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Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR FERTILIZATION RESEARCH IN PLANTS ON 2 OCTOBER 1992

The Production and Analysis of Interspecific Hybrids in *Alstroemeria* L.

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The genus *Alstroemeria* belongs to the family of Alstroemeriaceae together with the genera *Bomarea, Leontochir* and *Schickendantsia*. It comprises more than 60 species, which all originate from South America.

Nearly all present cultivars are the result of interspecific hybridization. Most of them are triploid or tetraploid, whereas species are diploid ($2n=2x=16$). The production of cultivars is often hindered by interspecific crossing barriers.

A histological study revealed that in all crossing combinations between three species (*A. pelegrina, A. aurea* and *A. psittacina*) an embryo had been formed. Abnormalities occurred only during seed development (post fertilization barriers): 7 days after pollination (DAP) the number of endosperm nuclei in the interspecific seeds was very low in comparison to the situation in normal seed development. In addition, the transition of the endosperm from nuclear to cellular failed to take place. This transition normally takes place at 12–18 DAP. The growth of the embryo was retarded and in many cases the embryo had already been aborted at 21–28 DAP. The embryo could be rescued when ovule culture was applied at 14 DAP. Young seeds were cut into halves and cultured in a liquid culture medium and incubated in the dark at 21°C under rotation. Optimization of the sucrose concentration of the culture medium and the moment of the start of the culture resulted in a high percentage of embryos which could be saved (80–100%). Although further growth of the interspecific embryos brought about some difficulties with respect to the formation of a rhizome, many plants were obtained. The hybrid character of the obtained plants was checked by cytological observation of mitotic metaphase chromosomes from shoot or root tips. Hybridity could be confirmed through recognition of species genomes viz. chromosome arm length ratio or the C-banding pattern visible on the chromosomes after Giemsa C-banding.

Pollen and Sporophytic Self-incompatibility of *Brassica oleracea*

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Pollination starts with a tissue–tissue interaction between the pollen grain and the stigmatic papillae and ends with fertilization. In the case of self-pollination in *B. oleracea*, this interaction leads to a physiological process called self-incompatibility: the pollen grain doesn't germinate or pollen tube growth is arrested, and fertilization is avoided.

In *Brassica* self-incompatibility is genetically determined by the S-genotype of the sporophyte, so the self-incompatibility reaction occurs when both the pollen donor and the pollen acceptor in the pollination event have an S-allele in common.

On the stigmatic side, two genes have been isolated which are linked to the S-locus and which are specifically expressed in the stigmatic papillae (Nasrallah, J.B. *et al.* (1991): *A. Rev. Pl. Physiol. Plant molec. Biol.* **42:** 393–422). Neither gene was identified as being expressed in the anther or the pollen.

We will focus on the pollen recognition component(s) involved in the interaction process leading to self-incompatibility. The involvement of components in the tryphine, as deposited in the exine (Heslop-Harrison, J. (1968): *New Phytol.* **67:** 779–786) is suggested by both the reaction time (within an hour after pollination) and the sporophytic control of self-incompatibility. Due to the occurrence of more than 60 S-alleles in *Brassica*, the interaction components present in the exine should have a high specificity. Proteins have the capacity to react specifically and can be deposited from the tapetum into the exine-held tryphine. So we have focused on the proteins in this tryphine (called 'exine proteins').

From the pollen of S5 homozygous plants, total exine proteins were extracted and used to raise a polyclonal antiserum. This antiserum turned out to

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detect proteins in the exine specifically, and has been used to screen a cDNA expression library, made from anthers during the whole of the development of the pollen in the anther, starting from the early uninucleate stage until anthesis. The screening resulted in the isolation of anther specific genes for which linkage analysis as well as the determination of the origin and nature of the transcripts is in progress.

Flavonols as Signal Molecules during Pollen Maturation and Germination

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Flavonoids belong to a large class of plant secondary metabolites which are primarily responsible for flower colour. They play an important role as signal molecules in a number of symbiotic plant–microbe interactions. They also play an important role in the development of the pollen. The basic skeleton of a flavonoid consists of two phenolic rings interconnected by three carbon atoms. Depending on the types and places of the substitutions they can be subdivided into different classes. In the first step of flavonoid biosynthesis the yellow chalcones are formed. This reaction is catalysed by the key enzyme of the pathway, chalcone synthase (CHS). After the onset of flavonoid biosynthesis by CHS, other classes of flavonoids will be synthesized such as flavones, flavonols, flavonones and the coloured anthocyanins. When low concentrations of flavonols (150–1500 nM) are added to *in-vitro* matured tobacco pollen the amount of pollen germinating increases 2–3 fold and the tubes are 2–3 times longer (Ylstra *et al. Pl. Physiol.* (in press). It was shown that only flavonols could stimulate the growth of the pollen tube and not other flavonoids. The flavonols are a common compound of the pollen exine, two of the three functional flavonols could be identified by thin layer chromatography analysis in tapetal anther extracts of tobacco. The tapetum is the innermost sporophytic cell layer of the anther, surrounding the developing microspores serving a nursery role.

In-vivo experiments show that flavonoids are essential for normal pollen development (van der Meer *et al.* (1992): *Pl. Cell* **4**: 253–262. Transgenic petunia and tobacco plants were made which do not produce flavonoids in the anther as a result of a *chs* gene block. This was achieved by the introduction of a *chs* gene in the reverse orientation (anti-sense method) driven by a promoter which is active in the tapetal cells of the anther. This results in aberrant pollen with reduced pollen tube growth. No further effects could be observed in other plant/flower parts. Transgenic petunia and tobacco plants which lack flavonoids are not able to produce normal fertile pollen. Self-pollinations on the transformed plants did not produce seed. It was observed that mutant pollen was arrested late in the development (after the first pollen mitosis).

The above results show that flavonoids serve an important function during pollen maturation and pollen germination. The specific action of flavonols, the low concentrations needed for the stimulative effect, together with the fact that flavonols are small molecules, and the separated sites of production and function (tapetal anther cells/pollen grains) suggest that flavonols act as signal molecules and can be regarded as plant hormones.

Structure of Fruit, Seed and Embryo in Hybridogenous Taxa of *Sorbus* L.

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The anatomies of fruits and seeds of *Sorbus thuringiaca* and *Sorbus hostii* were compared light microscopically after Technovit embedding.

The taxa differed considerably with regard to the anatomical structure of the hypanthium. Subepidermal collenchyma layers varied in thickness from 4 cells in *S. thuringiaca* to 6 in *S. hostii*. Parenchyma cells of the hypanthium of *S. thuringiaca* sometimes contained tannin whereas in *S. hostii* tannin-containing cells formed whole groups within the parenchyma. The separation between hypanthium and pericarp was marked by stone cells in both species. Stone cells in the pericarp were found in groups in *S. thuringiaca* and isolated in *S. hostii*. It can be concluded that the anatomical structure of the fruit is of high taxonomic value in the genus *Sorbus*.

Seed coats developing from the anatropous, bitegmic, crassinucellate ovules were very similar in the examined taxa. The epidermis of the outer integument formed thin-walled cells containing only mucilage substances. The subepidermal layer formed thick-walled cells, while deeper layers were characterized by thin-walled cells with large intercellular spaces. Inner integuments were pressed but a layer of exudate between the remnants of inner integument and nucellus epidermis was very distinct, the cells of which had the typical structure of glandular cells with enlarged nuclei and dense cytoplasm.

Embryo development was studied in *S. thuringiaca, S. hostii* and *Sorbaronia dippelii*. Up to 12% of the embryos of the hybridogenous taxa were characterized by abnormalities such as 3 or 4 cotyledons,

two radicles or two shoot apices. In *S. thuringiaca* a seed contained a globular embryo at the micropylar side and a large embryo with cotyledons and three small adventive, nucellar embryos in the chalazal region. In *S. dippelii* one embryo had formed two radicles, one at its micropylar side and one at its chalazal side. Somatic chromosome numbers ($2n=34$) and double chromosome numbers ($2n=68$) were observed in metaphase plates in the same embryo.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 30 OCTOBER 1992

Callose in Tobacco Cells

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Higher plant cells have been observed to deposit callose in the walls at defined stages of cell development or in highly specific cells. Callose is distinctly present during primary wall formation.

The involvement of callose was studied in four cell types of tobacco: pollen tubes of *Nicotiana tabacum* cv. Samsun clone 5, suspension cells of *N. tabacum* BY-2 Gô, regenerating protoplasts, and elongating reverted protoplasts both derived from suspension cells. The presence of callose was determined *in vivo* using decolorized aniline blue or in fixed pollen tubes using a monoclonal antibody against β(1–3)-glucans. Fluorescence microscopy and CLSM were applied to register callose depositions.

The pollen tube wall *in vivo* showed a non-homogeneous distribution of patched callose with a particular structure unlike conventional descriptions. These observations concorded with those made using the antibody. The fluorescence intensity varies along the tube resulting in a banding of fluorescence. Cessation of growth is accompanied by the deposition of callose in the extreme tip descending into the tubular part. These deposits in particular could have a filamentous appearance.

Suspension cells showed the known presence of callose at the site of the cell plate. However, callose appeared in the lateral walls as dispersed spots or as transversely oriented, parallel lines. They varied in number, size, density and/or length. Intermediate forms were present and all forms could simultaneously appear in the cell culture. The predominant presence of these callose structures up to the early log-phase suggests a transient character. Regenerating protoplasts initially showed callose spots and patches, but when elongation proceeded lines of callose became gradually apparent.

Mechanical wounding of cells resulted in typical, radiant callose formations that did not resemble the former observations. Moreover, the patterns of callose formations showed no similarity with the early deposition of the cellulose microfibrils in regenerating the protoplasts as studied *in vivo* with Calcofluor White.

These observations are new and unique. Nevertheless, there is a resemblance with callose formations in developing cotton hairs (Waterkeyn, L. (1981): *Protoplasma* **106**: 49–67). The presence of callose in the different cell types might indicate a crucial event of cell proliferation. However, there is still no consensus on how callose may function. The striking resemblance between the described observations allow us to suggest that callose may have a general function. The favoured hypothesis is that callose ultimately acts as a non-osmotic regulator of water flow (Eschrich, W. (1965): *Planta* **54**:283–300).

The Ultrastructural Development of Mucilage Cells in *Cinnamomum* (Lauraceae) and *Hibiscus* (Malvaceae)

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The ultrastructure of developing mucilage cells was studied in two different plant genera. *Cinnamomum* (Lauraceae) belongs to the so-called primitive dicotyledons. *Hibiscus* (Malvaceae), on the other hand, is a genus well known for its mucilage cavities.

The development of the mucilage cells in shoot apices and leaves of *Cinnamomum burmanni* can be subdivided into five arbitrary stages: (1) Cells differing from the surrounding tissue by the absence of deposits of proteins or crystals in the central vacuole and the presence of plastids showing a reduced thylakoid-system; (2) Cells in which a suberized layer has been deposited against the primary wall; (3) Cells in which a thin layer of mucilage has been deposited between the suberized wall layer and the plasma membrane; (4) Cells in which the cytoplasm is located at the centre of the cell at the cost of the central vacuole due to prolonged mucilage deposition; (5) Mature mucilage cells in which the degenerating cytoplasm is completely embedded in the mucilage.

Hibiscus schizopetalus contains two different types of mucilage cells in its tissues. The first type comprises mucilage cells in the shoot apex and leaf. These show a similar development to the mucilage cells described for *Cinnamomum*, but lack a suberized layer in their cell walls. These mucilage cells develop

as pairs. At maturity they fuse with each other by local breakdown of their common cell wall as a result of which small cavities/canals are formed. The other mucilage cells are solitary cells occurring in the adaxial epidermis of the leaves. These mucilage cells also lack a suberized wall layer and deposit mucilage on the lower side of the cell only. By prolonged mucilage deposition the cytoplasm is pushed upward and, at maturity, degenerating cytoplasm is located in the top of the cell.

Primordium Size and Packing in Relation to Numbers of Florets and Involucral Bracts in an *Asteracean* Flowerhead

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The development of the capitulum of *Microseris pygmaea* (Asteraceae, Lactuceae) was analysed with scanning electron microscopy and computer-assisted light microscopic measurements. Two inbred strains (A92 and C96) differing in the number of achenes per head and the number of inner phyllaries (=involucral bracts) were compared with inbred strains derived from F2 offspring of their hybrid selected for extreme capitulum sizes. The aim of the study is to explain how genetically determined differences among strains in numbers of florets and inner phyllaries per head arise during development. Receptacle size, floret primordium size and form of the receptacle were calculated from three-dimensional point measurements. The larger number of florets in A92 was primarily due to smaller floret primordia, while the difference in floret numbers between the lines selected from the hybrid was the result of a transgressive segregation for receptacle size.

Over a wide range of capitulum sizes, the number of inner phyllaries is 13 in all lines ('numerical canalization'). This is tentatively explained by the interaction between spiral phyllotaxis with a divergence angle of 137·5° and close packing of primordia on a circular receptacle. As a result, Fibonacci numbers of primordia (e.g. 13) are arranged in rings equidistant from the centre of the capitulum. The conditions under which this geometry arises were tested with a computer model developed in co-operation with D.R. Fowler and P. Prusinkiewicz (University of Calgary, Canada). Detailed measurements of radial distances and divergence angles of primordia in 70 capitula showed equidistant rings of peripheral floret primordia. In addition, significant changes in the divergence angle at ring boundaries were found. These indicate that dense packing of primordia plays a greater role than assumed in our model.

Comparative Wood Anatomy of the *Nephelieae* (Sapindaceae)

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The *Nephelieae* constitute a tribe within the Sapindaceae, and consist of twelve genera and 78 species. They occur in Africa (three genera), in South India (one genus), in South East Asia (six genera), in Australia, New Zealand and on some Pacific islands (two genera). The wood of most genera is characterized by a diffuse vessel pattern with both solitary vessels and radial vessel multiples of 2 to 4, simple perforation plates, alternate intervessel wall pits, 4–6-μm in diameter, fine helical vessel wall sculpturing, septate libriform fibres, scanty paratracheal parenchyma, uni (-bi) seriate rays and crystals present in ray cells as well as in fibres and parenchyma.

All genera can be distinguished using wood anatomical features. The main characters used for identification are: minute pits, which characterize *Dimocarpus, Litchi* and *Xerospermum*; marginal parenchyma, which characterizes *Pometia, Stadmania* and *Xerospermum*; aliform confluent parenchyma found only in *Nephelium*; crystals only in rays in *Cubilia* and crystals only in fibres and axial parenchyma in *Nephelium*.

The presence of helical vessel wall sculpturing in ten of the twelve genera, most of which occur in the tropical rain forest, runs counter to general ecological trends for vessel wall thickenings. They are absent in *Pappea* and *Stadmania*, two genera from dry savannahs.

Habit is related to vessel diameter and frequency. Large trees tend to have wider vessels with a lower frequency than small trees and shrub taxa.

Three phylogenetic analyses were carried out: one using wood anatomical features only, one with wood and additional features of pollen morphology and macromorphology, and one with pollen morphological and macromorphological features only. In all the analyses the African–Australian and the Asian groups were recognized and within the Asian genera two groups showed up, *Dimocarpus, Litchi* and *Xerospermum* on the one hand and *Pometia, Nephelium* and *Cubilia* on the other.

Tissue Culture of Tulip. Histological Aspects

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To investigate the propagation of tulip *in vitro*, scale tissue from bulbs of *Tulipa gesneriana* cv. Apeldoorn was used. Explants were cut perpendicularly to

the longitudinal axis of the scales and cultured on medium with 2,4-dichlorophenoxy acetic acid (2,4-D) and 6-benzylaminopurine (BAP) at 22°C.

After successive divisions meristematic centres arose from the relatively small cells of the vascular bundles. These centres gave rise to callus lumps at the upper cut surface of the explant. At a later stage of the culture, divisions in some cells of the ground tissue resulted in callus lumps as well. After a culture period of 7 weeks, club-shaped shoots arose from callus lumps and from meristematic centres at the cut surface of the explant. They developed into filiform, leaf-like or cup-shaped structures. Some shoots showed a notch at their base, debouching into a cavity. At the basal side of this cavity an apical meristem developed, which gave rise to primordia. In cup-shaped shoots an apical meristem was formed at the bottom of the cup. Most shoots, however, were entirely 'massive'; they did not develop an apical meristem.

For bulblet induction, explants with shoots were subcultured on a modified medium and incubated at 4°C for 12 weeks, prior to incubation at 22°C. Only shoots with a cavity produced a bulblet: primordia, originated from the apical meristem, developed into scales.

Immunodetection and Immunolocalization of Globulins in Developing Zygotic and Somatic Embryos of Maize

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Globulins are the most abundant proteins of mature zygotic embryos of *Zea mays* L. These proteins accumulate to high levels during embryo development and serve as storage proteins (Kriz (1989) *Biochem. Genet.* **27:** 239–251). The major globulins in maize are GLB1′ (M_r 67 000) and GLB1 (M_r 63 000) encoded by the *Glb1* gene; GLB2 (M_r 45 000) encoded by the *Glb2* gene; and two proteins of M_r 23 000 and M_r 26 000 (Kriz (1989) *Biochem. Genet.* **27:** 239–251).

To study their developmental expression and subcellular localization, globulins were immunodetected on blots and *in situ* with antibodies against maize globulins, which were kindly provided by Kriz (Belanger and Kriz (1989) *Pl. Physiol.* **91:** 636–643; Kriz *et al.* (1990) *Pl. Physiol.* **92:** 538–542). Immunoblot analysis showed that the first globulins to appear were GLB1′ and proGLB1′ (a short-lived precursor polypeptide of GLB1′, M_r 70 000), which were already present in embryos of 11 days after pollination (DAP). In embedded and sectioned embryos the *Glb1* encoded proteins were located in protein bodies, as expected for storage proteins. The accumulation started in the cells of the scutellar node. In 30 DAP embryos *Glb1* proteins were present in all cells of the scutellum with the exception of the epidermis and the provascular regions. In the embryo axis, *Glb1* proteins were present in the coleoptile, the coleorhiza and a part of the root cortex.

The histological development of somatic embryos that are attached to callus is very similar to the development of zygotic embryos of maize. Globular stage somatic embryos form a scutellum and meristems in maturation medium and regenerate into plants in regeneration medium (Emons & Kieft (1991) *Pl. Cell Rep.* **10:** 485–488). Immunoblotting showed that a *Glb1* encoded protein, probably proGLB1′, was present in the globular stage somatic embryos. However, during maturation in which somatic embryos develop a starch containing scutellum, one or two leaf primordia, and a root meristem (comparable with 11-DAP zygotic embryos), the amount of protein did not increase and no other globulins were detected. It was not possible to localize a *Glb1* protein in cells of somatic embryos. It is concluded that in somatic embryos the *Glb1* gene is active, but that the precursor polypeptide is not processed into GLB1 globulin. The precursor polypeptide might be present diffusely in the endoplasmic reticulum and not be detectable in the light microscope.

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON 20 NOVEMBER 1992

2,4-Dichlorophenoxyacetic Acid Affects Mode and Frequency of Protoplast Regeneration from *B. oleracea*

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During protoplast regeneration three phases can be distinguished: cell division, callus proliferation and shoot regeneration. In most cases a different type of culture medium or a specific hormone composition is used for each phase. In this study we examined the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the regeneration of cauliflower protoplasts by varying the hormone concentration during the first two phases: cell division and callus proliferation.

Hypocotyl protoplasts of the genotype BL12 divided in the presence as well as in the absence of

2,4-D and produced embryogenic calli. Omission of 2,4-D during both phases resulted in the formation of somatic embryos. These embryos were easily recognized at early stages of development by their red cotyledons, due to the production of anthocyanin. The presence of 2,4-D during cell division and/or callus proliferation drastically reduced the percentage of somatic embryos while anthocyanin stained cells disappeared. On the other hand an increase in the number of shoot-forming calli occurred at higher concentrations of the auxin. It is therefore concluded that 2,4-D directs the regeneration of protoplasts by suppressing somatic embryogenesis in favour of shoot morphogenesis. This is supported by histochemical analysis of the differentiating calli. Finally, it appeared that the regeneration of cauliflower protoplasts was most strongly affected during callus proliferation.

The Mode of Action of IBA on Root Formation on Thin-Stem Slices of Microcuttings of *Malus* is Partly via Conversion into IAA

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We have developed a test system for root formation *in vitro*. This system consists of thin stem slices (with a fresh weight of *c.* 1 mg) of *in-vitro* cultured apple (cv. M-9 Jork) shootlets. Incubation during 3 days on medium supplemented with 3·2 μM of indolebutyric acid (IBA) resulted in maximum root formation (*c.* 8 roots per slice). The mode of action of IBA on root formation was studied by relating the 'active auxin concentration' in the tissue to adventitious root formation. 'Active' auxin comprises the free IAA acid (IAAH) resulting from IBA conversion and possibly also the free IBA acid (IBAH). The amount of IBAH was 2·5% and that of IAAH 0·6% of the total IBA taken up from the medium. To assess whether or not IBA acts solely via conversion into IAA, we also incubated stem slices on medium with radioactive IAA. After uptake of IAA only 0·6% was extracted as the free acid. IAA was not converted into IBA. Since the uptake of IBA was fourfold higher than the uptake of IAA, an incubation on medium with IBA must lead to a higher IAAH concentration in the tissue than an incubation on medium with IAA. Preliminary results from experiments in which endogenous IAAH concentrations were measured after incubation of stem slices on medium with IBA or IAA (using an ELISA technique), support these findings. Incubation on medium with IBA and IAA leading to equimolar IAAH concentrations in the tissue, resulted, in the case of IBA, in significantly more roots than in the case of IAA (IAA induced a maximum of 4·3 roots per slice). From these results we conclude that IBA acts partly via conversion into IAA. Currently we are isolating genes involved in the induction of root formation. The function of those genes will be studied during *in-vitro* root formation.

The Effects of Bacterial Lipo-oligosaccharides on Developmental Processes in Plants

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The interaction between *Rhizobium* bacteria and leguminous plants results in the induction of a new plant organ, the root nodule. After invading these root nodules via infection threads, the bacteria start to fix atmospheric nitrogen into ammonia which is beneficial for the host plant. This symbiosis is highly host-specific in that each rhizobial strain is able to associate with only a limited number of host plant species. The molecular mechanism of the host-specific formation of the root nodule appears to be based on at least two stages of molecular signalling between the bacterium and the plant host (Spaink, H.P. (1992): *Pl. molec. Biol.* **20**). In the first stage, flavonoid inducers secreted by the plant root induce in a host-specific way, the transcription of bacterial genes which are involved in nodulation, the so-called *nod* genes. This leads to the second step of the signalling system: the production and secretion of lipo-oligosaccharide signal molecules by the *Rhizobium* bacteria. These signal molecules, which are acetylated forms of small fragments of chitin, have various discernable effects on the roots of the host plants. One of these effects is the dedifferentiation of groups of cells located in the inner cortex which leads to the formation of a (nodule) meristem (Spaink, H.A. *et al.* (1991) *Nature* **354**: 125–130; van Brussel, A.A.N. *et al.* (1992) *Science* **257**: 70–72). In this respect the effect of the bacterial signals resembles the effect of several well-known plant hormones such as auxins and cytokinins. However, one of the differences is that lipo-oligosaccharides are very host-specific in this activity: only signals produced by compatible bacteria are able to induce the nodule meristems. In addition, the lipo-oligosaccharides are also able to induce responses in suspension cultures of non-leguminous plants. In contrast, these effects do not require specific modifications of the oligosaccharide backbone. The molecular mechanism by which the bacterial signals induce cellular dedifferentiation in the plant is unknown. At present, the hypothesis that *Rhizobium* mimics signal molecules

which are also used by the host plants or even eukaryotes in general, is being tested. If such chitin-like molecules are indeed found in various plant species, a comparison of their chemical structures could answer the question of why the bacterial signal molecules are host-specific in their activity.

Hormonal Effects on Somatic Embryogenesis in Dicots with Pea as an Example

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It is widely accepted that the induction of somatic embryogenesis depends upon the application of strong, preferably chlorinated, auxins to competent tissues; one way of studying the signal transduction process involved is to analyse auxin-inducible transcripts/proteins in these particular systems, which may serve as causally related markers. We used two approaches to detect and characterize such markers. We applied a differential hybridoma screening to detect an 'early' auxin-inducible embryo-marker protein and we started from a protein of known function in bacterial, fungal and animal cell divisions, isolated the gene and found a correlation between its auxin-inducibility and its expression during somatic embryogenesis.

Hormone Levels and Response in Transgenic Plants

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Using specific analytical techniques such as HPLC with online fluorometric, diode array and mass spectrometric detection as well as immunochemical assays, the endogenous levels of auxins, cytokinins and their metabolites were analysed in various tobacco plants transformed with distinct chimeric T-DNA genes. Anthers harbouring a tapetum-specific *tap1-rol* B gene showed enhanced endogenous IAA-levels correlating for the first time the expression of *rol* B with an activated IAA biosynthesis. The indoxyl β-glucosidase activity of the *rol* B product, which was confirmed by kinetic LC–MS analysis, might be directly related to a Trp-independent IAA biosynthetic pathway, since concomitant with the increased IAA-level a decrease of the endogenous amount of indoxyl-β-glucose was observed. Tobacco plants that are transgenic for a transposon split 35S-ipt chimeric gene, have viviparous leaves. The formation of epiphyllous flower buds or vegetative buds is linked with a three- to fourfold increase in cytokinin concentration in the altered leaf tissue. Cytokinins appear to be transported to the tip of the leaf's midrib, where the shoot or flower bud is being formed.

MEETING OF THE SECTION FOR PLANT SYSTEMATICS AND GEOGRAPHY ON 11 DECEMBER 1992

Cnesmocarpon, Jagera and *Trigonachras* (Sapindaceae): Phylogeny and Systematics

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Among the unidentified material of the family Sapindaceae several new species were found. On account of a unique character: irritating (stinging) hairs on the fruits, they were tentatively placed in the genus *Jagera*.

The genus *Jagera* in its new circumscription probably can be divided into three groups: (1) *J. javanica* and *J. pseudorhus*; (2) *J. discolor* and two of the new species; (3) a third new species.

Van der Ham discovered that *J. javanica* and *J. discolor* have widely different pollen types with respectively, a scabrate, and a shallowly regulate ornamentation. He also found that *Trigonachras* spp. have exactly the same pollen type as *J. javanica*. This pollen type is unique in Sapindaceae–Cupanieae, and consequently a very close relationship between *J. javanica* and *Trigonachras* spp. was postulated.

At that point the question was no longer how should the genus *Jagera* be divided, but what are the relations between the *Jagera* spp., and between each *Jagera* species and the *Trigonachras* spp.? To solve this problem a cladistic analysis of all *Jagera* and *Trigonachras* spp. was performed.

For this analysis a datamatrix was drafted, including pollen morphological, leaf anatomical and macromorphological characters. This datamatrix was analysed with the program HENNIG86 with the options *i.e.* and all characters unordered. *Euphorianthus euneurus* served as outgroup.

The analysis leads to several conclusions:

(1) *J. javanica, J. pseudorhus* and *Trigonachras* spp. are very closely related indeed. *'Jagera' discolor* and the new species form the sister group of that pair of groups;

(2) All three groups are monophyletic, and of more or less the same taxonomic level and status;

(3) In view of the historical developments in Sapindaceae–Cupanieae these groups are genera;
(4) For *'Jagera' discolor* and the new species a new genus has to be described. This genus is named *Cnesmocarpon*.

The Genus *Diervilla* Miller (Caprifoliaceae) *sensu lato*

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The genus *Weigela* Thunberg has been considered by some authors as belonging to *Diervilla* Miller. The following points are subject to study:

(1) The delimitation of *Weigela* and *Diervilla*, and the position of some small, monotypic genera;
(2) The number of species of the genera, and the distinguishing characters;
(3) The relationship of the material cultivated in The Netherlands to the species distinguished in wild material, and the consequences for the nomenclature of cultivated plants.

The genera which are undoubtedly closely related to *Diervilla* (2(–3) species), next to *Weigela* (3–7 species), are *Macrodiervilla* Nakai and *Weigelastrum* Nakai. These two monotypic genera, rarely in cultivation, appear to have an intermediate position between *Diervilla* and *Weigela*. Some characters are shared with *Diervilla* and some others with *Weigela*. However, both genera also have characters not found in *Diervilla* or *Weigela: Macrodiervilla* and *Weigelastrum* both have coherent anthers, whereas the anthers of *Diervilla* and *Weigela* are always free, and *Macrodiervilla* has the unique character of seeds provided with long wings. A species described as *Diervilla suavis* Komarov (synonym: *Weigela suavis* (Komarov) Bailey) is apparently closely related to both *Macrodiervilla* and *Weigela*.

The combination of all species concerned into a single genus seems to be justified. This would require no new combinations, as all species have been described or combined in the past in *Diervilla*, which is the oldest name available. *Diervilla sensu lato* would consist of 7–12 species.

The merger would be of considerable influence on the nomenclature of cultivar material, as most plants in cultivation belong to *Weigela* (*c.* 200 cultivar names, vs. only 4 cultivar names in *Diervilla*). When comparing wild plants with cultivated plants, it is evident that hybridization and selection have blurred the delimitation of the species in cultivated plants, at least for part of the material. This seems to be enhanced by the often very close relationship between the *Weigela* species.

The Significance of Variation in Style Length for the Reproductive Biology of *Gentianella germanica*

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In one large and one small and recently founded population of the putative heterostylous *Gentianella germanica*, a study was made of the pattern of variation in style length and its effects on reproductive success after spontaneous and hand self pollination.

An analysis of the morphology of random samples of flowers in both populations revealed that although two groups of plants—with flowers that were long and short styled—could be distinguished by eye, overall variation in style length was normally distributed. By using as a criterion for long or short styledness the distance between the top of the anthers and the lowest part of the stigma surface (the 'anther–stigma distance'), flowers were considered to be 'long styled' when the stigma protruded above the anthers (distance>0 mm) and 'short styled' when the stigma was at or below the level of the anthers (distance $\leqslant$ 0 mm). With these two groups of flowers, a pollination experiment was performed to test the hypothesis that long styled flowers have reduced seed set after spontaneous selfing (as compared to hand selfing on the same plant) and short styled flowers have not. In the large population, the observed difference in spontaneous seed set between long and short styled plants was not significant. In the small population, it was, however. This difference between the two populations can be explained by the fact that the 'long styled' flowers had a significantly higher mean 'anther–stigma distance' in the small population.

Regression analysis on the data per individual plant revealed that seed set after spontaneous selfing decreases significantly as the distance between anthers and stigma in a flower increases. This indicates that style length is important for individual fitness at various rates of pollinator visitation.

Based on the data, it must be concluded that *G. germanica* is not heterostylous, since there clearly are not two separated style length morphs, while reciprocal cross pollination experiments among style lengths showed that there was no accompanying incompatibility system either.

It was found that the ratio of long styled to short styled plants in a population, estimated by eye, was a good indicator of the average 'anther–stigma distance' of that population. It was therefore interesting that the small and recently founded population had

relatively more short styled flowers (74%) and that a series of large populations all had relatively more (approx. 66% in all cases) long styled flowers (this agrees with the significantly lower mean 'anther–stigma distance' in the small population). It was argued that if style length is genetically determined, there may be a form of balancing selection between long and short styled plants in a population. The 'short styled' plants may be favoured during periods in which pollinators are scarce, or following founder events or population bottlenecks. Under normal circumstances, higher rates of inbreeding depression in progeny of the short styled plants may favour long styled plants, since these may be expected to have higher rates of outcrossing. This evolutionary mechanism will be of importance for the dispersal and the survival of the species in a fragmented landscape. Unfortunately, problems with germination of the seeds prevents a more thorough analysis of the genetic basis of variation in style length.

The Use of Molecular Markers in the Analysis of Evolutionary Relationships in the Genus *Microseris* (Asteraceae)

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The genus *Microseris* comprises 13 species, of which 11 are endemic to western North America. Ten of the species are diploid ($2n=18$), 3 are tetraploid ($2n=36$). A tetraploid species, *Microseris scapigera*, is the only representative of the genus found in Australia and New Zealand. Using nuclear RFLPs, the phylogenetic relationship of this species with its diploid relatives and the relationships among six Australian populations of *M. scapigera* were investigated. Phylogenetic trees were generated using PAUP. The separation of the diploid *Microseris* species into two monophyletic groups, one clade comprising the annual species and one clade comprising the perennial species (Wallace, R.S. & Jansen, R.K. (1990): *Syst. Bot.* **15:** 606–616) was confirmed as was the hypothesis that *M. scapigera* combines genomes from both clades (Chambers, K.L. (1955): *Contrib. Dudley Herb.* **4:** 207–312). Synapomorphies relating *M. scapigera* to its diploid relatives indicate *M. borealis* as its closest perennial relative, while the annual genome seems to be derived from an ancestor of the existing species. Six populations of *M. scapigera* show a phylogenetic progression which was not expected on morphological grounds. DNA sequence analysis of the nuclear ribosomal ITS1 suggests the possibility of recombination between the two genomes in *M. scapigera*.

MEETING OF THE SECTION FOR PLANT PHYSIOLOGY ON 11 DECEMBER 1992

Regulation of Seasonal Dormancy Patterns of *Sisymbrium officinale* seeds

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Studies on seasonal dormancy patterns of buried seeds have been mainly descriptive. Virtually nothing is known about the mechanism by which soil temperatures regulate this seasonal periodicity. Seeds of *Sisymbrium officinale* (L.) Scop. were buried in the field in November 1990 and at regular intervals portions were exhumed to test germination capacity under laboratory conditions. Germination was tested at 15°C. When germination capacity of exhumed seeds was tested under saturating conditions of light (15 min red light) and nitrate (25 mM KNO_3), both indispensable stimuli for germination of this species, a clear pattern of changes in dormancy was seen. Dormancy was broken in autumn–winter and re-induced in summer. To elucidate dormancy regulation in this species under natural conditions, a detailed analysis of the changes in sensitivity to some relevant germination factors was carried out.

Germination data fitted as logistic dose–response curves showed that sensitivity to light and nitrate varied with the seasons. Patterns of shifts in requirement for light and nitrate were remarkably similar. Sensitivity increased when both primary and secondary dormancy were alleviated and it was reversed during induction of secondary dormancy. During alleviation of primary dormancy in spring 1991, the fluence–response curves exhibited a biphasic character with responses occurring both in the very-low-fluence-range and in the low-fluence-range. The nitrate dose–response data could all be fitted as monophasic curves, although responses might have occurred in two distinct ranges as well. From interpretation of curve parameters, it is postulated that dormancy is regulated by changes in the number of phytochrome and nitrate receptors, in shifts in the binding characteristics of the receptors and/or in shifts in the response chain initiated by the ligand–receptor interaction. Somewhere in this response chain, biosynthesis of gibberellins (GAs) is stimulated. By use of the GA biosynthesis inhibitor tetcyclasis, it was indirectly proven that the capacity to synthesize GAs indeed varied with the seasons.

Sensitivity to GAs gradually increased from burial onwards and was not particularly related to changes in dormancy. Thus, except for the first few months of burial, GA sensitivity may not be regarded as a limiting factor in controlling dormancy in this species.

How Large is the Effect of CO_2 Enrichment on the Growth of Plants

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Since the observation that the atmospheric CO_2 concentration has risen over the last century and will continue to do so, a lot of attention has been paid to the direct effects of this higher concentration on the functioning of plants. Generally an increased total dry matter production is found, which is caused by an increased photosynthesis rate. If the stimulation of the dry matter production were to be predicted from the effect on the photosynthesis rate alone, the increase would be much larger than what is usually seen. What causes this reduction of the plant response to high CO_2?

If photosynthesis rate is considered to be the input of carbon (C) into the plant, then the output can be described in terms of respiration rate, relative growth rate (RGR), allocation to the different plant parts and C concentration. For *Plantago major* and *Urtica dioica* grown on nutrient solution with ample supply of nutrients, the effects of elevated atmospheric CO_2 on many or all of these processes have been studied under climate chamber conditions and at 350 and 700 ppm CO_2.

As for many other species a 20–80% increase in total dry matter was observed within 3 weeks. This effect, however, was solely due to an increase in the RGR during the first 1–2 weeks of the treatment. Thereafter, the RGR returned to the control level. A similar response was observed for the shoot photosynthesis rate. This effect was counteracted by a doubling of the shoot respiration rate and a decrease in the specific leaf area (SLA) in *P. major*. In neither of the species did a change of the allocation between the different plant parts or of the root respiration rate occur. The most important effect is that on the shoot respiration rate. This effect was observed also at the end of the experiment when the stimulation of both the RGR and the photosynthesis rate no longer occurred.

The effect of a doubling of the ambient CO_2 concentration caused a transient effect on individually grown plants of *P. major* and *U. dioica*. In longer term experiments or in shading or mutual shading, high CO_2 grown plants may even be confronted with less favourable growing conditions. This would reduce the effect of CO_2 on the dry matter accumulation. An alternative question to be dealt with when studying the effect of elevated CO_2 on plant growth would then be: How do plants maintain a 20–80% increase in total dry matter production throughout a full life cycle or growing season?

Effects of Nitrogen Supply and Culture-pH on Coniferin Production of *Linum flavum* Phosphate Limited Continuous Cultures

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Cell suspensions of *Linum flavum* are able to produce considerable amounts of the secondary metabolite coniferin. The purpose of this work is to find relationships between growth conditions and this coniferin production. Continuous cultures, where growth conditions are well defined and stable during prolonged periods of time, are the appropriate culture system.

The authors have already demonstrated a clear difference in coniferin production between phosphate- and glucose-limited continuous cultures, production being significantly lower in the latter case.

When a phosphate-limited continuous culture was switched to a five times lower but non-limiting nitrogen supply (potassium nitrate 1·90 g litre^{-1}, ammonium nitrate 1·65 g litre^{-1}), the coniferin production increased linearly from 8·5% to approximately 18% of the dry weight. Total biomass, protein content and respiratory ATP production did not change after the decrease of the nitrogen supply. Also, there was no correlation between the production of coniferin and the external pH of the cell suspension.

This experiment was repeated with a two-step decrease in nitrogen supply (first to 40%, then to 20% nitrogen) in a culture with a somewhat lower growth rate. This time, no relation between the treatments and coniferin production was found. The latter only correlated with small pH changes that occurred due to electrode drift. The significance of this correlation is still unknown.

Flooding and Adventitious Root Formation—Hormonal Regulation of a Morphological Adaptation

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In terrestrial plants flooding causes a strong limitation of the uptake of atmospheric oxygen, due to the very slow gas diffusion rate in water. Combined with shoot elongation, the formation of adventitious roots with large aerenchymatous lacunae is an adaptive response, which enables the plant to restore gas exchange between root tissue and the atmosphere. The genus *Rumex* is used as a model, because of the wide variety in the extent of adventitious root formation in the different species. Species from frequently flooded habitats show larger amounts of new roots with more aerenchyma than species from high elevated, rarely flooded sites. However, in both types of species the timing of adventitious rooting is similar. Within two days after submergence of the root system the first adventitious roots are visible at the base of the shoot. The aim of this study is to determine the hormonal regulation of this rooting process, which may reveal the reason for differences in the amounts of adventitious roots between closely related species.

In a series of experiments it is shown that after application of ethylene or auxin, adventitious root formation occurs to an extent similar to that under flooded or hypoxic conditions. Ethylene might be involved in adventitious rooting during flooding, since a substantial increase in ethylene concentration is commonly found in submerged plant tissues. The maximum rooting response is found after application of ethylene concentrations which correspond well with estimated concentrations in flooded *Rumex* shoots.

Auxin may accumulate especially in waterlogged plants at the base of the stem because basipetal auxin transport is inhibited in the oxygen deficient root system. This increase in concentration is possibly the initiating factor for adventitious rooting. Evidence for this supposition was obtained by an experiment in which adventitious rooting during hypoxic root conditions was prevented by applying an auxin transport inhibitor (naphthylphthalamic acid) to the shoot.

Experiments which will reveal changes in transport and production of ethylene and auxin in flooded plants are in preparation, as are determinations of endogenous concentrations of both hormones.

A Cost-Benefit Analysis of Investment of Carbon and Nitrogen in Roots and Shoots for Inherently Fast- and Slow-Growing Species

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Do inherently slow-growing species function closer to their optimal growth and metabolic rate and are they therefore more fit in low-nutrient environments than inherently fast-growing species, as suggested by Chapin? At low nitrogen supply, the following results were obtained: (1) model studies indicated that both fast- and slow-growing species allocated their carbon and nitrogen in such a way over roots and shoots as to maximize both relative growth rate and nitrogen productivity; (2) no inherent differences in the rates of photosynthesis, shoot and root respiration between species; (3) no inherent differences between species in the photosynthetic nitrogen use efficiency and utilization of the photosynthetic capacity at subsaturating light intensities; (4) fast-growing species had relatively more biomass in their roots than slow-growing species.

It is concluded that slow-growing species are not more fit in nutrient-poor environments because they 'behave' closer to their optimal growth or metabolic rate, nor because they fix carbon dioxide more efficiently with respect to internal leaf nitrogen concentration, nor because they respire (roots and shoots) less. The contradiction between the general dogma, 'a relatively high investment of biomass in roots is of competitive advantage in nutrient-poor environments', and the present results (species from nutrient-poor environments invest relatively less biomass in their roots than species from productive environments) is discussed.

Anatomical Differentiation and Chemical Variation in Relation to Plant Adaptation

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Plant species adapted to nutrient-poor environments have an inherent low potential relative growth rate (RGR, mg-plant g-plant^{-1}day^{-1}) in comparison with species from ruderal areas. It is assumed that there is a link between the low RGR and the possibility of growing under stress, which may be caused by an extra investment in secondary compounds. Increased accumulation of cell-wall components associated with a low RGR was indicated in previous studies.

The aim of the present study is a further investigation of RGR-correlated investment in cell-wall components for closely related plant species and possible RGR-correlated anatomical differentiation.

The 14 grass species were grown under optimal conditions in a growth room. With chemical analyses we determined total nitrogen and carbon content. Light microscopy and picture analyses with an IBAS image processing system were used to analyse the anatomy of the leaf sections.

Species with a low RGR had a higher carbon content (and accumulated more lignin) than those with a higher RGR. The organic nitrogen content was positively correlated with the RGR.

The number of sclerenchyma cells correlated inversely with the RGR. The thickness of the epidermal cell walls was the same for all grasses. The size of these cells, however, was positively correlated with the RGR. For the mesophyll cells no RGR-correlated effects were found.

Leaves of low-RGR species contain relatively more cell-wall material than high-RGR species, possibly partly based on an increased number of sclerenchyma cells.

Localization and Regulation of Thiophene Biosynthesis in *Tagetes patula*

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Root cultures of *Tagetes patula* accumulate high amounts of thiophenes (Croes, A.F. *et al.* (1989): *Planta* **179:** 43–50). The most abundant thiophene is 5-but-3-en-1-ynyl-(2,2')bithiophenyl (BBT). In feeding experiments with [^{35}S]-labelled BBT we have shown that almost all naturally occurring thiophenes in *Tagetes* can be formed by modifications of the side chain. This suggests that side-chain modification occurs after the formation of the heterocyclic thiophene rings.

We have identified the monothiophene 2-but-3-en-1-ynyl-5-penta-1,3-diynyl thiophene (BPT) as a key intermediate in the biosynthetic route towards BBT. This result indicates that the position and sequence of thiophene ring formation are strictly regulated and not more or less randomly determined as was suggested previously (Jente, R. *et al.* (1981): *Phytochemistry* **20:** 2169–2175).

By cell fractionation it was shown that thiophenes accumulate in the microsomal fraction. Combined cell fractionation and radiolabelled precursor feeding will enable us to find out in which cellular compartments thiophenes are formed.

If thiophene biosynthesis can be pin-pointed to specific cell fractions, examination of these fractions may facilitate the isolation of key enzymes and the study of their metabolic and molecular regulation.

Ethylene and Flooding Resistance. I. An Ecophysiological Approach with *Rumex* as a Model

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As an ecophysiological research group, the authors are concerned with the occurrence and distribution of plant species in riparian areas of the Rhine river system in the Netherlands. During the last decades, plants within these areas have been confronted with infrequent and unpredictable floods during the growing season due to improved drainage in the upper Rhine area. The down-stream flood plains are characterized by differences in elevation, leading to a distinct flooding gradient and a typical plant zonation.

In order to elucidate this zonation, investigations were started with eight species of the genus *Rumex* which occurred in the river area, each having a specific position in the flooding gradient. The central hypothesis is that the *Rumex* zonation is mainly determined by the resistance of the different species to flooding. As a consequence of the ecological approach, experiments, both in the field and laboratory situation, are conducted on all life stages of these species.

Adaptations of *Rumex* spp. towards flooding are aerenchyma formation and enhanced shoot elongation. Ethylene plays a central role in the initiation and regulation of most of these adaptive responses. Future research will concentrate on the mechanism of ethylene-mediated shoot elongation and aerenchyma formation, and the interaction of ethylene with other plant hormones in these responses.

Ethylene and Flooding Resistance. II. Application of an Advanced Laser-Driven Photoacoustic Cell in Ethylene Measurements on Flooded *Rumex* Plants

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The regulation of some important adaptations of the genus *Rumex* (sorrel or dock) towards flooding is mediated by ethylene. During flooding, ethylene tends to accumulate in plant tissues due to the very slow diffusion rate of gases in water and increasing ethylene production rates. High ethylene concentrations in the shoots of flood-adapted *Rumex* spp. induce fast elongation of the petioles and leaves.

Additionally, ethylene seems involved in stimulating the formation of a highly aerenchymatous root system. These adaptations enable the flooded plant to reach the water surface and aerate the submerged tissues, both leaves and roots.

The changes in ethylene concentration and production during submergence are very fast: an accurate high resolution technique is a prerequisite for research. Therefore, an extremely sensitive laser-driven photoacoustic cell, combined with a flow-through system, was applied. It proved possible to measure ethylene concentrations as low as 0·006 ppb, or production rates as low as 10 pl h^{-1}. Because measurements take place continuously, changes in ethylene concentrations or production rates can be detected within minutes.

Ethylene and Flooding Resistance. III. The Role of Ethylene in Shoot Elongation of *Rumex* Plants in Response to Flooding

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Rumex spp. that occur in the lower parts of Dutch river forelands can survive partial or even total submergence for weeks. This resistance may be explained by physiological and morphological adaptations, such as rapid shoot elongation. This process is regulated by the gaseous hormone ethylene. The release of this hormone can be continuously measured with a very sensitive laser-driven photoacoustic detection system. During waterlogging or submergence, flooding resistant *Rumex* plants show an increased ethylene production rate. Also an accumulation of the hormone within the plants occurs, especially during total submergence, due to the 10 000-fold slower diffusion of gases in water compared to air. This results in a high internal ethylene concentration, which causes a rapid shoot elongation response. In this way contact with the atmosphere can be restored. *Rumex* spp. that occur in higher rarely inundated parts of the river forelands are sensitive to flooding, and do not show this rapid shoot elongation response.

Interactions between Electron Transport Pathways in Potato Callus Mitochondria

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Mitochondria isolated from potato tuber callus (*Solanum tuberosum* cv. Bintje) contain a cytochrome pathway and an induced cyanide-resistant pathway for quinol oxidation. Both pathways function in respiration not only with succinate or malate as substrate, but also with cytoplasmic NADH or NADPH as substrate. The kinetics of this branched respiratory chain were studied experimentally with a Q-electrode during titrations of succinate respiration in purified potato callus mitochondria with an inhibitor of a quinol-oxidizing pathway (myxothiazol), inhibiting the cytochrome pathway, and an inhibitor of the quinone-reducing pathway (malonate), inhibiting succinate dehydrogenase. Titration experiments with *malonate* show that in state 3 a linear dependence is obtained between the redox state of the Q-pool and the respiration rate. In state 4 the dependence is non-linear, probably due to engagement of the cyanide-resistant pathway. Respiration activity of the cyanide-resistant pathway is only obtained at high reduction levels of the Q-pool (>80%). Measurements of the respiratory rate and the reduction of the Q-pool in titration experiments with *myxothiazol* show a more or less linear dependence of the rate on the redox state of the Q-pool in both states.

The obtained kinetic characteristics of the enzymes are used for further theoretical study of the branched respiratory pathway in potato callus mitochondria, using a model for the kinetics of the Q-pool. In this model the behaviour of the complete system is derived from the kinetics of the individual enzymes.

Effect of Nitrate on the Proton Gradient and Membrane Potential of the Tonoplast Membrane of Lettuce

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For many plant species nitrate is one of several solutes that accumulate in the vacuoles to maintain cell turgor. Accumulation of nitrate in the vacuoles may rise to high concentrations (up to 100 mM) and is inversely related to the concentration of organic acids.

Vacuoles already contain a large proton gradient after isolation, but they do not have a membrane potential. In this condition they do not take up nitrate. Influx of nitrate in the vacuoles is only possible when a membrane gradient is generated at the expense of ATP. Indeed, nitrate uptake is stimulated by ATP. This indicates a coupling between nitrate and ATPase.

Nitrate also inhibits ATPase activity, as shown abundantly in the literature. However, nitrate can

also stimulate proton pumping by ATPase, provided that a membrane potential is present. This depends on the order of addition: nitrate inhibits ATPase when the anion is added to the assay before addition of ATP. Nitrate initiates proton pumping when ATP is present at the start of the assay.

To study a situation similar to that in the vacuoles, vesicles were used with an existing proton gradient, with or without an imposed membrane potential. The change in the proton gradient or membrane potential were monitored with quinacrine or oxonol fluorescence quenching, respectively.

Addition of nitrate to the vesicles results in a transient increase in the proton gradient followed by a dissipation. Addition of PP_i recovers the proton gradient completely. The appearance of the transient increase depends on the nitrate salt used. The slope of the dissipation depends on the nitrate concentration and obeys Michaelis–Menten kinetics. The dissipation is probably due to proton leakage, resulting from ATPase inhibition by nitrate.

Addition of nitrate to vesicles with an imposed membrane potential causes a dissipation of the proton gradient. Addition of PP_i does not recover this gradient.

Analysis of Root Respiration of Potato (*Solanum tuberosum* L.) as related to Growth, Ion Uptake and Maintenance of Biomass

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Knowledge of the processes depending on respiratory energy is necessary for a quantitative understanding of plant growth. Few literature data are available on the assessment of the amount of respiratory energy utilized by growth, ion uptake and maintenance processes. Here data for potato are presented and compared with (1) the available data (Veen (1981): *Pl. Soil* **63:** 73–76; Van der Werf *et al.* (1988): *Physiol. Pl.* **72:** 483–491; both for monocotyledons) and (2) estimates of the specific costs of these basic processes obtained by calculation or by direct measurements. Although there is some variation between species, the variation is less than a factor of 4. Calculations suggest that it is not clear whether maintaining solute gradients correlates with the costs for maintenance or ion uptake; this might vary for the different species.

Photosynthesis and Chlorophyll *a* Fluorescence in the Facultative CAM-Plant *Sedum spectabile*

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Photosynthesis and chlorophyll *a* fluorescence were measured during the day on *Sedum spectabile* var. Brillant plants which were in either C_3- or CAM-mode. Nursery-grown plants were placed in a climate chamber under summer conditions (28/15°C; 12 h light, PAR 250 μmol m^{-2} s^{-1}; RH 30%). CAM was induced by withholding water for 3 weeks prior to the experiments. C_3-plants were watered every day.

Chlorophyll *a* fluorescence was measured with a PAM-fluorometer. In both plants, the ratio F_v/F_m was 0·8 or higher, which pointed to a high efficiency of photochemistry of PSII. Photochemical fluorescence quenching (q_p) was high in the well-watered (C_3) plants, indicating a high Calvin-cycle activity. This was also reflected in high photosynthesis rates, measured as CO_2-fixation and O_2-evolution.

CAM-plants showed a lower q_p compared to the C_3-plants, and also maximum photosynthetic capacity (measured as O_2-evolution) was reduced to 25% of that of the C_3-plants. CO_2-uptake was 0–5% of the well-watered plants since the stomates of the CAM-plants are closed during the day. Non-photochemical quenching (q_N) was higher in the dry plants, indicating an accumulation of reducing equivalents.

Abscisic Acid-Binding Protein Activity in *Arabidopsis thaliana* Seeds

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Abscisic acid (ABA) is thought to be one of the inducers of desiccation tolerance and dormancy in seeds. Using single mutants impaired either in ABA synthesis (deficient mutants, *aba*) or in responsiveness to ABA (insensitive mutants, *abi*), several aspects of the physiology of *Arabidopsis thaliana* have been investigated. However, so far nothing is known about the proteins capable of binding abscisic acid.

The authors have developed a method to screen for abscisic acid-binding protein (ABA-BP) activity using an indirect immunoassay (IIA). Using this assay buffer soluble protein extracts from mature wild type were compared with *aba* and *abi3-1* seeds of

A. thaliana. Extracts from developing seeds, aged from 14 to 16 days after pollination (DAP), of these three genotypes were also compared. At this stage of seed development in wild type the tissue is highly sensitive to ABA.

Comparing mature seeds no striking differences were found between the three genotypes, although the ABA binding activity of the insensitive mutant was approximately half that of wild type or *aba*. In the comparison of the 15 DAP seeds, however, *aba* seeds showed a fivefold higher ABA binding activity than wild type or *abi3-1* seeds. This result can be explained as a receptor up-regulation as described in animal receptor literature. If so, it might indicate that the plant balances the ABA-deficiency by enhancing ABA sensitivity through more receptive units.

Chloramphenicol as a Tool to Study the Regulation of Respiration in Cell Suspensions of *Petunia hybrida*

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Chloramphenicol (CAP) is an inhibitor of the mitochondrial protein synthesis and blocks the synthesis of *a.o.* cytochrome oxidase and ATP-synthase. When *Petunia hybrida* suspension cells were inoculated in fresh medium with chloramphenicol (CAP), the activity of cytochrome *c* oxidase, and the respiration via the cytochrome pathway of isolated mitochondria decreased, while in untreated cells these parameters were more than doubled in 2–3 days. However the *in-vivo* respiratory activity of the cytochrome pathway of CAP-treated cells showed a similar course in time as that of untreated cells, even in the presence of an uncoupler: a large rise during the first 2–3 days followed by a decline. This leads to the conclusion that respiration via the cytochrome pathway, even measured in the presence of an uncoupler, is not the capacity of this pathway.

CAP had little effect on the uninhibited respiration and the cyanide-resistant, alternative pathway of the *Petunia* cells. However the engagement of the alternative pathway was increased in CAP-treated cells.

Furthermore, the results suggest that, although new synthesis of proteins occurs directly after inoculation, a large overcapacity must be present of (mitochondrial encoded) cytochrome pathway elements.

Plasticity in Growth and Morphology in Relation to Light Quality in Two Ecotypes of *Plantago lanceolata*

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Plants from open and shaded habitats were grown under equal photon flux densities but different ratios of red to far-red light (R/FR). Since plants from both populations differed in size, plants were compared at a common weight. Total plant weight was not influenced by the light treatment. However, light conditions simulating vegetation shade (i.e. low R/FR) changed morphology and promoted formation of long, upright growing leaves with long petioles. This increase in plant height, generally interpreted as an adaptive response to shading, was associated with a decline in leaf number. Plants raised in low R/FR had a higher specific leaf area (SLA) than plants raised in high R/FR, although the effect diminished over time. Significant differences in plasticity were found between the populations: the leaf elongation was larger in plants from the dense vegetation, whereas increase in SLA was larger in plants from the open habitat. At the end of the experiment leaf area had decreased in low R/FR in the sun plants, whereas it had increased in shaded plants. Apparently both populations react to light conditions, comparable to their natural habitat, by changing their allocation patterns.

Characterization of the Binding Properties and Isolation by Affinity Chromatography of the Binding Protein of the Fungal Phytotoxin Fusicoccin

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Fusicoccin (FC) is the phytotoxin produced by the fungus *Fusicoccum amygdali* Del. Infection of plants with the fungus leads to a wilting disease, caused by a constant opening of the stomata. The most striking effects of FC are: stimulation of the plasma membrane H^+/ATPase and hyperpolarization of the plasma membrane. The FC action is probably mediated by specific receptor sites for FC, the plasma membrane-bound fusicoccin binding protein (FCBP). The binding properties of [^{3}H]-dihydrofusicoccin to oat (*Avena sativa*) root plasma membranes were examined. The aim was to isolate the FCBP by affinity chromatography, using a

(covalently linked) biotinylated fusicoccin derivative (FCBio) together with a column of immobilized avidin. The following data were obtained from binding experiments with [^{3}H]-dihydrofusicoccin (at 30°C):

Association rate constant (K_{on}): $0{\cdot}5 \times 10^5$ M^{-1} s^{-1}.
Dissociation rate constant (K_{off}): $2{\cdot}0 \times 10^{-5}$ s^{-1} ($t_{1/2}$=9·3 h).
Dissociation constant (K_d): 0·7 nM for ^{3}H-dihydroFC; 70 nM for FCBio; 73 nM for FCBio–Avidin.
B_{max}: 65 pmol mg^{-1} plasma membrane protein (0·65% if molecular weight of the FCBP= 100 kDa). Hill constant:1.

From these data it can be concluded that isolation of the FCBP using an FCBio and avidin affinity system is possible. The avidin–biotin technology can also be useful in further research, when using other avidin conjugates.

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 of endogenous GAs and the biological activity and metabolism of applied GAs.

At first, an inventory was made of free GAs, in sprouts of cooled and uncooled bulbs (*Tulipa gesneriana* L. cv. Apeldoorn). By combined gas chromatography–mass spectrometry (GC–MS) and GC-selected ion monitoring (SIM), GA_4, GA_9, GA_{24} and GA_{34} were detected. During dry storage the amount of GA_4 had increased from 3 to 6 ng sprout^{-1}, compared to bulbs at the beginning of the treatment in October. However, this had occurred during the cold as well as during the non-cold treatment.

The biological activity of GA_4 and GA_9, and the not detected but in many plants active GA_1, was tested by applying them *in vitro* to sprouts of uncooled and cooled bulbs. Sprouts of uncooled bulbs did not respond to the tested GAs. The sensitivity to applied GAs increased during the cold treatment and the three GAs were equally effective in stimulating stem elongation.

Fructan Depolymerization and Redistribution during Vernalization and Chicon Growth of Witloof Chicory (*Cichorium intybus* var. *foliosum*)

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Chicory is a biennial producing a taproot in the first growing season for storage. The dry matter of the taproot consists at the end of the growing season of 60–80% carbohydrate, which is mostly polymerized to fructans. Depolymerization of the fructans in the root takes place during the vernalization preceding the second growing season. Roots with a high nitrogen content do contain less carbohydrates, but have a higher rate of depolymerization resulting in a higher content of transportable carbohydrate after vernalization. During chicon growth at high temperatures in the dark, the so-called forcing, depolymerization of fructans continues at a higher rate than during vernalization. This depolymerization rate is still higher in roots with a high nitrogen content. Because of the absence of photosynthesis, the regrowth fully depends on the reserves in the root. The redistribution of carbohydrate is not as much related to the amount of depolymerized fructan as to the formation of sucrose from the fructose liberated by depolymerization.

Carbohydrates and Flower Bud Opening in 'Madelon' Roses

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Flowers of cut Madelon roses often fail to open. Addition of sucrose to the vase solution results in bud opening comparable to that of intact plants (control). The effect of sucrose addition on opening and carbohydrate content is dependent on the number of leaves on the stem. Madelon roses were grown in growth chambers (>60 W m^{-2}). Plants were harvested in several stages of flower bud opening. After cutting, roses were transferred to two different light conditions: >60 W m^{-2} (HL) or 3 W m^{-2} (LL). Fresh and dry weight, photosynthesis and (dark) respiration of these plants were compared to control. The HL treated roses displayed flower bud opening and photosynthesis comparable to control roses, while LL treated roses showed delayed flower bud opening and no net photosynthesis. The amount of carbohydrate in 'cutting stage' flower buds was probably not sufficient to complete bud opening. In LL roses carbohydrates necessary for flower bud

opening must be imported from elsewhere, probably from the leaves.

A New Red-Light Insensitive Mutant of Tomato

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A recessive mutant C66 of tomato (cv. GT) which has a slightly longer hypocotyl than wild type (WT) under white light (WL) has been studied. While continuous low fluence rate red light (R) is relatively ineffective in hypocotyl inhibition, both blue light (B) and far-red light (FR) are just as effective as in WT. Short pulses of R or R followed immediately by FR, given every 4 h, demonstrate that the phytochrome response via its low fluence mode on hypocotyl growth is not detectable in C66 during the first 2 days of treatment, whereas the anthocyanin synthesis response, is reduced compared to WT. The high irradiance component shown in fluence rate–response relationships for anthocyanin biosynthesis in WT during a 24 h irradiation is more or less absent in C66. In R/FR-reversible anthocyanin biosynthesis during a 24 h dark period after 12 h pretreatment with R or B, the response was dramatically less in the case of R pretreatments in C66. Adult light-grown plants of C66 respond to reduction in R:FR ratio and end-of-day FR treatments by stimulation of elongation growth. In contrast to the R-insensitive phytochrome-deficient tomato *aurea* mutant, WL-grown C66 plants are not yellow and have a similar chlorophyll level to WT plants.

The Role of Polyamines in Flower Bud Initiation of Apple

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Polyamines have been implicated in the process of flower initiation in several species including apple. External applications of the polyamines putrescine, spermidine and spermine were shown to increase the number of apple flower buds. In the following experiment, the internal levels of polyamines in buds on trees kept at conditions more or less favourable for flower initiation were determined. The level of arginine, a precursor of polyamines and one of the main constituents of the soluble N-fraction of apple tissue, was also determined. Differences in flower-inductive conditions were created by spraying with gibberellins (GA in apple generally being inhibitive to the process of flower bud initiation), and by varying branch-orientation, the horizontal position being more favourable for initiation than the vertical. Though large differences in flowering between treatments were observed, little, if any, difference in polyamine concentration was found. Arginine levels varied more with treatments, the concentration being higher in unsprayed buds and in buds on vertical branches. Differences in polyamine or arginine concentration between treatments only partly corresponded to the most inductive treatments. The response of bound polyamines to the above treatments is still being investigated.

A Simulation Model for the Dormancy Cycle of Seeds in the Seed Bank

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A model has been developed to simulate the annual dormancy cycle of buried seeds of light-requiring summer annuals.

The simulation model is based on a physiological model concerning the action of phytochrome in the seed. Dormancy is related to the amount of a hypothetical phytochrome receptor, that fluctuates in an annual pattern. An increase in the amount of receptor, which means relief of dormancy, results in a widening of the range of temperatures over which germination can occur. A decrease in the amount of receptor, which means induction of dormancy, results in a narrowing of this range. Temperature is the driving force for the changes in the amount of receptor. Germination is triggered by light. The active phytochrome (Pfr) that is generated disappeared from the seed by dark reversion. The time period during which Pfr is *able* to activate the germination process after irradiation decreases with increasing temperature and with a decreasing amount of phytochrome receptor. The time period during which Pfr is *needed* to activate the germination process, the escape time, decreases with increasing temperature. The model quantifies the germination that results from these conflicting processes as a function of the temperature and the amount of phytochrome receptor.

There was a close fit between simulated results and data from a field experiment with seeds of *Polygonum persicaria*.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 10 FEBRUARY 1993

Development of Vegetation Patterns on the Dollard Salt Marsh During a Period of Extensified Management

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The study area is located in the Ems–Dollard Estuary in the north-east of the Netherlands. The tidal marsh (*c.* 700 ha) has developed from land reclamation works. The marsh became a nature reserve in 1981. The management was extensified (neglect of the drainage system and reduction of stocking densities) to create a salt marsh with a higher species and pattern diversity and suitable breeding habitats for birds. The vegetation development was studied by two successive mappings (1:2000) of five dominant species in four study sections in 1983 and 1991. The five selected species determine to a large extent the structure of the vegetation. The area of bare soil was also mapped. The maps were analysed using GIS.

Grazing was more intensive at the more elevated parts close to the dike than in the lower lying outer areas.

Spartina anglica was found at the lower parts of the salt marsh. Its initial homogeneous area of distribution was broken up into smaller areas during the study period. *Scirpus maritimus* was dominant at the edge of the marsh, but it showed a significant decrease there. *Phragmites australis* was also found at the lower parts. Its distribution increased, also towards more elevated areas. Both the distribution and abundance of *Aster tripolium* have strongly increased, especially onto more elevated parts of the salt marsh. *Elymus repens* was found at more elevated areas. Its distribution remained more or less constant, but the species remained dominant only at well drained sites.

The decrease of *S. anglica* and *S. maritimus* is probably caused by exploitation of below-ground parts by greylag geese (*Anser anser*) in autumn and winter. The decrease of *E. repens* and the increase of *A. tripolium* in the more elevated areas, as well as the increase of bare soil are probably due to waterlogging conditions caused by the neglect of the drainage system.

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The Effect of Drought on the Development of the Pioneer Vegetation after the Drawdown of a Shallow Lake

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Since 1975 much of the emergent vegetation in the Grote Plas, part of the Oostvaardersplassen, a shallow lake in the Zuid–Flevoland polder in the Netherlands, degenerated to open water. To restore the emergent vegetation cyclic water level management with periodic drawdowns was started in 1987. Due to a height gradient (40 cm) the subsoil was exposed at different periods. The area that was exposed in the early spring of 1987, became dominated by *Typha latifolia*. In the early spring of 1988 an area was exposed that became dominated by *Chenopodium rubrum – Rumex maritimus*. The areas that emerged in the late springs of 1987 and 1988, became dominated by *Senecio congestus*. Previous seed-bank studies revealed that these various plant communities could not be explained by differences in the seed bank. The different plant communities could be related to the different weather conditions during the periods of drawdown.

Under wet conditions, in the climate chamber and glasshouse, seedlings of *T. latifolia, S. congestus, C. rubrum* and *R. maritimus* emerged and survived. When the conditions remained wet, as in the spring of 1987, *T. latifolia* became dominant. *S. congestus* and *R. maritimus* grew well, but *T. latifolia* grew much higher. *C. rubrum* grew badly. Under dry conditions, as in the spring of 1988, all four species emerged, but only small numbers of *C. rubrum* and *R. maritimus* survived. When the dry conditions continued these two species could grow to maturity. *C. rubrum* thrived much better than under wet conditions and could become dominant over the other species. Adults of *T. latifolia* and *S. congestus* grew badly and most plants died within 4 months. Very dry periods, as in the late spring and early summer of 1987 and 1988, could kill all seedlings. When such a dry period was followed by rainfall all species except *T. latifolia* emerged again. In the absence of *T. latifolia, S. congestus* could become dominant when the conditions remained wet.

The experiments showed that the zonation of plant communities might be explained by the prevailing weather conditions after drawdown.

Accumulation of Nutrients and Biomass during Primary Succession on the Dunes

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On the island of Schiermonnikoog, chronosequences of primary succession can be found, ranging from bare sand to forest vegetation on a soil profile of about 200 years old. Two chronosequences were studied, one on the higher parts of the dunes, and another on former beach plains which are situated between the major dune ridges. The dune sequence showed a much lower salinity and a lower soil moisture content than the plain sequence in each stage of succession. The vegetation on the plains succeeded from *Glaux maritima* and *Juncus gerardii* in the earliest stages, via *Phragmites australis* to a *Betula pubescens* community in the oldest stage in the sequence. The dune vegetation succeeded from *Ammophila arenaria* via *Hippophae rhamnoides* to a mixed forest of *B. pubescens* and *Prunus serotina*. Changes in stand structure, nutrient distribution in plant soil compartments and various soil factors were investigated in different successional stages ranging from herbaceous to forest vegetation.

Moisture content increased, while lime content, pH and salinity decreased along both chronosequences. In a fertilization experiment, in an early stage of plain and dune succession, it was found that nitrogen was the major limiting nutrient for above-ground production (Olff, H. (1992): *On the mechanisms of vegetation succession*; Ph.D. thesis, University of Groningen). Nitrogen accumulated in the soil–plant system in both sequences, but faster on the dunes, which may be due to the nitrogen-fixing shrub *H. rhamnoides*, which was only present in the dune sequence. Just a slight increase in the C/N ratio of the organic layer in the plain sequence was found, and no significant increase in the dune sequence. This difference illustrates the close connection between the accumulation of nitrogen and biomass during these primary successions. In contrast, the C/P ratio of the organic layer showed a remarkable increase in both sequences.

Both N and P accumulated in the above-ground vegetation along both sequences. The pool size of K in the above-ground biomass however did not change. The investigations of stand structure showed that the canopy became taller with progress of succession. The root biomass in the upper 20 cm of soil did not increase, which might be interpreted as a consequence of the increasing importance of light competition.

Allocation Patterns in Herbaceous Climbing Plants

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Climbing species make use of the structure of the surrounding vegetation as this provides the necessary support for climbing. A climbing strategy has consequences for the allocation of biomass of the various plant parts within these species. It is known that woody lianas and vines from tropical forests allocate a smaller fraction of their above-ground biomass to supporting tissue, *i.e.* stems and petioles, than non-climbing trees. As a result a relatively higher amount of biomass can be allocated to photosynthetically active leaf tissue (Putz, F.E. (1983): *Biotropica* **15**: 185–189).

Herbaceous climbing species from (temperate) grasslands, show an allocation pattern that is in contrast to that mentioned above. These species (*Vicia* sp. and *Lathyrus* sp. and *Galium aparine*) allocate a relatively larger amount of above-ground biomass to supporting tissue and, hence, a smaller amount to leaf tissue than non-climbers. The reduction in leaf biomass is partly compensated by a relatively high rate of photosynthesis per unit of leaf dry weight and by an increased allocation of support tissue to stem length and thus plant height.

In dense grassland-vegetations where competition for light is severe, the herbaceous climbers appear to be more plastic in their response to shade than non-climbers. If the photon flux density decreases, the climbing species show a considerable increase in stem length. The amount of length per unit of stem dry weight increases significantly more than for the non-climbing species. In this way climbers are able to grow above and eventually on top of the canopy of the neighbouring vegetation in order to intercept better light conditions.

Ecology, Distribution and Syntaxonomy of *Fritillaria meleagris* in The Netherlands and Surrounding Countries

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Fritillaria meleagris is largely restricted to seasonally-flooded rather nutrient-rich alluvial soils. Here, it occurs in meadows, woodlands and communities

dominated by tall forbs. The life cycle of the species comprises six phases. After germination (in February/March), a sterile phase of about 4–5 years finally results in a so-called candlestick form. The subsequent fertile phase may last another 25 years. The annual cycle of the species can be divided into seven phases with two active periods in spring and autumn. Winter flooding of the habitat not only constitutes an important mechanism for seed dispersal, but also results in low soil temperatures in early spring, preventing a fast development of non-bulbous competitive species.

F. meleagris is a European lowland species, with a nowadays widespread but scattered distribution. The two major partial areas include the north-west and the south-east of the continent. In the northern part of its area, the richest stands are found in the river valleys of the Loire, the Elbe and the Overijssel Vecht. In The Netherlands, where *Fritillaria* until half a century ago was considered to be not indigenous, the number of sites inhabited by this species has considerably decreased during this century from 82 h-squares before 1950 to 52 since 1950 (estimation in 1992: 25 h-squares).

Syntaxonomically, a distinction must be made between grasslands and other formations. In The Netherlands, all grasslands with *F. meleagris* belong to the *Fritillario–Alopecuretum pratensis*, that can be assigned to the alliance *Arrhenatherion elatioris* (class *Molinio–Arrhenatheretea*). Within this association, three subassociations can be distinguished, viz. *cynosuretosum, typicum* and *calthetosum*. The subassociation *cynosuretosum* shows affinity to the *Cynosurion cristati*; the subassociation *calthetosum* can be seen as a transition towards *Calthion palustris*. The communities dominated by tall forbs belong to the *Filipendulion*, the woodland communities to the *Alno–Padion*. On an international level, *F. meleagris* can be regarded as a character-species of the *Molinio–Arrhenatheretea*, provided that for each individual area the species is indicating syntaxa of lower syntaxonomical ranks within this class.

Spatial Gradient Analysis of West African Rain Forests

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Forest gradients have been described in relation to environmental factors like moisture, temperature, light or nutrients. When exact data on the latter factors were not available, geographical or topographic conditions such as elevation or slope exposure have been used as an index to rank the forests (Whittaker, R.H. (1967): *Biol. Rev.* **42:** 207–264). In West Africa forest composition is changing from the coast inland and thus, the spatial position (x and y coordinates) of a forest controls its position on the gradient. An appropriate map of the forest gradient should indicate both the direction and the rate of compositional change.

The gradient approach to forest compositional change relies on three principles: (1) that both the biotic and the environmental control model apply to the species' distribution; (2) the individuality of the species' response; (3) the continuity of vegetational change.

The results of forest inventories carried out in the 1960s and the 1970s in south-east Liberia and south-west Côte d'Ivoire were ordinated using DCA (detrended correspondence analysis). Only data from 53 of the largest tree species were used for the ordination. Each sample consisted of a systematic 1% sample of a given forest area of *c.* 50 000 ha. The first axis ordination scores of the forests were plotted on the map and contour lines every 20 axis units were interpolated using the Kriging spatial interpolation technique. Cross-sections were made to compare the forest gradient to the rainfall gradient and relief. A correlation of the species' scores with those found by Hall, J.B. & Swaine, M.D. (1981: Distribution and ecology of vascular plants in a tropical rain forest. Forest vegetation in Ghana. *Geobotany* 1, Junk Publ., The Hague) in Ghana provided an opportunity to check the precision of the results.

It is concluded that contours of ordination scores provide a more precise map of the forest gradient than forest types. Rather than classifying a given forest within a forest type, its tree species composition is more accurately summarized by its ordination score. The DCA ordination table, in combination with the DCA score contour map, simultaneously provides information about the ecological optimum and range of a large number of emergent tree species (van Rompaey, R.S.A.R. (1993): *Forest gradients in West Africa. A spatial gradient analysis,* Ph.D. thesis, Agricultural University Wageningen, The Tropenbos Foundation, Wageningen).

Tropical Rain Forest Types in a Sandy Watershed in Guyana

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Tropical rain forest is a term that may cover a wide array of forest types. In Guyana many forest types are clearly related to edaphic factors. Also, within the so-called mixed forests associations can be distinguished. Quantitative data are lacking, however.

An inventory was made of the vegetation and soils of a watershed area in the interior of Guyana, South America. In the watershed area of 480 ha, 252 plots of 0·05 ha were laid out on 21 lines 100 m apart. Pilots were situated 100 m apart on the lines. All trees, with DBH>20 cm, on the plots were measured and identified. Soil type, slope, 'forest type' (in the Guyanese sense), and soil drainage type were noted. Data for the tree species and plots were classified with TWINSPAN, and ordinated with DECORANA.

In the plots 111 tree species were found. With TWINSPAN seven main forest 'associations' were found. Classification and ordination produced very comparable results. The main classification and ordination were based upon the differences in species occurrence on white sands (Haplic Arenosols) and on brown sands (Haplic Ferralsols and Ferralic Arenosols). Further differentiation could be made with respect to soil hydrology (drainage status). Many species showed a clear preference for either one soil type or soil drainage class. The main forest types described (with dominant species between brackets) were:

(1) Dry evergreen forest on Haplic Arenosols (*Eperua grandiflora* and *E. falcata*);
(2) Palm-Swamp forest on Histosols (*E. falcata, E. rubiginosa* and *Jessenia bataua*);
(3) Creek forest on alluvial Gleyic Arenosols and Dystric Fluvisols (*E. falcata* and *Catostemma* sp.);
(4) Poorly drained mixed forest in low lying small creek heads on Ferralic Arenosols and Haplic Ferralsols (*E. rubiginosa* (strongly dominant), *Eschweilera sagotiana, Chlorocardium rodiei, Mora gonggrijpii*);
(5) Well-drained mixed forest on Ferralic Arenosols and Haplic Ferralsols (*Chlorocardium rodiei, Eschweilera sagotiana, Dycimbe altsonii*).

The forest types identified did not correspond with the forest associations currently accepted for Guyana, as first described by Fanshawe (1952: The Vegetation of British Guiana: A preliminary review. Paper No 29 IFI, University of Oxford). Forest types such as 'Greenheart forest' and 'Morabukea Forest' are both mixed forests on well drained soil with a local dominance of Greenheart (*C. rodiei*) and Morabukea (*M. gonggrijpii*) respectively. The subset of species co-occurring with both dominant species is the same, however.