6 days after starting the culture provided desiccation tolerance after 2 more weeks of cultivation when the following additional prerequisites were observed: (a) slow drying over a period of at least 24 h before desiccation to 5% moisture content; (b) rehydration in humid air for several hours before imbibition of the dry somatic embryos. The latter requirement prevents imbibitional damage. It is recommended that drying and the further cultivation of the embryos into plantlets, occur under sterile conditions.

According to the literature on desiccation tolerance of both plants and animals, certain disaccharides are involved in the protection of membranes at low water activity. Disaccharides closely interact with the phosphate-head group of phospholipids, thereby preventing or postponing the desiccation-induced formation of gel-phase lipid.

We analysed the soluble carbohydrates in ABA-treated and control embryos and found that sucrose

contents doubled as a result of the addition of ABA. Furthermore, we found that ABA evoked synthesis of raffinose. The impact on the other soluble carbohydrates, the monosaccharides glucose and fructose, was minimal. Concentrations of glucose and fructose were low during embryo development. Sucrose and raffinose also are the two major soluble carbohydrates in mature carrot seeds. When high sucrose concentrations were applied during embryo culture, the embryos contained higher levels of endogenous sucrose. With 8% sucrose in the culture medium and without the addition of any ABA, some embryos became desiccation tolerant. We conclude that ABA alters carbohydrate metabolism during somatic embryogenesis, and suggest that the elevated levels of sucrose and raffinose may play a role in the ABA-induced desiccation tolerance.

An Electronic Control Unit for the Automatic Measurement and Registration of Photosynthetic CO_2 Response Curves

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Over the last few years there has been considerable interest in the impact of carbon dioxide on photosynthesis, plant growth and development. The response of plants to changing CO_2 concentrations is studied in large scale projects (global climatic changes and long-term elevated atmospheric CO_2 concentrations), as well as in smaller research projects (CO_2 enrichment in greenhouse and growth chambers). Even in plant micro-propagation and post harvest research, special attention is paid to the CO_2 concentration in culture containers or the conservation room. In order to avoid the time-consuming and often inaccurate manual control of changing CO_2 concentrations in gas exchange experiments for deriving photosynthetic CO_2 response curves, an electronic gas diluter was designed which allows control of environmental CO_2 concentration around an enclosed measuring object (whole plant, leaf, fruit, etc.).

The unit provides a pre-programmable time-course setting of CO_2 concentrations which allows the automatic recording of CO_2 response curves. Any concentration between zero and ambient CO_2 level can be attained. If higher than ambient concentrations are necessary, a gas bottle with the maximum desired CO_2 concentration is used.

The principle for the production of defined CO_2 concentrations is based on the electronic switching of a gas flow between a scrubbing column and a by-pass followed by a buffer vessel. The produced outlet CO_2 concentration is directly related to the duty cycle of the valve activation.

When the unit was connected to a portable ADC-LCA gas measuring unit, an automatic recording of the photosynthetic CO_2 response curve of a rose leaf between zero and 1200 ppm CO_2 was obtained in about 1 hour.

Isolation and Characterization of Mutants of *Tagetes erecta* with Altered Thiophene Contents

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Two mutants of *Tagetes erecta* with altered thiophene spectrum were identified in a population of 316 M_2 plants by HPLC-analysis of thiophene contents in root samples from all plants. The M_2 population of *Tagetes erecta* was created by causing wild-type seeds to mutate by adding aqueous ethyl methane sulphonate and permitting the resulting M_1 plants to self-fertilize. Two new peaks were prominent in the mutant HPLC spectrum at retention times that were slightly longer than those of the known thiophenes butenenylbithienyl and α-terthienyl.

Co-segregation for the presence of the new compounds was observed in the second generation, obtained after selfing of M_1 plants. No segregation was observed in the third generation, obtained after selfing of two selected M_2 plants. The results indicate that the selected M_2 plants were homozygous for the mutation and that the mutation resided in one gene.

Different organs from 3-week-old wild-type and third-generation mutant plantlets were analysed for thiophenes by HPLC. The new compounds were detectable in roots and hypocotyls of mutant plants but not in any organs of wild-type plants. The concentration of known thiophenes was slightly lower in mutants compared to wild-type plants. The mutant plantlets were smaller than wild-type plantlets and the fresh weights of root system, shoot and hypocotyl were 1/3 to 1/2 of the fresh weights of the corresponding wild-type organs.

Feeding experiments with [^{35}S]sulphate showed that sulphur is incorporated in the unidentified new compounds, as well as in known thiophenes. This result supports our hypothesis that the compounds are intermediates in thiophene metabolism. Identification of the metabolites is underway.

Further analysis showed that the new compounds were also produced in sterile root-cultures initiated from mutant plants in Erlenmeyer shake flasks. This makes the mutants suitable for studies on thiophene metabolism in an in-vitro system with sterile root cultures. Differences in gene expression between mutant and wild-type plants are being studied with the aim to identify genes that are involved in thiophene metabolism.

Response of Photomorphogenetic Tomato Mutants to Changes in Phytochrome Photoequilibrium During the Daily Photoperiod

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Four genotypes of tomato (*Lycopersicon esculentum* Mill.) in the genetic background 'Ailsa Craig' were used: *aurea* (*au*), deficient in the labile type of phytochrome (P); high pigment (*hp*); the double mutant *au,hp* deficient in labile P; and the isogenic wild-type (WT). Seedlings were initially grown in 16-h light (170 μmol m^{-1} s^{-1} photosynthetically active radiation PAR)/8-h dark cycle for 7 days. The plants were then transferred to cabinets with the same cycle, but higher irradiance (PAR = 250 μmol m^{-1} s^{-1}) which had a red light (R): far-red light (FR) quantum ratio (R:FR) of 6·9 and maintains a P photoequilibrium (FR-absorbing form of P [Pfr]/R-absorbing form of P [Pr] + Pfr, which is abbreviated as φ, of 0·72). After a further 10–14 days the plants were transferred to cabinets with a similar irradiance and cycle, but one cabinet had additional FR, which is not photosynthetically active, reducing the R:FR to 0·13 and maintaining the φ value at 0·37). A remarkable increase in plant height as a result of an increase in the length of all internodes for all the genotypes in the cabinet with the reduced R:FR was observed. A small concomitant increase in leaf length was measured. As the *au* and *au,hp* mutants deficient in labile P respond to a reduction in the R:FR it is proposed that the stable type of P mediates this response. Anthocyanin was only detectable in the young growing leaves of the WT and *hp* mutant in the high R:FR cabinet suggesting that the potential for anthocyanin synthesis is correlated with the presence of the labile type of P. The kinetics of anthocyanin decrease in the young growing leaves were investigated in the *hp* mutant and suggest a very rapid cessation (within the first day) of flavonoid biosynthesis upon transfer to lower R:FR.

The Effect of Low Oxygen and High Carbon Dioxide Atmospheres Upon the Respiration Activity and Ethylene Production of Minimally Processed Leek and Endive

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In order to extend the shelf-life of minimally processed vegetables, they are packed and preserved under modified atmospheres. The composition of the atmosphere inside the package influences to a great extent the physiological processes that gradually lead to product deterioration.

We investigated the effect of low oxygen and high carbon dioxide atmospheres on respiration activity and ethylene production of minimally processed leek and endive stored in closed containers at 4°C.

The minimally processed leek or endive was put in containers that were flushed for 3 h with low oxygen (10%, 5% or 2% O_2; 0·5% CO_2; N_2 q.s.) or high carbon dioxide (5%, 10% or 15% CO_2; 20% O_2; N_2 q.s.) atmospheres. After closing the containers, carbon dioxide and oxygen concentrations were measured during the following 10 days. Ethanol and ethylene levels were also determined.

For both leek and endive all altered atmospheres reduced respiration activity to about half of the activity found in non-flushed containers.

For endive ethanol production occurred sooner in the non-flushed containers than in all others. No ethanol was detected in the low oxygen containers. Ethanol was produced at an earlier stage by the leek in the low oxygen containers and at a later stage by the leek in the high carbon dioxide containers.

In the containers with endive under high carbon dioxide atmospheres, the ethylene levels were at least twice as high as those found in the non-flushed containers. However, for leek, higher carbon dioxide levels resulted in a reduced ethylene production. The low oxygen atmospheres had an even more pronounced effect.

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Bulb Formation in Lily is Under Control of Abscisic Acid

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Lily plantlets regenerated *in vitro* on scale explants are an excellent model system to study storage-organ formation. We carried out our examinations in *Lilium speciosum* cv. Rubrum nr. 10. Plantlets regenerated under standard conditions consisted of scales and leaf-bearing scales. We studied how various plant hormones applied via the tissue culture medium affected the leaf/scale ratio.

Abscisic acid (ABA) completely blocked leaf formation. In agreement with this, leaf formation was promoted and scale formation blocked by fluridone, an inhibitor of ABA-synthesis. ABA added simultaneously with fluridone restored scale formation demonstrating that the effect of fluridone was indeed caused by a blocking of ABA synthesis. Other hormones (GA_{4+7}, auxins, ethylene, cytokinins) had no or no consistent effect on scale formation. Increasing the

concentration of sucrose or osmoticum affected the leaf/scale ratio as increasing the concentration of ABA did.

Our observation on the role of ABA in bulb formation contrasts with other findings which indicate that this hormone does not regulate the accumulation of storage material in developing seeds.

Growth Rate and Plant Development of Tomato *Gib* Mutants in Relation to Endogenous Gibberellic Acid

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Fast-growing species allocate more biomass to their leaves and have a higher leaf area relative to their plant weight (leaf area ratio, LAR) than slow-growing species (Poorter, H. & Remkes, C. 1990, *Oecologia* **83:** 553–559). Tomato *gib* mutants have a reduced gibberellin (GA)-biosynthesis (Koornneef *et al.* 1990, *Theor. Appl. Genet.* **80:** 852–857). These plants showed increasingly retarded stem and leaf extension and lower relative growth rates (RGR) with increasing GA-deficiency. This lower RGR was mainly the result of a lower LAR and a lower specific leaf area (SLA). Root growth relative to shoot growth, however, was clearly promoted.

Photosynthetic rates of the mutants were equal to that of wild-type when expressed per unit leaf area, but lower when expressed per unit leaf weight. The rate of root respiration was lower in the mutants.

The transpiration was higher in the mutants, but did not affect the turgor of the leaves.

The mutants had a higher percentage dry weight in the stems, but not in leaves and roots. We found no consistent quantitative differences in chemical composition between mutants and wild-type plants, in contrast to such differences found when slow and fast growing wild species were compared (Poorter, H. & Bergkotte, M. 1992, *Plant Cell Envir.* **15:** 231–240.

The reduced size of the GA-deficient mutants is the result of smaller cell numbers in stems and leaves and, in internodes, also of smaller cell dimensions.

Application of GA to the roots of mutants converted them phenotypically to the wild-type.

Although the GA-deficient tomato mutants have much in common with slow-growing wild species, further studies must reveal if these similarities are, in the latter case, also connected with low endogenous GA levels. In addition, the differences in chemical composition between the two types of slow-growing plants needs to be explained.

Carbohydrate Partitioning in Tomato Leaves as Affected by Temperature

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An increase in soluble sugar content in tomato leaves was observed when tomato plants (*Lycopersicon esculentum* Mill., cv. Abunda) were exposed to low temperatures (6–10°C) and low light intensity (85 μmol/m² s) for a period of two weeks (Brüggemann *et al.* 1991, *Planta* **106:** 179–187). By measuring the temperature dependence of photosynthesis, respiration and carbohydrate partitioning we tried to find the reason for this increase in soluble sugar content in leaf tissue under the given conditions. By using a temperature gradient cuvette system it was possible to study temperature dependence of carbohydrate partitioning in intact leaf tissue. Photosynthesis and respiration were determined by measuring oxygen evolution and consumption at saturated CO_2 concentrations with an oxygen electrode. Sugar and starch concentrations were determined colorimetrically (Fales, F.W. 1951. *J. Biol. Chem.* **193:** 113–124).

In the temperature range 6–30°C, light saturated photosynthesis decreases with decreasing temperature, but under light-limiting conditions, photosynthesis was not affected by temperature. Respiration increased exponentially with increasing temperature from 6 to 30°C. At low light intensity (85 μmol/m² s) and saturating CO_2 concentration, the total carbohydrate production was similar over a broad temperature range between 14 and 30°C. At lower temperatures, starch formation decreased. The formation of soluble sugars was not much affected by temperature.

The unaffected photosynthesis on the one hand and a strong decrease in respiration with decreasing temperature at the other hand will lead to an increase in the total amount of carbohydrate compounds at low temperatures and low light intensity. The differential effect of temperature on sugar and starch formation will result in an increase in soluble sugar concentration in tomato leaves under chilling conditions.

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IBA Conversion into IAA Causes Root Formation on Apple *In Vitro*

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Micropropagation of plants consists of two phases: (a) the multiplication of shoots by cytokinins; (b) the formation of roots by auxins. The most commonly

used auxin for root formation is indole butynic acid (IBA). After uptake, auxins can be conjugated with amino acids or alcohols. As only the free auxin acid is active, conjugation leads to inactivation of the hormone.

We have studied the conversion of IBA into the physiologically active free IAA. The role in root formation of IAA derived from IBA was assessed by comparing the concentration of free IAA after application of IBA and IAA in the culture medium. As a model system for root formation, thin stem slices of in-vitro cultured apple shoots were used. In this system IBA and IAA induced eight and five roots, respectively. These roots were formed on a small number of cells so that a relatively large number of cells was involved in the regeneration process. Our results strongly suggest that conversion of IBA into IAA at least partly causes the abundant formation of roots on apple *in vitro*.

Effect of Gibberellic Acid on Sugar Transport into Petals of 'Madelon' Rose Flowers During Bud Opening

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In cut flowers of 'Madelon' roses, flower buds often fail to open properly. Adding a carbohydrate source, e.g. sucrose, to the holding solution results in a better opening. Flower-bud opening is characterized by the expansion of the petals. Besides water, this process requires energy, osmotic compounds and intermediates for synthetic processes. These functions depend on carbohydrate availability. There is evidence of competition for carbohydrates among the petals.

Individual petals from several stages of flower-bud opening were placed on well plates containing different solutions. Weight and surface area were measured after an 8-day experimental period on the wells. The petals were divided into three groups according to requirements to obtain a size more or less comparable to their size in the final stage as intact flowers. The number of petals which only need water to expand increased during flower-bud opening. The increase was due to petals from the outer-most whorl. At the same time, the number of petals, which depended on water + carbohydrates + gibberellic acid (GA) decreased when flower opening progressed. During flower opening, the petals first lost their dependence on GA, then temporarily required water + carbohydrates, and eventually lost their need for carbohydrates.

GA served as an activator for the active component of the uptake of carbohydrates by the petals. The passive (diffusion) component of carbohydrate uptake was relatively weak in these petals.

Free and Bound Polyamine Content During the Cold-Induced Elongation of the Tulip cv. 'Apeldoorn'

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Dry-stored tulip bulbs cv. 'Apeldoorn' require a cold period of 12 weeks at 5°C to ensure satisfactory shoot elongation during subsequent growth at higher temperatures (17–20°C). It has been reported that polyamines might play a role in the process of cell elongation (Schoonjans, L. 1991, Ph.D. Thesis, K.U. Leuven). Therefore, we studied the free and bound polyamine content in the lower and upper internode of the shoot, during the growth of previously cooled (12 weeks 5°C) and non-cooled bulbs (12 weeks 17°C) tulip bulbs cv. 'Apeldoorn'.

In the pre-cooled and thus elongating bulbs, the free putrescine, spermidine and spermine content per grammes dry weight decreased considerably in both internodes. In the lower internode, this decrease coincided with the increase in the internode length. In the upper internode, however, the decrease in the free polyamine content per gramme dry weight was nearly completed before the onset of the elongation, except that of putrescine.

In the non-cooled and thus not-elongating bulbs, the free spermidine and spermine content decreased to a much lesser extent, whereas the putrescine content per gramme dry weight even increased. This occurred in both internodes.

The changes in the bound spermidine and spermine content were opposite to those of the free polyamine contents, but they were not quantitatively correlated. The changes in the bound and free putrescine content showed a similar pattern.

Future research is aimed at investigating whether the cold-related decrease of the free polyamine content per gramme dry weight results in an increase in the membrane permeability. In that way, solute uptake and elongation growth may be facilitated.

Influence of the Water Potential of the Incubation Medium on Assimilate Fluxes in Immature Pea Cotyledons

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At present, the water potential of the seed-coat apoplast is considered to be an important factor

controlling assimilate fluxes in developing legume seeds. Therefore, we have studied the influence of the water potential of the incubation medium on influx and efflux of sucrose and amino acids in isolated cotyledons of developing pea seeds. We have also measured the changes in the water relations of the cotyledons during incubation in a hypotonic medium.

The water potential of freshly isolated cotyledons was *c.* 1·0 MPa. During incubation of stage-1 and stage-2 cotyledons in a hypotonic medium their volume increased by 16 and 5·6%, respectively, while the calculated turgor pressure increased from 0·2 MPa to 0·6 MPa for both developmental stages.

In stage-1 cotyledons the influx of labelled L-valine, solely mediated by a linear uptake system, was unaffected by the water potential of the incubation medium. In stage-2 cotyledons the influx of labelled L-valine was only slightly reduced in a hypotonic medium.

In contrast, efflux of endogenous amino acids was stimulated by incubating the cotyledons in a hypotonic medium. The stimulation of the efflux, however, was restricted to early developmental stages. Incubation in a hypotonic medium reduced the sucrose influx in stage-1 cotyledons, but had no effect on sucrose uptake by stage-2 cotyledons.

These observations imply that an increase in turgor cannot be the reason for the observed effects of the water potential of the incubation medium on effluxes and influxes. It is more likely that the osmo-sensitivity is related to stretching of the plasma membrane.

Carbon Metabolism and Morphogenesis of Greenhouse Roses as Affected by Light Quality

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Light strongly affects growth and flower production in greenhouse roses. Low light intensities reduce the number of sprouting buds and increase the percentage of flowerless or blind shoots. At low light intensities, the young flower-bud—initiated in every sprouting bud—is likely to abort in an early stage of its development, probably due to a lack of assimilates. In many plant species morphogenesis and carbon partitioning are influenced by changes in light quality, especially by alterations in the ratios of blue to red (B:R) and red to far-red light (R:FR). In the present experiments we investigated the effects of light quality on the growth, flowering and carbohydrate content of newly formed shoots of rose cv. Mercedes at a suboptimal light intensity for flower formation.

A reduction in B:R enhanced elongation growth and dry weight increase in newly formed shoots. Filtering out all blue wavelengths of the spectrum of white fluorescent tubes (yielding orange light) resulted in an increase of 43% in length and 18% in dry weight of the shoots in a period of 6 weeks. Photosynthesis at equal light intensities of white or orange light was similar. Total plant weight also was not affected by changes in light quality, indicating that the differences in shoot development are the result of differences in assimilate partitioning. Although orange light stimulated assimilate partitioning to new shoots, the percentage of shoots with a flower was not affected. Thus, there seems to be no direct relationship between shoot dry weight increase and flowering. This conclusion is supported by a second experiment in which rose plants were grown in white fluorescent light and in which part of the plants received an end-of-day treatment with far-red light. Without affecting dry weight increase, end-of-day FR drastically reduced the number of flowering shoots from 42 and 18% to 15 and 2% for the two shoots that were allowed to develop at each rose plant.

So far, carbohydrate analysis of shoots developing in white or orange light have shown that reducing the B:R ratio of the light causes a build-up of starch in the leaves of the newly formed shoots. At present we have some indications that orange light inhibits the export of sucrose from the leaves resulting in starch accumulation. Experiments with $^{14}CO_2$ are currently being undertaken to study the short-term effects of blue light on the partitioning of ^{14}C-sugars within the leaf and within the plant.

Effects of Fruit Growth on Photosynthesis in Cucumber

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In *Cucumis sativus* L. net photosynthesis of leaf number 12 increased until 12 days after its unfolding and then decreased gradually.

The number of fruits per plant varied independently of the number of leaves per plant. Net leaf photosynthesis did not respond to the number of fruits on a plant during the first 16 days of fruit growth. Subsequently, a substantial reduction in photosynthesis was found only when all fruits were removed. This reduction was accompanied by a decrease in the rate of transpiration, indicating a higher stomatal resistance.

Neither starch nor mono- and disaccharides accumulated noticeably when the number of fruits was reduced, suggesting that there was no end-product inhibition of photosynthesis.

Dry matter content of the leaves increased and specific leaf area decreased when the number of fruits was reduced.

When modelling cucumber production, effects of fruit growth on leaf photosynthesis can be ignored.

Osmotic Potential and Carbohydrate Contents in the Corolla of the Rose cv. Madelon

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Flower-bud opening in the rose cv. Madelon is often not successful. Adding a preservative (containing sugars and bactericides) to the vase solution improves bud opening. However, many buds still fail to open properly, especially when flowers are harvested too unripe. Consumers often do not add preservatives, and harvesting unripe flower-buds is still common practice. As both practices are not likely to change, more knowledge concerning flower-bud opening is necessary to prevent further problems. From former research the positive effect of carbohydrate supply on flower-bud opening is known. As carbohydrates may play an important role in osmoregulation, some factors influencing osmoregulation have been studied. The osmotic value of the pressed sap from petals of roses on the plant increased during bud opening, while the osmotic value of cut roses, placed in water, increased to a much lesser extent. The soluble sugar content measured in corollas during bud opening on the plant was higher than that of corollas from roses placed in water.

During vase life, the expansion of the outer petals is sometimes still relatively successful, but further development of the inner petals stops. Removing the outer petals leads to a better expansion of the inner petals, thus indicating a competition for water and/or sugars between the petals of one corolla. The soluble sugar content of the inner petals increases when less competing petals are present in the corolla. Therefore, it is likely that a carbohydrate flow occurs from the stem and leaves into the flower bud during development in water.

Isolation and Purification of Plasma Membrane-Bound NADH: Fe III Chelate Reductase from Iron-Deficient Tomato Roots

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Plasma membranes from roots of iron-deficient tomato plants were isolated by two-phase partitioning as described (Brüggeman *et al.* 1990, *Physiol. Plant.* **79:** 339–346). After a 500 mM KCl wash, membrane proteins were solubilized with 0·25% Triton X-100 at a protein detergent ratio of 1:2·8 (w/w). These proteins were subjected to native PAGE in a 7·5% gel containing 0·1% Triton X-100. A small vertical section was cut and stained for enzymatic activity to identify the region containing the enzyme. In this region the rest of the native gel was cut in horizontal slices and these were electro-eluted. The collected fractions were assayed for enzyme activity and the active fraction subjected to IEF.

The procedure allowed an increase in specific activity from 760 (triton-soluble) to 4600 (eluate) nmol/(mg protein min). Polypeptides of *c.* 104, 74, 62, 56, 58, 35 and 31 kD comigrated with the activity. The enzyme has a pI of 5·5, as we always found one strong band at pI of 5·5 after enzyme staining. In several cases, minor staining was also obtained at pI 5·7, and in one case one more band was seen at higher pI. Possibly, these pI's represent degradation products or minor isoforms.

In preliminary experiments the eluate was run with a salt gradient on a Mono Q column using FPLC, resulting in the separation of three non-active protein fractions from a very active fraction of NADH: Fe III chelate reductase. We assume, that this fraction contains only the purified enzyme.

Growth Rate, Plant Development and Water Relations of the *sitiens* Tomato Mutant in Relation to Abscisic Acid

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The growth rate of plants is determined by the allocation of assimilates between shoots and roots and predominantly by its leaf area relative to plant weight. Applied abscisic acid (ABA) is known to influence biomass allocation in plants.

The *sitiens* ABA-deficient mutant in tomato (*Lycopersicon esculentum* Mill. cv Moneymaker) was used to study if and how ABA is involved in the allocation of assimilates in intact plants. The relative growth rate (RGR) of *sitiens* was about 25% lower than that of the wild-type, as a result of a decreased specific leaf area (SLA) and a lower shoot/root ratio. *Sitiens* showed a much higher transpiration rate and lower hydraulic conductivity in the roots. This caused a significantly lower leaf water-potential and turgor, resulting in reduced leaf extension. Application of ABA via the roots resulted in phenotypical reversion to the wild type.

The influence of ABA on allocation and relative growth rate seems to be primarily the result of altered

water relations in the plants rather than an effect on sink strength of different plant organs.

Relative Growth Rate Correlated with Biomass Allocation into Cell Wall and Cytoplasm: A Py-MS Study

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The grasses *Brachypodium pinnatum* (L.) Beauv., *Briza media* L., *Corynephorus canescens* (L.) Beauv., *Cynosurus cristatus* L., *Dactylus glomerata* L., *Deschampsia flexuosa* (L.) Trin., *Festuca ovina* L., *Holcus lanatus* L., *Lolium perenne* L., *Phleum pratense* L. and *Poa annua* L. have large differences in potential relative growth rate (RGR), with maximum RGR ranging from 113 mg/g/day for *C. canescens* to 272 mg/g/day for *P. annua*. The RGR appeared to be positively correlated with the specific leaf area (SLA), the ratio between leaf area and leaf weight (Poorter, H. & Remkes, C. 1990, *Oecol.* **83:** 553–559).

Chemical differentiation between the species was analysed by analytical pyrolysis combined with mass spectrometry (PyMS) as described before (Niemann *et al.* 1991, *J. Anal. Appl. Pyrolysis* **19:** 213–236).

The PyMS spectra of all grasses showed similar characteristics with fragments from the polysaccharides cellulose, hemicellulose and pectin, from phenolic acids, protein, guaiacyl- and syringyl-lignin and steroids. Special grass components are the aliphatic wax esters with molecular ions *m/z* 592, 620, 648, 660, 676, 704, 732 and 760 and a carbon-chain length ranging from C_{40} to C_{54}.

Multivariate data analysis was performed for the mass ranges *m/z* 30–220 which is mainly determined by pyrolysis fragments of the biopolymers, and *m/z* 300–740 which is mainly determined by thermal desorbtion.

In both mass ranges species-specific variation and group correlations were found. The latter appeared to depend on the RGR. Species with a low potential RGR contained relatively more cell wall material like lignin, cellulose and hemicellulose, hydroxyproline-rich proteins and wall-bound ferulic acid. Species with a high potential RGR showed relatively more cytoplasmic elements like protein and sterols and were richer in aliphatic wax esters.

Relative enrichment of cell wall elements in slow-growing species could explain the low SLA found for such species. Growth hormones like the gibberellins may be responsible for the regulation of RGR.

Physiological Characterization of a High Pigment Mutant of Tomato

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A high pigment (*hp*) mutant, which shows exaggerated phytochrome responses, and three other genotypes of *Lycopersicon esculentum* Mill. cv. Ailsa Craig: the *aurea* (*au*) mutant deficient in the bulk light-labile phytochrome (PI) pool, the *au,hp* double mutant and their isogenic wild-type were used in this study. Measurements of phytochrome destruction in red light (R) revealed that the higher responsiveness of the *hp* mutant is not caused by a higher absolute phytochrome level or a reduced rate of phytochrome destruction. Fluence-response relationships for anthocyanin synthesis after a blue-light pretreatment were studied to test if the *hp* mutant conveys hypersensitivity to the far-red light (FR)-absorbing form of phytochrome (Pfr). However, the response range for the *hp* mutant and wild-type was identical, although the former exhibited a 6-fold larger response. Moreover, the kinetics of anthocyanin accumulation in continuous R were similar in the wild-type and *hp*-mutant seedlings, despite the latter accumulating nine-fold more anthocyanin. Therefore, the *hp* mutation appears to affect the state of responsiveness amplification. Escape experiments showed that the anthocyanin synthesis after different light pretreatments terminated with an R pulse was still 50% FR reversible after 4–6 h darkness, indicating that the Pfr pool regulating this response must be relatively stable. However, fluence-rate response relationships for anthocyanin synthesis and hypocotyl growth induced by a 24-h irradiation with 451, 539, 649, 693, 704 and 729 nm light showed no or a severely reduced response in the *au* and *au,hp* mutants, suggesting the importance of PI in these responses. We therefore propose that the capacity for anthocyanin synthesis (state of responsiveness amplification) could be established by PI, while the anthocyanin synthesis is actually photoregulated via a stable Pfr pool. The *Hp* gene product is proposed to be an inhibitor of the state of responsiveness amplication for responses controlled by this relatively stable Pfr species.

The Morphogenesis of Poplar and the Pipe Model Theory

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The development of the vascular system can be seen as identical to plant morphogenesis, because it precedes

and strongly resembles the growth and initiation of plant organs.

Each leaf is supported by specific vascular bundles. Visualizing such a specific vascular bundle as a 'pipe', the plant can be considered to be built of as many pipes as there are leaves. The basal end of the pipe is rooted in the soil and the other end bears a leaf or a leaf-like organ. The data seem to indicate that the mature length of a leaf is related to the cross-sectional area of the pipe.

If this picture is correct, it is expected and indeed found that the cross-sectional area of the stem, as an approximation of that of the vascular cylinder, is proportional to the length of the attached leaf in the primary part of the plant and proportional to the sum of lengths of the full-grown leaves above the point of measurement in the secondary part.

The cross-sectional area of the pipe per unit leaf length depends on irradiance (I) and nitrate dosage (N); the lower I and the higher I/N the wider the pipes. The results indicate that wider pipes are correlated with a larger root system.

The rooted pipe with its appendage is the determinate, developmental unit of the morphogenetic process. The continuous, 'indeterminate' growth of a branch is caused by the determinate initiation of new 'procambial pipes' branching off from the preceding 'mother-pipe' in the orthostichy.

The pipe model theory was devised by Shinozaki *et al.* (1964, *Jap. J. of Ecology* **14**: 97–104) on the basis of the distribution of leaves and wood in a forest stand. Independently, we arrived at the same theory by growth analysis.

Efficiency of the Uptake and Utilization of Phosphorus for Growth by Tropical Tree Seedlings

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The regeneration of a tropical rain forest starts with tree seedlings. Nutrients, light and herbivory play an important role in this stage of life. On the white and brown sands of Guyana, nutrients, especially phosphorus, are scarcely available for plant growth. Tree species, growing on the white and brown sands of Guyana, are expected to be efficient in the uptake and utilization of phosphorus for growth.

As a part of a study on the uptake of phosphorus, we investigate the morphology of the root system, mycorrhiza and the ability to absorb phosphate from rock phosphate.

The use of phosphorus for growth is investigated by measuring the relative growth rate (g/(g/day)), the plant phosphorus productivity (g/(gP/day)) and translocation.

Tree species are grown at different levels of phosphorus supply in a tropical greenhouse in the Netherlands. Due to a low relative growth rate and the high phosphorus content of the seeds, *Lecyhtis corrugata* did not respond to a high supply of phosphorus.

The Role of Gibberellins in the Cold Requirement of Tulip

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The involvement of gibberellins (GAs) in the regulation of stem elongation and flowering has been implicated in cold-requiring plants, including tulip. To investigate their role in tulip, research is directed towards qualitative and quantitative analyses, metabolic studies and comparative biological activities of endogenous GAs. At first, an inventory is being made of GAs including the conjugated forms, in sprouts of tulip bulbs. Four known and four GA-related compounds were detected by combined gas chromatography–mass spectrometry (GC–MS) in purified extracts from sprouts of 6- and 12-week-old cooled tulip bulbs (*Tulipa gesneriana* L. cv. Apeldoorn). The presence of GAs 4, 9 and 24 could be demonstrated, in estimated amounts less than 100 ng/g fresh weight. Traces of GA_{34} were indicated by GC-MS-selected ion monitoring. Most detected GAs and GA-related compounds were found in the free as well as in the conjugated form and occurred in sprouts of both cold and non-cold treated bulbs. Consideration of the hydroxylation patterns of the ent-gibberellane ring structure indicates two families of GAs: one whose members are non-hydroxylated (GAs 9 and 24) and another with 3-hydroxylated GAs (GAs 4 and 34). Current research is directed towards quantitative analyses using ^{2}H-GAs, in relation to the cold pretreatment of the bulbs. The biological activity of the identified GAs is being confirmed by applying them *in vitro* to sprouts of uncooled bulbs.

The Effect of the Root Protein Content on the Carbohydrate Metabolism During the Forcing of Chicory

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During the forcing of chicory in the dark, the production of a shoot or chicon is completely dependent on reserves in the taproot and the uptake of minerals from the nutrient solution. After growth of the root in the field part of the polyfructan inulin is hydrolysed and converted to sucrose during vernalization. The capacity of this process, which continues during

forcing, is very important for the chicon production. Nitrogen deficiency can also be a limiting factor for chicon growth, that may lead to an accumulation of sucrose, and a negative feedback on the conversion of fructose.

The redistribution of protein from the root during forcing is proportional to the protein content. A longer vernalization period or a higher forcing temperature causes a proportional increase in protein redistribution. The participation of nitrogen uptake in the total nitrogen transport to the chicon is relatively small.

Despite a lower carbohydrate content in protein-rich roots, the availability of the carbohydrate reserves for transport is higher. As a result of a higher rate of depolymerization of inulin and conversion of fructose, the increase in sucrose content during vernalization is larger in roots with a high protein content. In accordance with the higher sucrose content the decrease in total amount of carbohydrates during forcing is larger in protein-rich roots.

The apparent recovery of root dry matter in the chicon decreases with the protein content of the root, caused by a higher consumption of carbohydrates for respiration. The consequence is an only slightly higher chicon dry matter production on protein rich roots, but a different chicon dry matter composition.

Polyclonal Chicken Egg Yolk Antibodies in the Analysis of the 3′,5′ cAMP Metabolism in Higher Plants

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Cyclic AMP antibodies from chicken egg yolk enabled us to construct affinity columns for a rapid though exhaustive purification of cAMP from crude plant extracts. The cAMP antibodies, which very selectively show high affinity for 3′,5′ cyclic nucleotides, were bound to commercial Affi-Gel 10 support (Biorad). The cAMP content of samples, purified over these affinity columns, could be measured immediately by means of UV detection at 258 nm after 'ion suppression'-HPLC.

Using ^{3}H adenylimidodiphosphate (AMP P(NH)P) as a specific substrate for adenylate cyclase activity (Yount *et al.* 1971, *Biochemistry* **10:** 2484–2489), the formation of ^{3}H-cAMP was radiometrically and spectrophotometrically monitored after sequential chromatography on AG 50W-X4 cation exchange resin, immunoaffinity columns and 'ion suppression'-HPLC (Van Onckelen *et al.* 1982, *Physiol. Plant.* **55:** 93–97). Use of cAMP affinity columns significantly enhances the extraction yield and hence increases the sensitivity of the adenylate cyclase assay.

Adenylate cyclase activity was measured in *Nicotiana tabacum* cv. Petit Havana SR1 leaf protoplasts using different concentrations of lysolecithine in order to allow penetration of substrate into the protoplasts. Except for an apparent optimum at 0·25%, addition of this detergent resulted in an inhibition of activity. This might be due to a lysolecithine stimulated phosphatase activity, resulting in adenosine formation, an adenylate cyclase inhibitor in animal systems. The optimum at 0·25% could be explained as a latent adenylate cyclase activity in the chloroplast fraction that partially surmounts the inhibition effect of adenosine.

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Membrane Potential Gradients and Associated Symplastic Isolation of Sieve Element/Companion Cell Complexes Along *Lupinus* Stems

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Symplastic isolation of the SE/CC-complex in phloem strips of *Lupinus luteus* stems was examined by means of intracellular injection of membrane impermeant dyes, membrane potential measurements and electrical cell–cell coupling experiments. The longitudinal membrane potential gradient of the SE/CC-complexes was determined in consecutive internodes. Symplastic isolation of the SE/CC-complex was concluded from: (a) abrupt membrane potential discontinuity between SE/CC-complex and phloem parenchyma, (b) absence of dye transport between SE/CC-complex and phloem parenchyma, (c) electrical resistance between SE/CC-complexes and phloem parenchyma was seven times higher than between adjacent phloem parenchyma cells. An apex-directed membrane potential gradient of the SE/CC-complexes was found along the stem. The membrane potential difference—and thus retrieval activity—was maximal at the basis of the stem, where the most important sources are present.

Effects of an Increased Atmospheric CO_2 Concentration on the Growth and the Florescence of Bean Plants (*Phaseolus vulgaris* L.)

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The increases in global atmospheric CO_2 concentration have stimulated an interest in the direct effects of CO_2 on plant growth and generative reproduction. In this study, the effects of CO_2 on growth, C-partitioning and

florescence were observed on bean plants (*Phaseolus vulgaris* L.) cv. Label, grown from seedling to the end of flowering in two controlled-environment chambers that maintained CO_2 concentrations at 350 and 700 ppm.

During the entire period of growth an increased CO_2 concentration had no effect on the total leaf area, though the total leaf biomass was increased by 64%. This resulted 60 days after planting (DAP) in an augmented specific leaf weight (SLW) from 17·2 g/m^2 at 350 ppm to 26·0 g/m^2 at 700 ppm. Sixty DAP, the root and the stem biomass were increased by 41 and 34% respectively with CO_2 enrichment. Sixty DAP plants grown at 700 ppm CO_2 had a total vegetative biomass that was augmented by 54% in comparison to the control plants.

The C-partitioning between the roots and the above-ground biomass did not change with CO_2 level so that the root/shoot ratio remained unaffected.

The florescence started 34 DAP at 350 ppm CO_2 and 1 day earlier at 700 ppm. The florescence was lengthened by an increased CO_2 concentration. The end of the flowering-time in the control experiment was noted 53 DAP, while the florescence under high CO_2 conditions continued at least 9 days longer. The appearance of each individual flower was recorded. The percentual distribution of the flowers over the different nodes was unaffected by CO_2. Until 39 DAP, the number of formed flowers was augmented with increased CO_2 concentration. As the mean number of daily formed flowers and the length of florescence were increased, the total number of flowers was augmented with 60% in a CO_2 enriched environment.

Expression of a Pollen-Specific Gene and its Involvement in Pollen-wall Synthesis

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A pollen-specific cDNA clone (pNTP303) was isolated to study the relationship between gene expression and pollen development. NTPc303 was obtained through differential screening of a cDNA library prepared against mRNA from mature tobacco pollen. Characterization of this clone, by Northern blot analyses and in-situ hybridization, showed that NTPc303 is exclusively expressed in pollen from monocots and dicots. The expression of this clone arises in tobacco pollen after the first haploid mitosis, accumulates thereafter and is expressed also during the first hours of germination. *De novo* transcription of NTP303 in germinating pollen was confirmed by in-vivo labelling of the newly formed RNA in combination with liquid hybridization procedure to an antisense pNTPc303 transcript.

The nucleotide sequence analyses of NTPc303 showed a full length frame of 1960 bp and the carboxy terminal of the protein has the characteristics of a leader peptide. The open reading frame encodes for a protein that shows homology to ascorbate oxidase and laccase. Both enzymes are thought to be involved in cell wall formation. So it is likely that NTP303 is highly conserved during evolution and has a main function in pollen-tube wall synthesis, a process with a long evolutionary history.

Mechanism of Polarity in *Elodea*: Redox Regulation of PM-ATPase. NAD(P)H-Ferricyanide Reductase is not Involved

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The light-induced activation of the plasmamembrane bound H^+ ATPase (pm-ATPase) results in acidification of the unstirred layer near the morphologically lower epidermis of the *Elodea* leaf. Previously we proposed that this effect of light is mediated by reducing equivalents produced in the chloroplasts. In-vivo experiments indicated that reducing equivalents regulate the pm-ATPase via a pm-bound reductase. Results of in-vitro experiments on pm-ATPase and pm-proton pump activity, using inside-out pm vesicles, seem to support this model.

From in-vivo and in-vitro measurements of pm-bound ferricyanide reductase activity, it is concluded that this enzyme does not play a role in redox regulation of pm-ATPase.

A model is developed for the redox regulation of pm-ATPase independent of pm-bound ferricyanide reductase.

Plasma Membrane ATPase of Mung Bean Hypocotyls

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It has been suggested that Mung bean plasma membranes contain an unusual ATPase (Kasamo, K. 1986, *Pl. Phys.* **80:** 818–824; Kasamo, K. 1987, *Pl. Cell Phys.* **28:** 19–28; Mito *et al.* 1988, *Pl. Cell Phys.* **29:** 875–882). The enzyme was reported to be comprised of three major polypeptides of Mr 105, 67, 55 and 57 kDa, instead of the more usual 100 kDa polypeptide found in other higher plants. It was also reported that the

phospholipids which stimulated enzyme activity were different from those normally associated with plant ATPases, and that K^+ had an inhibitory rather than a stimulatory effect.

Therefore, we were interested in the properties of the Mung bean ATPase enzyme and so we have determined pH optimum, enzyme kinetics, enzyme latency, influence of K^+ and some other ions and inhibitors.

In other experiments, where we focused on the changes in ATPase activity along the hypocotyl axis, we found a lower enzyme activity (hydrolytic and proton pumping) in segments immediately above the root compared with that found in segments 4 cm above the root and segments 1·5 cm below the hypocotyl hook. These differences were also confirmed by the determination of the kinetic constants (V_{max} and K_m) of the ATPase activities.

In addition to the above experiments, we have analysed the lipid composition of the plasma membranes isolated from the different Mung bean hypocotyl segments.

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The Effect of the Inhibition of Photosynthesis or Respiration on Sugar Distribution in Salt-Stressed Barley Plants

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It is known that during salt stress (150 mM NaCl) glycophytic plants tend to accumulate sugars in response to the ionic stress, for osmotic adjustment within the cell.

As sugar metabolism is directly linked to the functioning of photosynthesis (production) and of respiration (consumption), both processes were inhibited separately at the level of the second fully expanded leaf of barley. This inhibition was obtained by covering this leaf with thin aluminium foil and thus darkening the leaf (for inhibition of photosynthesis) or by rubbing a 1 mM DNP solution over both sides of the leaf (for inhibition of respiration).

The effects of these manipulations were clearly seen during analysis of sugar content and sodium accumulation in that leaf. The inhibition of photosynthesis lowered the sugar content drastically in non-stressed plants, but when under salt stress the sugar levels raised again by import from other leaves. Sodium accumulation in this leaf was inhibited up to 80% as compared with non-darkened leaves. The inhibition of respiration increased the sugar content and as a result approximately 50% more sodium could be accumulated in this leaf.

Further research is needed on the level of evapotranspiration, to see how this process interferes with the sodium accumulation. We would then be able to integrate the biochemical and biophysical aspects of sodium transport and accumulation within one leaf of the plant studied.

Nitrate Accumulation in Three Spinach (*Spinacia oleracea* L.) Varieties in Relation to Organic Osmotics

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Under poor light conditions, as used during winter production of greenhouse vegetables, the nitrate concentration in spinach plants (variety 'Vroeg Reuzenblad') shows a diurnal rhythm. During the dark period, the nitrate content is higher than during the day. A shortage of osmotic solutes in the shoot is the cause of the nitrate accumulation. In 'Vroeg Reuzenblad' the demand of the shoot for nitrate for osmotic purposes may regulate the nitrate uptake by the roots.

In three other spinach varieties, differing in the degree of nitrate accumulation, viz. Glares, Subito and Spinoza, the fluctuation of the nitrate concentration during the day/night cycle was studied. Plants were grown at limiting light conditions and harvested 21 and 31 days after sowing, at the beginning and at the end of the light period. In leaf blades, petioles and roots the nitrate, sugar and malate concentrations were determined.

In these three varieties, the fluctuation of the nitrate concentration occurs both in leaf blades and roots and is compensated for by the fluctuation of the sugar and malate concentration. The sum of the measured osmotics accounts for a fixed, but per plant part different, percentage of the total osmotic potential. Currently, we investigate the nitrate uptake in relation to the demand for nitrate for both growth and osmotic purposes in these varieties.

The Effects of Increased Levels of Atmospheric CO_2 on Dry Matter and Nitrogen Partitioning in *Plantago major* and *Urtica dioica*

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The effects of increasing the atmospheric CO_2 concentration for 350 to 700 ppm on growth, dry matter partitioning and nitrogen allocation have been studied in *Plantago major* ssp. *pleiosperma* and *Urtica dioica*. The experiments were performed in growth chambers using nutrient solutions as the growth medium.

A positive response of the Relative Growth Rate (RGR) to high CO_2, due to an increase in the Net

Assimilation Rate (NAR) was found for both species. However, this response was more profound during the first week of the treatment, especially in *P. major*, where the RGR dropped to control level in the third week of exposure to high CO_2.

Dry matter allocation was influenced within the leaves only. This is shown by a decrease of the Specific Leaf Area (SLA) and the absence of any effect on the root/shoot ratio (R/S). With respect to the allocation of nitrogen, the two species showed a marked difference. In *P. major* the shoot N concentration was reduced, whereas in *U. dioica* no such response occurred. In the roots no effects of increased atmospheric CO_2 concentrations were observed in either of the species.

To test whether the absence of any CO_2 effect on the N concentration in *U. dioica* could be due to its nitrophilic character and the ability to accumulate nitrate, the soluble and insoluble reduced nitrogen concentration were also determined for this species. The CO_2 concentration did not affect these N pools either. Therefore, a differential nitrate accumulation pattern does not seem to occur due to the CO_2 treatment.

In *U. dioica* starch accumulation in response to the increased CO_2 level did occur. This accumulation cannot fully account for the observed decrease of the SLA. Correction of the N concentration for starch content by expression on a structural dry matter basis does not influence the result, because the starch concentrations are too low to seriously affect it.

Why Does H_2S Not Reduce Growth in Monocots?

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H_2S can reduce the growth of higher plants at concentrations of 0·1 µl/l and higher (De Kok, L.J. 1989, Thesis, Groningen). Growth reduction occurred in several dicots. For instance, when *Spinacia* and *Cucurbita* plants were fumigated for 14 days with 0·75 µl/l H_2S a reduction in dry matter production of 69 and 36% was found, respectively. For *Zea*, however, no reduction in dry matter production was found after a fumigation period of 14 days under these conditions. In order to find out whether the absence of growth reduction by H_2S can be shown for more monocots, the influence of fumigation with low concentrations of H_2S on growth and metabolism was determined for *Zea*, *Hordeum* and *Sorghum*.

For all three monocots considerable SH accumulation was found after a 48-hour fumigation period with 0·4 µl/l H_2S, showing that H_2S was metabolized. Growth, measured as relative growth rate and leaf elongation, however, was not affected at all. Neither was in-vitro NADH oxidation capacity changed, by fumigation with H_2S, as found for *Spinacia*. So far, in-vitro NADH oxidation capacity is the only physiological parameter that shows a correlation with growth reduction by H_2S (De Kok, L.J. 1989, Thesis, Groningen).

In further experiments with *Zea* the leaves above the sheath of the first leaf were cut off. Under these conditions fumigation with H_2S resulted in a decrease in leaf elongation. It is, therefore, concluded that in monocots, H_2S is only phytotoxic when it can reach the vegetation point.

Effects of an Elevated CO_2-Concentration on Growth and Development of Two *Lolium perenne* L. Cultivars in Monoculture

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There is plenty of evidence that the steady increase of the atmospheric CO_2-concentration has important consequences on the growth of plants. Some of these effects were studied on two cultivars of *Lolium perenne* L., cv. Vigor (2*n*) and cv. Condesa (4*n*).

Plants were grown in monoculture under natural conditions of light, temperature and humidity, with control of CO_2-concentration (350 and 700 ppm) and water supply.

In five consecutive clips (with intervals of 24 days) above-ground biomass was collected, while below-ground biomass was gathered at the beginning and the end.

Both spring and summer growth were stimulated in the elevated CO_2-treatment, which was explained by a rise in photosynthetic activity. Leaf dry weight and leaf area augmented by 68 and 19%, respectively. The difference in stimulation between leaf dry weight and leaf area is due to a reduction in specific leaf area (SLA), which means that the density and/or thickness of the leaf has increased. Root growth was spectacularly stimulated (251%). As a result of this alteration in allocation pattern, the root/shoot ratio rose (110%).

In both treatments cv. Vigor had more leaves and consequently a greater leaf area than cv. Condesa, while the latter had a slightly higher root/shoot ratio. Comparison of the cultivars showed no profound differences in stimulation by CO_2, apart from the higher photsynthetic rate of cv. Vigor. This, however, did not result in a higher net production in comparison with cv. Condesa. Explanation of this incongruity is hypothetical and might include differences in dark respiration and light compensation point.

Photosynthetic Characteristics of a Transgenic Graft

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The photosynthetic capacity of a leaf is regulated by the co-ordinated action of environmental and internal parameters (Smart *et al.* 1991, *The Plant Cell* **3**: 647–656). Transgenic grafts, obtained from shoots regenerated on transformed calli, are a useful tool to study the effect of an 'overproduction' of, for instance, endogenous cytokinins.

The transgenic graft used contains the recombinant Ti plasmid pGV 2488. This plasmid harbours the structural octopine-type *ipt* gene with its polyadenylation site and the *Eco*RI-*Hind*III promoter fragment of the *Pisum sativum* gene encoding the small subunit of the ribulose-1,5-bisphosphate carboxylase (P*ssu-ipt* graft) (Beinsberger *et al.* 1991, *Plant Cell Physiol.* **32**: 489–496). Analysis of the endogenous phytohormone content of transgenic P*ssu-ipt* grafts shows an increased cytokinin concentration in the older leaves as compared to younger ones (Beinsberger *et al.* 1992, *Proceedings of the 14th International Conference on Plant Growth Substances*, Amsterdam, in press).

Electron microscopic study of chloroplasts of the P*ssu-ipt* graft shows slightly swollen thylakoids, in comparison with the untransformed control plants (SR1). Low temperature fluorescence spectroscopy shows a very slight red-shift of the PSI-emission band in the graft compared to the SR1 plant. There is a significant difference in the 685/730 and the 695/730 ratios between the P*ssu-ipt* graft and the control plants. The ratios drop by almost 50%. Quenching analysis indicates that in SR1 plants, the q_P increases with the age of the leaf (from 0·901 to 0·977). An inverse tendency is observed in the P*ssu-ipt* graft. In old leaves with 'chlorosis' regions, the q_P drops dramatically to values below 0·5. The q_P of dark green old leaves fluctuates in between. The q_{NP} does not change significantly.

Measurements of the capacity of the whole electron transport chain (PS1 + 2) and of the partial systems (PS1, PS2) show a significant reduction in the leaves of the P*ssu-ipt* graft compared to the SR1 leaves. For PS1 + 2 a 70%, for PS1 a 60% and for PS2 a 45% reduction in electron transport capacity is observed.

These results indicate that an increase in endogenous cytokin content does affect photosynthesis, at least on the level of the ultrastructure of the plastids, on the electron transport capacity and on the energy distribution.

Photoinhibition and Photosynthetic Rate Correlate with Productivity in Ryegrass Genotypes

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Causes of genetic variation in productivity within a species are not well known. We examined whether photosynthesis contributes to varietal differences in growth rate. Cloned plants of three cultivars and one selection of *Lolium perenne* L. differing in productivity were studied. CO_2 exchange rate (CER) and chlorophyll fluorescence were measured as affected by a shift in light intensity. The resistance of the four ryegrass clones to photo-inhibition is positively related with their yield, as determined in both greenhouse and field experiments. Under optimal conditions, no differences in CER were found. However, the CER of low-light (0·14 mmol $m^{-2} s^{-1}$) adapted plants of high yielding clones was less sensitive to conditions inducing photo-inhibition (1·4 mmol $m^{-2} s^{-1}$ and/or a 7 K temperature drop). Transpiration rate was not affected. Photosynthetic rate after *c.* 2·5 h of high light (switch from 140 to 1400 mol $m^{-2} s^{-1}$ PPFD) is directly proportional to the ratio of variable to maximum chlorophyll fluorescence (low PPFD), indicating photo-inhibition of photosynthesis. We conclude that resistance to photo-inhibition varies between genotypes of ryegrass, and is an important characteristic contributing to varietal differences in dry matter production. We conclude that photo-inhibition and photosynthesis contribute to variation in growth rate between ryegrass genotypes.

The Influence of Low Temperature on the Membrane Lipid Composition and Flowering Capacity of Tulip Bulbs

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To produce flowers of good quality, tulip bulbs (cv. Apeldoorn) need a cold period of 12 weeks. This study was performed to determine if the amount of cold the bulbs had received was sufficient to induce good flowering. Four series of bulbs were transferred every 6 weeks to 5°C (starting 4 September until 3 January). The change in lipid composition was followed to determine the influence of the developmental stage of the shoot on the changes in lipid composition during the cold treatment.

After harvesting of the bulbs, the shoot was not really dormant. During the storage period at 5°C or 17°C the shoot still showed some growth. The total lipid content/fresh weight increased in the four series only at 5°C but not at 17°C.

The relative composition of the phospholipids, which consisted of PC, PE, PI, PG and DPG, showed a cold-induced change which was the same for all four series.

The most important change in the fatty acid composition was an increase in the ratio linoleic acid/linolenic acid (18:2/18:3) which occurred at 5°C, but not at 17°C. This increase at 5°C only took place in the first two series. In the last two series, which were transferred to 5°C on 21 November and 3 January, the ratio 18:2/18:3 did not increase during the cold treatment.

During the low temperature treatment the lipid composition showed the same change in the four series, except for the ratio 18:2/18:3 which showed in the first two series only a cold induced change. However, all four series showed good flowering. It can be concluded, therefore, that a change in the ratio 18:2/18:3 is not related to the flowering capacity.

Spectroscopic Characterization of Photo-Inhibited Photosystem II and Kinetic Resolution of the Triggering of the D_1-Reaction Centre Protein for Degradation

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Photo-inhibition of isolated thylakoids is normally characterized by inactivation of Photosystem II (PS II) electron transport and proteolysis of the D_1-protein (Andersson, B. & Styring, S. 1991, *Current topics in Bioenergetics* **16:** 1–81). At low temperatures, the mechanism of inactivation for PS II electron transport can be experimentally studied without interference of secondary effects as D_1-protein degradation does not take place (Aro *et al.* 1990, *Biochem. Biophys. Acta* **1019:** 269–275).

Electron magnetic resonance (EPR) spectroscopy has been applied to characterize the sequential events leading to inhibition of PS II electron transport and triggering of the D_1-protein for degradation. PS II-enriched membranes were illuminated by strong white light at 2°C for periods up to 3 hours. Two principle kinetics of inactivation and damage were observed.

(1) Inactivation with a half-time of 1–1·5 h in case of electron transport through PS II, the induction of the S_2-multiline EPR signal, the chemically induced EPR signal $Q_A^-Fe^{2+}$ and lowering of the Fv/Fm fluorescence ratio. This is explained by over-reduction of the first quinone acceptor (Q_A) leading to impairment of its function.

(2) Inhibition with a half-time of 3–4 hours in case of EPR Signal II_{slow}, inhibition of the primary charge separation reaction and release of manganese from its site in the oxygen evolving system.

We were also able for the first time to follow the kinetics for the triggering of the D_1-protein for degradation. This triggering followed the slower kinetic phase and is likely to be the result of conformational changes in the protein induced by singlet oxygen. Evidence for this mechanism was obtained from previous anaerobic photo-inhibition experiments (Vass, I. *et al.* 1992, *Proc. Natl. Acad. Sci. USA*, in press) in which it was shown that centres with over-reduced Q_A form chlorophyll triplets during illumination. In the absence of oxygen these triplets are essentially harmless but when oxygen is present, as in our experiments, the chlorophyll triplets react with oxygen to form singlet oxygen (Asada, K. & Takahashi, M. 1987, in: *Topics in Photosynthesis* **9:** 227–288).

Additionally, a dark-stable cationic radical ($g = 2{\cdot}0031$ and 10–11 G wide) was progressively induced during the inhibition and was tentatively attributed to a carotenoid cation. We speculate that this radical was induced in the small fraction of centres which were able to reduce Q_A but which had impaired or inefficient electron donation from the oxygen evolving system.

The Effect of Day-Length on the Metabolism of Sucrose in Stolon Tips of *Solanum demissum*

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One of the main environmental factors which control the formation of tubers in potato is day-length. Long nights will favour tuber-induction. From in-vitro experiments it is known that high sucrose levels in the medium can be tuber-inducing. Therefore, we are interested in the changes in the levels and metabolism of sucrose in the stolon tips of intact plants.

Once delivered to a stolon tip, sucrose will be split into either glucose and fructose of UDP-glucose and fructose. The first reaction is irreversibly catalysed by alkaline—or acid invertase, the latter reaction is reversible and is catalysed to sucrose synthase.

Under 24-h day-length conditions at a moderate temperature *Solanum tuberosum* will form tubers. Therefore, we decided to do our experiments with a wild *Solanum* species. *Solanum demissum* is an absolutely short-day dependent *Solanum* species. A clone was multiplied *in vitro* and plants were grown in a climate room under short-day (SD: 10 h photosynthetic active radiation (PAR)) and long-day (LD: 10 h PAR + 6 h incandescent light) conditions. Tubers were formed under SD conditions, whereas no tuber formation was observed under LD conditions. Three

times a week stolon tips (2 cm) were collected from LD and SD plants. After fresh weight determination the tips were frozen in N_2(l) and freeze-dried. The dry-weight was determined and the tips were homogenized. As tuber-induction is a continuous process (Vreugdenhil and Struik, 1989, *Physiol. Plant.* **75:** 525–531) stolon tips from the same plant harvested at the same day cannot be pooled. It appeared that only when stolons have also got similar dry-matter contents (DM%) pooling is acceptable.

Homogenates were split, one half was used for sugar analyses, in the other half the sucrose synthase activity was determined. For sugar analysis 80% MeOH/20% H_2O was added to the samples and they were 10 min heated at 75°C. Thereafter the samples were dried again and sucrose, glucose and fructose were determined enzymatically. The glucose concentration of the tips decreased upon tuberization, the fructose concentration decreased also, but more drastically. This resulted in a sharp increase of the glucose/fructose ratio from about 0·7 to 10–40.

With some minor changes sucrose synthase activity determinations were done according to Xu *et al.* (1986, *Biochem. Biophys. Res. Comm.* **141:** 440–445). The breakdown of sucrose was monitored by the release of UDP-glucose. PGM and *Leuconostoc* G6P-DH (NAD) were used and UDP-glucose release was indirectly detected by the change in absorption at 340 nm.

Compared to stolons (DM% ≈ 7) tubers of about 0·1 g fresh weight (DM% ≈ 11) from the same plant show an increase (> 10 times) in sucrose synthase activity. The activity in stolons is very low.

The decrease in glucose content after tuber initiation can be explained by the large increase in sucrose synthase activity. The change of the glucose/fructose ratio might be caused by a shift from acid invertase—to sucrose synthase activity. In the apoplast or in the vacuole fructose will be less readily phosphorylated than in the cytosol.

MEETING OF THE SECTION FOR FERTILIZATION RESEARCH IN PLANTS ON 6 SEPTEMBER 1991

Flow Cytometric Determination of Relative Nuclear DNA Contents in Tomato Seeds

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Relative nuclear DNA contents in tissues of tomato (*Lycopersicon esculentum*) seeds were measured with a flow cytometer. With the use of the fluorescent dye DAPI, relative amounts of DNA per nucleus expressed as C values were quantified with high accuracy. Flow cytometric determination of DNA levels in embryos of fully maturated dry seeds revealed large amounts of 2C DNA signals, indicating that most cells had arrested mitosis at the pre-synthetic G_1 phase of nuclear division. After imbibition in water, an augmentation of the 4C signal in the embryonic root-tip region was found. This increase could be ascribed to cells entering the synthetic phase of nuclear division leading towards the doubling of chromosomal material. In the root-tip cells, 4C/2C ratios raised 1 day after imbibition in water though radicle emergence started 2 days later. Apparently, DNA synthesis preceded germination. Only a small rise of 4C DNA levels was found in the rest of the embryonic tissues. In whole dry seeds, DNA histograms revealed both a 2C signal and a considerable 6C peak, the latter originating from the endoreduplicated endosperm.

A priming period of 14 days in PEG-6000 considerably enhanced the rate and uniformity of germination. In the ungerminated seeds, the 4C DNA signal of root-tip cells started to increase after 3 days incubation in PEG. The ratio of 4C/2C steadily increased during the 14 days priming period, though did not reach the level obtained after hydration in water. Upon priming, the 4C/2C ratio was constant after redrying the seeds towards the original moisture content, indicating that the chromosomal material in the root cells had stably ceased nuclear division at G_2 phase. The present results indicate that the beneficial effects of priming on seedling performance are associated with the action of replicative DNA synthetic processes prior to germination. The specific location of DNA synthesis implicates that, in tomato seeds, the root-tip region is most active in responding to a shift in physiological conditions. Presently, we are analysing effects of physiological conditions on tissue-specific protein patterns in tomato seeds.

Application of Flower Biological Techniques in Research on Interspecific Hybridization in Lily and Tulip

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For environmental reasons, it is of great importance for the present-day flower-bulb grower to introduce genes for resistance from unrelated species in the assortment. Crossings between species are often not

possible because of crossing barriers. For lily, various techniques have been developed for circumventing crossing barriers, like the cut-style method, the grafted style method, in-vitro pollination, ovary-slice, ovule and embryo culture (Tuyl, J.M. van *et al.* 1991, *Plant Science* **74:** 115–126).

Last year we started to develop biological techniques for circumventing crossing barriers in tulip. Research was carried out on the growth of pollen tubes, the localization of crossing barriers and the development of in-vitro pollination, ovary-slice and ovule culture.

The growth of pollen tubes was analysed in the compatible combinations of *Tulipa gesneriana*, cultivars 'Christmas Marvel' and 'Leen van de Mark'. The pollen tubes grew down in the ovary in about 11 days. Crossing barriers have been analysed in incongruent crosses between both cultivars of *T. gesneriana* with *T. praestans* 'Zwanenburg'. In the combination *T. gesneriana* × *T. praestans* the pollen tubes stopped about 2/3 of the way into the ovary. A pollen tube had entered about 1% of the ovules. In the reciprocal combination the growth of pollen tubes had stopped already in the stigma.

For in-vitro pollination, flower buds were placed on medium 5–7 days before anthesis. The influence of sucrose, hormones, macronutrients were tested. Although in-vitro pollination techniques must be improved, at this time it appears that viable seeds can be obtained.

Further we have started to develop procedures for ovary-slice and ovule culture. For this purpose, flowers were pollinated on the plant, after which at different stages of development, ovary-slice and/or ovule culture were carried out. The later ovary-slice and ovule culture are started, the better the ovules will germinate.

Changes in the Microtubular Cytoskeleton during Microspore and Pollen Development in *Brassica napus* L.

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Microtubular (MT) configurations were visualized immunocytochemically from late tetrade stage till mature pollen stage using polyclonal anti-tubulin (IgG fraction) and goat-anti-rabbit-FITC-conjugates and by transmission electron microscopy (van Lammeren *et al.* 1985. *Planta* **165:** 1–11). For light microscopy the DNA was stained simultaneously with DAPI.

At late tetrade stage of microspore development, a fine network of equally dispersed MTs marked the encapsulated microspores. In young free microspores, MTs were found to be mainly perinuclear. During mid-microspore stage, bundles of MTs were observed throughout the whole cell. Late microspores contained single MTs in the region between the single large vacuole and the eccentric positioned nucleus. The nuclei were connected with the plasmamembrane via MTs. Pre-prophase bands were not observed. During the prophase of microspore mitosis, the nucleus was surrounded by MTs arranged in a criss-cross pattern. In all following stages of division, resulting in the formation of a large vegetative cell and a small generative cell, MTs were only observed in the mitotic spindle and in the phragmoplast. During cytokinesis, the phragmoplast moved from a position between the nuclei to the periphery. Initially, both nuclei were connected with the cell plate via MTs, but during the final stage of wall synthesis only connections to the generative nucleus were observed. This phenomenon might influence the direction of phragmoplast movement and thus induce the lense shape of the generative cell.

The young bicellular pollen grain contained MTs in the generative as well as in the vegetative cell. During the mid- and late bicellular stage, the number of MTs in the vegetative cell decreased, whereas the generative cell formed bundles, each consisting of 5–15 MTs. During pollen mitosis MTs were observed in the spindle and phragmoplast of the dividing generative cell but not in the vegetative cell. The mature tricellular pollen grain contained MTs only in the cytoplasm of the sperm cells. A high background-labelling was observed in the cytoplasm of the vegetative cell which hints at a pool of free tubulin to be used for the following events of pollen germination and pollen tube growth.

Gene Expression During Early Pollen Development of Tobacco

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Pollen development with its morphologically well-described stages is a good model system to study developmental processes. Earlier research carried out by our group (Schrauwen *et al.* 1990 *Planta* **182:** 298–304) revealed that during development, the mRNA population changes composition and amount of individual mRNA's. Some of the RNA's present during late development are involved in germination. Most pollen-development research is carried out on mature pollen.

This project, however, focused upon early development at the uninucleate stage. During this period, most RNA's are involved in the development processes. In this phase, a unique asymmetric division takes place in the microspore resulting in binucleate pollen with a vegetative and a generative

cell. Therefore, the uninucleate stage is a good point to focus on the gene expression mechanism involved in these processes.

A cDNA library was synthesized against mRNA from tobacco microspores in Lambda ZAP. The library exists of $1{\cdot}3 \times 10^6$ recombinants. The average insert length was 670 basepairs. By differential screening with cDNA probes of RNA from microspores and leaf, 90 microspore-positive clones were isolated. The tissue specificity was analysed for two clones on a Northern blot, no hybridization signal was found with RNA of root, stem or pistils.

The preliminary results of the microspore cDNA library show that this library is of a promising quality. Therefore the work will be continued with a division of the isolated clones into different hybridization groups. Some members of the different groups will be characterized further. The tissue specificity of these clones and their presence during pollen development will be determined. In-situ localization will be used to find the exact start of transcription and to detect a possible signal in the surrounding tapetum. The corresponding genomic clones will be isolated and promoter analysis will be carried out.