Acta Bot. Neerl. 38(1), March 1989, p. 85–103

Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON 11 MARCH 1988

PROPAGATION OF TULIP (CV. 'APELDOORN') BY MEANS OF BULB-SCALE TISSUE: SOME ASPECTS

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The system of culturing bulb-scale pieces as described by Nishiuchi (1979) was followed. Apeldoorn bulbs were harvested on five dates in May and June. Slices of scale were cut freshly after harvest as well as after storage of the bulbs for 1–5 weeks. The slices were cultured at *c.* 22°C on Murashige and Skoog medium with 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP). About six weeks after the start of the culture callus lumps arose on some of the slices; shoots often emerged from these callus lumps.

Fresh bulbs: The fourth harvest (18 June) gave the highest percentage of explants with shoots; the fifth harvest (29 June) gave none.

Stored bulbs: Bulbs of the fourth harvest (storage sequence: 1 week 34°C, 1 week 30°C, 3 weeks 30 or 23°C) gave the highest percentage of slices with shoots when stored for 2–3 weeks. Bulbs of the fifth harvest (storage sequence: 2 weeks 30°C, 3 weeks 30 or 23°C) yielded a more or less constant percentage of slices with shoots when stored for < 1 week. For both harvests the difference between 30 and 23°C in the second part of the storage sequence was not significant.

As for the formation of shoots, Nishiuchi (1979) has reported a better yield, but pretreatment of the bulbs was different. However, even when 'Apeldoorn' bulbs grown in Japan and bulbs grown in Holland were subjected to the same treatment, Japanese bulbs gave a better yield of shoots.

Nishiuchi, Y. (1979): *J. Jpn. Soc. Hort. Sci.* **48:** 99–105.

NAA UPTAKE, TRANSPORT AND METABOLISM DURING FLOWER BUD DEVELOPMENT ON THIN-LAYER EXPLANTS OF TOBACCO *IN VITRO*

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Thin-layer explants from flower stalks of *Nicotiana tabacum* L. cv. Samsun developed flower buds between 9 and 14 days after the start of culture. For this regeneration, the presence of a cytokinin and an auxin in the medium was necessary. Maximum bud numbers are obtained when 1 μM of 6-benzylaminopurine (BAP) and 1-naphthalene acetic acid (NAA) are used.

The exogenously applied NAA was taken up by the explants at a very high rate, proportional to the concentration in the medium. Because of this high uptake from a limited volume, the

concentration of NAA in the medium diminished very rapidly, and, as a consequence, the uptake rate declined. Due to this process, it was observed that the less the volume of medium present, the higher the initial concentration of NAA necessary to yield maximum bud numbers.

Within the explants, the NAA was found to be transported polarly to the basal edge. As a consequence of accumulation at the basal edge, maximum bud numbers were found here at lower medium concentrations than on the other parts of the explant. This difference was not found when transport inhibitors were present in the medium.

NAA taken up was rapidly metabolized into a number of conjugates. The conjugation was specifically induced by the auxin, and therefore the increase in internal concentration of free NAA levelled off after 6 h of culture.

BAP was also metabolized into a number of conjugates and it also induced its own conjugation. There was a major difference in the uptake rate of the two hormones. During the first day of culture on 1 μM of both hormones, 139 pmol of NAA were taken up but only 13 pmol of BAP. In addition, no NAA leaked out of the tissue, while most of the BAP taken up leaked back into the medium during the second day of culture. These processes led to a ratio of 5 between free auxin and free cytokinin inside the tissue.

From the results, it is clear that the concentration of hormones in the tissue cannot be simply derived from the external concentration. For a better understanding of the processes that are induced in tissues *in vitro*, the actual concentrations during the relevant period should be determined. This is certainly the case when results obtained under different incubation conditions are compared.

TRANSFORMATION OF ROOTSTOCKS OF ROSE WITH *AGROBACTERIUM RHIZOGENES*

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'Infectious hairy-root' is a disease that has been found under natural conditions on a number of rosaceous hosts. The disease is caused by the soil bacterium *Agrobacterium rhizogenes*. *A. rhizogenes* is related to *A. tumefaciens* which induces tumours on plants.

In *A. rhizogenes* a plasmid, named the root inducing plasmid or Ri plasmid, is present. The genes that are transferred to the plant and are responsible for hairy root induction are located on this Ri plasmid together with the virulence functions, which are involved in the transfer. In addition, chromosomal virulence regions are essential for virulence on the host plant (Koukolikova-Nicola 1986).

We report an *in vivo* test for virulence of two wild-type *A. rhizogenes* strains, LBA 9365 (pRi 8196, mannopine type, Petit *et al.* 1983) and LBA 9402 (pRi 1855, agropine type, Pompoui, M. *et al.* 1983) on two different rootstocks; *Rosa canina* 'Inermis' and *Rosa chinensis* 'Major' ('Indica Major') used in The Netherlands (De Vries & Dubois 1987). Stems of young greenhouse grown plants were wounded with a pin. *A. rhizogenes* was inoculated with a tooth-pick. The mannopine type strain gave a relatively fast reaction: all plants tested showed adventitious root formation within 4 weeks after inoculation. The agropine strain was less virulent: after 6 weeks about 50% of the inoculations resulted in root formation at the site of inoculation.

Outgrowth of the adventitious roots was obtained by surrounding them with soil. All the roots tested so far were positive in the opine test.

Experiments are now focused on the inoculation of *in vitro* plantlets and the regeneration of shoots from transformed roots.

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Petit *et al.* (1983): *Mol. Gen. Genet.* **190:** 204–214.
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CONDITIONS FOR SOMATIC CYBRIDIZATION OF *DAUCUS CAROTA* L.

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Hybrid seed production of carrot (*Daucus carota* L.) cannot be achieved via cross breeding due to the practical impossibility of hand-emasculation to prevent self-pollination. Therefore, the introduction of cytoplasmic male sterility (CMS) in a cytoplasmic male fertile (CMF) line would be highly desirable. The introduction of CMS via cross breeding, however, is complicated by crossing barriers and recurrent backcrossing and selection. Furthermore, CMS might be transferred through somatic cybridization in only one generation.

Therefore, conditions were established for callus induction and the subsequent culture of calli and cell suspensions. Cell suspension-derived protoplasts were isolated, cultured and, via somatic embryogenesis, regenerated to plantlets. Protoplasts of the donor CMS line were gamma-irradiated for inactivation of the nucleus and stained with fluorescein diacetate (FDA). Protoplasts of the recipient CMF line were treated with iodoacetate for inactivation of the cytoplasm and stained with rhodamine isothiocyanate (RITC). Protoplasts of both lines were electrofused and heterokarvons were identified by green-yellow (FDA) and red (RITC) fluorescence. Irradiation of protoplasts of the CMS line never resulted in sustained cell divisions and iodoacetate-treated protoplasts of the CMF line sometimes gave rise to aggregate formation. In the electrofusion dishes some aggregates continued to grow, giving rise to calli which eventually differentiated into embryoids, shoots and plantlets.

Putative cybrids are being analysed on the basis of their chromosome number, nuclear DNA content, and mitochondrial-DNA restriction fragment pattern. The ultimate selection for CMS will be carried out after flowering.

THIOPHENE PRODUCTION BY *TAGETES PATULA* IN A PILOT PLANT AIRLIFT LOOP REACTOR (ALR)

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Sterility turned out to be the major problem in operating a 165 dm^3 Airlift Loop Reactor (ALR) for a period longer than 1 week. During the experiment the cells remained viable, respiratory active, looked healthy and remained heterogeneously aggregated. Although at a lower rate than in a 1·6 dm^3 continuously stirred tank reactor (CSTR), thiophenes were released into the medium during the experiment. The time pattern of the accumulation and release of thiophenes resembled that in

flasks and a 1·6 dm^3 CSTR. Thus the production of thiophenic metabolites by *T. patula* was not essentially curtailed upon a 100-fold scale-up.

MORPHOGENESIS OF SOMATIC EMBRYOS FROM *DAUCUS CAROTA* L. IN RELATION TO CYTOSKELETON DEVELOPMENT

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Somatic embryos from carrot suspension cultures were observed at different developmental stages by transmission and scanning electron microscopy (SEM). Semi-thin sections of polyethyleneglycol (PEG) embedded embryos were labelled for tubulin with monoclonal antibodies and fluorescein isothiocyanate (FITC) to investigate the three-dimensional arrangement of the microtubular cytoskeleton during somatic embryogenesis.

After induction of embryogenesis by 2,4-dichlorophenoxyacetic acid (2,4-D), the embryos developed to the globular stage within 5 days, and via the heart-shaped stage to the torpedo-shaped stage within 11 days. During early development the amount of cytoplasm increased, cells divided frequently and the proembryo enlarged. Organogenesis then started with the formation of cotyledons and the hypocotyl. Cells in the hypocotyl elongated as embryos developed from heart- to torpedo-shape.

Electron microscopy revealed tubulin or microtubules at the cellular level (see also Van Lammeren *et al.* 1987). Microtubules were often present in bundles close to the cell wall: the cortical microtubules. The three-dimensional orientation was difficult to recognize. With semi-thin sections, however, bundles of cortical microtubules arranged in parallel were found in the protoderm and in the elongated cells in the torpedo-shaped embryos. The latter showed an orientation perpendicular to the length axis of the cells. The bundles appeared to be arranged in helical structures. At the root tip and in the subepidermal and central region of the cotyledons random orientations of microtubules dominated. These tissues showed isodiametric cells preferentially.

The results indicate that cell shaping is correlated with the arrangement of the microtubules.

Van Lammeren, A.A.M., Kieft, H., Provoost, E. & Schel, J.H.N. (1987): *Acta Bot. Neerl.* **36:** 125–132.

IN VITRO REGENERATION OF *ROSA*

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New biotechnological methods are of great promise for the genetic improvement of cultivated plants. Application of the methods to roses, however, is hampered by the lack of methods of *in vitro* regeneration.

At IVT, regeneration of the rose is studied using explants from *in vitro* grown cultivars and species. Somatic embryoids developed on a callus from leaf explants of *Rosa hybrida* 'Tendresse' on Murashige and Skoog (MS) medium with 2 μM 6-benzyladenine (BA) and 0·5 μM 1-naphthylacetic

acid (NAA). Adventitious bud regeneration occurred in root cultures of *Rosa canina* 'Inermis' on MS medium with 25 μM BA and 3 μM giberellin-A_3. Future research will aim at increasing the frequency of regeneration and the further development of plantlets from somatic embryoids or adventitious buds.

A SHORT PERIOD OF CULTURE ON INDUCTION MEDIUM IMPROVES REGENERATION OF *CHRYSANTHEMUM* EXPLANTS

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Usually nutrient components of the medium and phytohormones are used as the tools to control regeneration processes in plant tissue culture. Little attention is paid to the period during which the explants or cells are exposed to a medium.

Within the scope of a study on somaclonal variation in *Chrysanthemum*, internodium explants of cv. Parliament were cultured on a regeneration induction medium for different periods of time, namely 0, 1, 2, 4 and 9 days, continuously. The regeneration medium was Murashige and Skoog (MS) medium with 3% sucrose. Four micromolar kinetin (K) and 12 μM indole-3-acetic acid (IAA). After the period of regeneration medium the explants were transferred to MS medium containing only 0·5 μM IAA. Two days on regeneration medium yielded optimum results, on average 3·1 transplantable plantlets were obtained per explant. Lengthening the period on regeneration medium led to an increase in the number of adventitious buds by up to about 50 per explant in the continuous treatment. None of these buds, however, developed into a plantlet. Thus, the results show that the length of the period of exposure to the regeneration medium contributed very much to the success of regeneration of internodium explants of *Chrysanthemum*.

SELECTION FOR RESISTANCE TO BACTERIAL CANKER IN TISSUE CULTURES OF TOMATO

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A promising way to obtain disease-resistant plants is the use of *in vitro* selection at the cellular level. Selection may be carried out by adding a toxin, produced by a plant pathogen, to tissue cultures of the plant. Cells selected for resistance to the toxin may give rise to resistant plants.

We investigated the production of a toxin by *Clavibacter michiganensis* subsp. *michiganensis* (*Corynebacterium michiganense* subsp. *michiganense*), the causal agent of bacterial canker of tomato, and the possible use of this toxin as a selective agent in tissue cultures of tomato. A toxic factor, a glycopeptide capable of mimicking the wilting symptoms of the disease in a bioassay with plant cuttings, was isolated from culture filtrate of the bacterium. This toxic factor was found to behave in a non-specific way since partial resistant and susceptible genotypes responded similarly in the bioassay. The specific activity of toxic factors isolated from strains of *C. michiganensis* differing in aggressiveness was similar. No correlation was found between the quantitative production of this factor *in vitro* by the strains and their aggressiveness. Therefore, the role of the toxic factor in pathogenesis is not clear.

The toxic factor inhibited growth of cell suspension cells plated on a solid medium and decreased the viability of tomato protoplasts according to a dose–response curve when added to the culture

medium. Since this factor was found to be toxic at the cellular level, it can be used as a selective agent.

APPLICATION OF OVARY- AND EMBRYO-CULTURE TECHNIQUES IN *LILIUM*

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Many methods have been studied in order to overcome pre-fertilization barriers in interspecific hybridization programs with *Lilium* species: application of growth regulators, heating the style before pollination, cut-style pollination and application of mentor pollen. These methods are applied successfully in hybridization programmes. After fertilization, embryo abortion and/or endosperm degeneration can cause barriers in different stages of development. Using the embryo rescue technique, embryos can be saved by *in vitro* culture, starting 40–70 days after pollination. By using nurse endosperm Asano (1980) could rescue embryos of 0·3–0·4 mm in length, starting 35 days after pollination. To improve the embryo rescue technique, an ovary and ovule culture technique is being investigated, starting as early as 5 days after pollination.

For the induction of gynogenic haploids in lilies, Prakash & Giles (1986) used the medium of Gamborg & Larne (1968) + 400 mg l^{-1} glutamine, 50 mg l^{-1} aspargine, 100 mg l^{-1} serine, 0·1 mg l^{-1} 1-naphthalylacetic acid (NAA), 0·1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 10% sucrose, 0·8% agar at pH 5·8. They also found that young anthers on the medium had a positive influence on the swelling of the ovules. For their embryo rescue Asano & Myodo (1977) used the medium of Murashige & Skoog (1962) + 0·001 mg l^{-1} NAA, 2% sucrose at pH 5·0. In our experiment the effects of the medium (Prakash & Giles 1986; Asano & Myodo 1977) and the presence of anthers (continuously, 1 week, absent) on compatible and incongruent combinations were investigated. Three flowers per treatment were used; 5 days after pollination the ovaries were cut into 3–4 mm thick slices; four slices per disc and six discs per treatment were used. Incubation took place at 24°C in the dark. After 42 days the swelling of the ovules was scored. Subsequently, the swollen ovules were excised and cultured on the same medium, from which the hormones were omitted and the sucrose content was reduced to 2%. No anthers were placed on the latter medium and the ovules were incubated in the dark until embryos emerged. After 4 weeks these embryos were transferred to the medium described by Asano & Myodo (1977).

The results show a positive influence of the medium on the anthers. The medium of Prakash & Giles (1986) gave more swollen ovules than the medium of Asano & Myodo (1977). A high concentration of sucrose and a high pH were favourable. The treatments with the most swollen ovules produced the largest number of growing embryos. One plantlet was obtained from the interspecific cross. It can be concluded that the ovary culture method is promising for preventing embryo abortion.

Further research will be focused on improving the ovary culture medium, localizing the sites for crossing barriers, determining the time of embryo abortion during the development, and increasing the efficiency of the pollination technique.

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INFLUENCE OF PRETREATMENT AND AUXIN CONCENTRATION ON CALLUS PRODUCTION FROM ANTHERS OF *LOLIUM PERENNE* AND *LOLIUM MULTIFLORUM*

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Anther culture in *Lolium* species has been reported but the anther response was rather low and the majority of the regenerated plants were albinos (Clapham 1971, Pagniez & Demarly 1979, Stanis & Butenko 1987, Rose *et al.* 1987). These problems interfere with a commercial application of anther culture in *Lolium*.

The aim of this study was to increase the anther response in *L. perenne* and *L. multiflorum* by optimizing anther pretreatment and auxin concentration in the induction medium. Furthermore, a suitable regeneration medium was selected to achieve higher regeneration percentages.

To investigate the influence of anther pretreatment, spikes were kept at 4°C for 0–4 weeks before plating the anthers on solid culture medium: Linsmaier & Skoog (1965) salts with 12% w/v sucrose, 100 mg l^{-1} inositol, 6 g l^{-1} agarose, 20 mg l^{-1} glycine, 5 mg l^{-1} nicotinic acid, 5 mg l^{-1} pyridoxine HCl and 1 mg l^{-1} thiamine HCl. This medium was supplemented with 1 mg l^{-1} NAA for *L. multiflorum* and 2 mg l^{-1} NAA for *L. perenne*. This pretreatment significantly raised the anther response in both *L. perenne* and *L. multiflorum*, the optimum duration of pretreatment was between 2 and 3 weeks, respectively.

The influence of auxin concentration was investigated by plating anthers on the above-mentioned culture medium with NAA or 2,4-D in different concentrations. It was found that callus formation was possible without an auxin for both *L. perenne* and *L. multiflorum*. However, anther response in *L. perenne* was enhanced by 2,4-D (1 mg l^{-1}) and NAA (2 mg l^{-1}).

For shoot formation, microspore-derived calluses were transferred to three different regeneration media. On one of these three media (2/3 × Linsmaier & Skoog salts with 1·4% sucrose, 60 mg l^{-1} inositol, 6 g l^{-1} agarose, 1 g l^{-1} casein hydrolysate, 1·2 mg l^{-1} glycine, 0·3 mg l^{-1} pyridoxine HCl, 0·3 mg l^{-1} nicotinic acid, 0·06 mg l^{-1} thiamine HCl, 1·2 mg l^{-1} IAA and 0·2 mg l^{-1} kinetin) green as well as albino shoots of both *L. perenne* and *L. multiflorum* were formed. However, the ratio albino: green shoots was still unsatisfactory.

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COMPARISON OF PLANT REGENERATION FROM ROOTS TRANSFORMED BY *AGROBACTERIUM RHIZOGENES* AND NORMAL ROOTS OF THE POTATO CV. 'BINTJE'

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Transformed (Ri) root clones were obtained after infection with *Agrobacterium rhizogenes* AR 15834 of leaf segments and tuber discs of the potato cultivar Bintje. A variation in the growth rate and pattern between Ri root clones was observed.

Cytological analysis and optimization of the plant regeneration procedure were carried out for the Ri root clones and control roots. Cytological differences were observed between transformed and normal roots; a greater number of smaller cells, a zone of radial cells in the pericycle, a larger root cap, and statocytes with small statoliths were typical traits of transformed roots. Vigorous growth rate, multiple branching and diminished geotropism are ascribed to these traits.

After optimization of the plant regeneration procedure, about 60% of the transformed root segments produced shoots whereas the response in control root segments was 100%. For plant regeneration, transformed root clones required a diminished auxin 2,4-dichlorophenoxyacetic acid (2,4-D) level as compared with control roots (0·025 mg l^{-1} and 0·1 mg l^{-1}, respectively). The phenotype of Ri-regenerated plants had typical Ri transformation traits, e.g. diminished apical dominance, shorter internodes, crinkled leaves and abnormal tubers (oblong and deep-set eyes), whereas the phenotype of regenerated plants from normal roots was similar to that of control plants. In all cases, Ri and normal regenerated plants were tetraploid similar to control plants ($2n=4x=48$). Thus the Ri phenotype is correlated with the presence of genes of T-DNA integrated into the plant genome (Taylor *et al.* 1985).

The potency of *A. rhizogenes* Ri plasmids as a virulence vector in binary plant transformation system (Simpson *et al.* 1986, Sukhapinda *et al.* 1987) with the additional advantage of genetic stability of the regeneration process suggests suitability of the system for genetic manipulation.

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TRANSFORMATION OF DIPLOID POTATO GENETYPE HH 260 WITH *AGROBACTERIUM* TO PRODUCE GENETIC MARKER LINES FOR SOMATIC CELL GENETICS

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Genetic markers are indispensable tools in fundamental breeding research, e.g. somatic cell genetics and gene localization. In potato, genetic markers are scarce. Attempts were therefore made to introduce genetic markers using *Agrobacterium* vectors. These vectors are capable of introducing randomly into the host cell genome one or more copies of T-DNA-carrying marker genes. The transformed cells may develop into transformed roots, which can be cultured as individual root clones. Shoot regeneration is possible from transformed root clones, thus enabling the establishment of a series of plant clones, each carrying the T-DNA marker genes on a different chromosome.

Stem internodes of the diploid potato geneotype HH 260 were incubated with *A. rhizogenes* LBA 9365 (pRi 8196), LBA 9402 (pRi 1855), or with *A. tumefaciens* LBA 1060 KG. The latter *Agrobacterium* strain is a binary vector containing the wild type Ri plasmid 1855, and the plasmid pBI121 which carries the genes for kanamycine resistance (kan^R) and β-glucuronidase (GUS) between T-DNA border sequences. Transformed root clones were obtained after selection for hormone autotrophy (LBA 9365, LBA 9402, LBA 1060 KG) and kan^R (LBA 1060 KG). Twenty-four established root clones were used for plant regeneration; two of them developed shoots spontaneously after decreasing the saccharose concentration in the culture medium, ten other clones showed shoot regeneration after induction with different plant hormones. The regeneration procedures were carried out in the absence of kanamycine. Most of the regenerants had a normal shoot phenotype and a transformant root phenotype (i.e. branched, hairy roots with decreased geotropism and high growth rate). The ploidy level of the original genotype HH 260 was maintained in 10 out of the 12 regenerants.

The transformation characteristics were not found in all regenerants and their original root clones. In only 50% of the root clones transformed with LBA 9365 or LBA 9402 was opine production observed. This is in contrast to transformation with LBA 1060 KG and culture of the resulting root clones under selection pressure for kanamycine, after which all the marker genes were retained. Only one regenerant from the latter group of six transformed root clones showed expression of these marker genes. Three regenerants had lost the GUS-activity, two lost opine expression and one of these two also lost kanamycin-resistance.

From the regenerated transformed plant clones, protoplasts were obtained at high concentrations ($1{\cdot}0$–$7{\cdot}7 \times 10^6$ p.p.s. g^{-1} leaf material). This makes these plant clones suitable as genetic marker lines for application in interspecific somatic hybridization with *Nicotiana plubaginifolia* to produce monochromosomal hybrids to be used for gene localization.

CONSTRUCTION OF A GENETIC LINKAGE MAP OF POTATO USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs)

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A genetic linkage map of potato is under construction using RFLPs as a source of genetic markers. These markers should allow saturation of the genetic map since they are numerous and ubiquitous.

RFLPs were detected by Southern blotting. Thirteen probes, selected from a potato tuber- or leaf-cDNA library, were tested on genomic DNA of two heterozygous, diploid *Solanum* lines, digested with the restriction enzymes BamHI, EcoR1 or HindIII. Nine probes showed possible useful polymorphisms after hybridization to genomic DNA digested with EcoR1 or HindIII.

Two probes were tested on 12 F_1-hybrids. Complex segregation patterns were obtained, indicating that the resulting polymorphisms were of polygenic origin. Because of the complexity of the patterns, analysis and mapping of these RLFP-markers will be laborious.

New probe versus enzyme combinations will be tested to find more simple restriction patterns. In addition, the production of interspecific, monochromosomal somatic hybrids of transformed potato plant clones (carrying the gene for kanamycin resistance) with *Nicotiana plumbaginifolia* is in progress and will be used for further localization of the RFLP markers.

ACCUMULATION OF ETHYLENE AND CARBON DIOXIDE IN DIFFERENT TISSUE CULTURE SYSTEMS AND THE EFFECT ON QUALITY OF ROSE AND *GERBERA* PLANTS

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Carbon dioxide and ethylene concentrations were determined in various sterile culture systems with *Gerbera* plantlets. High concentrations were found in closed (sealed) systems. Elevated carbon dioxide and ethylene concentrations were also found in semi-closed systems of types that are frequently used in commercial practice.

Treatment of rose and gerbera plantlets with 0–5% carbon dioxide and 0–$0{\cdot}9\ \mu l^{-1}$ ethylene showed that in both species increased ethylene concentrations were detrimental, while increased carbon dioxide concentrations were clearly beneficial.

In addition, it was found that phytotoxic vapours were produced by polypropylene tissue culture containers.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 3 JUNE 1988

THE INFLUENCE OF WOUNDING ON THE PLANE OF CELL DIVISION

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Thin explants of leaves of *Nautilocalyx lynchii (Gesneriaceae)* were cultured on microscopic slides on agar medium. The epidermis cells of the explants are highly vacuolated. Most of these cells divide after 3 days. Mitosis is preceeded by the migration of the nucleus to the centre of the cell and by the formation of a phragmosome (PS) which meets the cortical 'division site' and so gives an early indication of the future plane of cell division. The PS is visible in living cells as a thin cytoplasmic septum across the cell. It is formed between 9 h and 2 h before prometaphase by a process of flattening and fusion of cytoplasmic strands.

In the present study two successive wounds were made in different planes, the first at the time of explantation, the second after 1–3 days. Most cells near the second wound divided in a plane related to this wound irrespective of the time of wounding. This indicates that the plane of cell division was not stably fixed until shortly before mitosis.

A model in accordance with these results will be discussed. In this model the exact division site at the cell cortex is determined during and by the completion of the PS.

CHANGES IN THE ORIENTATION OF CELLULOSE MICROFIBRILS IN CELLS OF TOBACCO EXPLANTS

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The orientation of cellulose microbfibrils at the inner surface of the wall of cortical cells of tobacco explants was examined using cryo-sections. In cells of freshly cut explants the direction of cellulose microfibrils is transverse to the longitudinal axis of the cell. The longitudinal axes of pit-fields are also oriented tranverse to the longitudinal axis of the cell.

Within 6 h after the onset of the experiment most cells show curved or longitudinally oriented cellulose microfibrils. The underlaying lamellae with transverse cellulose microfibrils is still seen.

After 12 h of culturing all cortical cells show cellulose microfibrils running parallel to the longitudinal axis of the cell forming a thick layer. Also the longitudinal axes of the pit-fields are parallel to the longitudinal cell axis.

It may be concluded that cellulose microfibrils in cortical cells of tobacco explants are indirectly and passively controlled by cortical microtubules (Heath's hypothesis in Heath & Seagull, 1982).

Heath, I.B. & Seagull R.W. (1982): *The Cytoskeleton in Plant Growth and Development*. Lloyd, C.W. (ed.): Academic Press, London.

CO-DISTRIBUTION OF MICROTUBULES AND MICROFILAMENTS IN PROTOPLASTS OF *NICOTIANA PLUMBAGINIFOLIA* AND *TAGETES MINUTA*

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Protoplasts from cell cultures of *Nicotiana plumbaginifolia* and *Tagetes minuta* were used to study the cortical cytoskeleton. Rhodamin and FITC-labelled probes were used to detect microtubules and microfilaments simultaneously. For ultrastructural studies the dry cleaving technique was used. In protoplasts, both microtubules and microfilaments show random patterns. Occasionally microtubules and microfilaments are parallel. In dry cleave preparations, microfilaments can be seen parallel to microtubules for distances up to 1·5 μm. Microtubules are often observed in bundles, up to 10 microtubules per bundle. Microtubules in bundles are connected by cross-bridges, 25–50 nm wide.

Occasionally microfilaments connect coated pits to each other and to microtubules. Large differences exist between various cells. To study possible differences during the cell cycle presently cell cultures are synchronized.

THE P-PROTEINS OF CUCURBITACEAE

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P-proteins form the filamentous structures that are electron microscopically visible in the sieve tube lumen. Though they are generally assumed to protect the phloem against leakage, e.g. after wounding, their function remains basically unknown.

Phloem exudate was collected from stems and fruits of pumpkin, *Cucurbita maxima*, and of cucumber, *Cucumis sativus*. The exudate is very rich in proteins, up to 30 mg ml^{-1}, from which about 80% are P-proteins. The PP1 (M_r 96,000) and PP2 (M_r 26,000) fractions were isolated using procedures described by Read and Northcote (1983a and b). Exudate and subfractions were submitted to PAGE gel electrophoresis. Purified PP1 fractions were used to raise mouse monoclonal antibodies. In Elisa tests sera show a high affinity for the PP1 protein.

Read, S.M. & Northcote, D.H. (1983a): *Eur. J. Cell Biochem.* **134:** 561–564.
— (1983b): *Planta* **58:** 119–127.

A CELL WALL LAYER LIMITING DIGESTION IN GRASSES

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Investigations with light and scanning microscopy of plant material digested *in vitro* by rumen fluid have shown morphologically unchanged tissues which reacted positively with phloroglucinol (AP) and chlorine–sulphate (CS) (Akin *et al.*, 1984, Akin *et al.*, 1985). These tissues were identified

as subepidermal and vessel sclerenchyma and they show very thick cell walls. Nearly complete digestion of sclerenchyma was observed in barley straw leaving a warty layer as the undigestable residue (Engels & Brice, 1985).

Stem internodes from maize bearing the kernel were harvested in mid July (17/7) and mid October (13/10). Thin sections (100 μm) of the 13/10 harvest were stained with AP. Subepidermal and vessel sclerenchyma and stem parenchyma showed an intensive cell wall staining. In sclerenchymal walls the location of the middle lamella–primary wall was just visible because it sometimes stained more intensively than the adjacent secondary wall. Sections digested *in vitro* and stained by AP thereafter showed only very thin and intensively coloured cell wall residues, indicating probably the complete removal of the secondary wall. A 0·1 M KOH treatment at room temperature followed by AP staining resulted in nearly colourless secondary and yellow-orange primary cell walls. If a delignification with $KMnO_4$, modified after Goering & Van Soest (1970), was followed by AP the secondary walls were completely colourless. The primary walls still showed an intensive yellow-orange colour suggestive of the presence of $KMnO_4$ resistant 'lignin'. The same tissues in sections from the 17/7 harvest did show negative staining after both KOH and $KMnO_4$ treatments. These were found to be digested completely. Comparable results were found in stem sections made from *Panicum laxon* and *Poa trivialis*. It is concluded that the middle lamella–primary wall is the origin of limitation in cell wall digestion built up during maturation of the plant stem tissues.

Akin, D.E., Brown, R.H., & Rigsby, L.L. 1984. *Crop Sci.* **24:** 769–773.
—, Willemse, M.T.M. & Barton, F.E. II. 1985. *Agronomy J.* **77:** 180–182.
Engels, F.M. & Brice, R.E. 1985. *Current Microbiol.* **12:** 217–224.
Goering, H.K. & Van Soest, P.J. 1970. *Agric. Handbook* **379:** 1–20.

ENZYMATIC SACCHARIFICATION OF THE WOODY CELL WALL

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Ultrastructural changes in cell walls were investigated upon autohydrolysis and successive enzymatic saccharification of a number of Japanese hardwoods, among which the enzymatic susceptibility after autohydrolysis differs substantially. The nature of secondary wall lignin was studied using UV spectrometry. Enzymatic susceptibility of various cell wall layers was observed in ultra thin sections hydrolysed with enzyme (crude cellulase). Wood meals were chemically analysed using conventional methods.

In species with a low Klason-lignin content, UV absorbance of the secondary walls of fibres was low. In these species, the methoxyl content and the syringyl/guaiacyl ratio were high. The ratio of absorbance at 280 nm to absorbance at 273 nm was small in the secondary walls of those species, which easily became susceptible to enzyme upon autohydrolysis and also upon ultra thin sectioning.

The secondary walls of vessels in all species were more difficult to delignify with autohydrolysis and successive extractions than the secondary walls of fibres and axial parenchyma cells. The secondary walls of vessels were not susceptible to enzyme after hydrolysis or ultra thin sectioning. The vessel wall lignin was, therefore, considered to be rich in guaiacyl units and to be densely packed.

Ultraviolet absorbance and the ratio of absorbance at 280 nm to absorbance at 273 nm of xylem parenchyma walls were widely different among hardwood species as in the fibre walls. Consequently, the content and nature of lignin appear to be different among hardwood species.

GROWTH RING PATTERNS AND WOOD ANATOMY OF DOUGLAS FIR (*PSEUDOTSUGA MENZIESII* (MIRB.) FRANCO) AND PENDUNCULATE OAK (*QUERCUS ROBUR* L.) IN RELATION TO VITALITY

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For a number of years there have been reports of alarming decline in the vitality of Dutch forests. Air pollution, weather conditions and insect pests are pointed out as possible causes. A study of the wood of Douglas fir and pendunculate oak has been initiated to determine the onset and extent of increment reduction and any effect that might exist on wood structure and quality.

Growth ring analysis of Douglas fir from several stands of different age and vitality showed a growth reduction in less vital trees. This growth reduction can be abrupt or slow. In the latter case the beginning of the reduction was in the late Fifties or early Sixties. Increment reduction coincides with a reduction in the relative amount of latewood, of the maximum density and of tracheid length. Relations between tracheid length and ring width, as reported in the literature, with an optimum ring width for maximal tracheid lengths, were not found. There is only a reduction in tracheid length in the outermost narrow rings of non-vital trees. Tracheid length is reduced by about 25% as compared with tracheids in vital trees. The effect of the reduction in the relative amount of latewood, of the maximum density and of tracheid length on the wood quality, will be minimal because the changes occur only in the narrower, outermost growth rings.

In the last 5 years oaks were regularly defoliated by insect pests and, despite a great regeneration ability of oak, many trees seem unable to recover. Data on insect pests over the last 40 years were compared with ring width patterns of oaks from three stands. In one stand, in which no insects were recorded in the last few years, there was no relation between ring width patterns and long-term reports on insect pests for The Netherlands as a whole. In two other stands, where insects had been observed frequently in recent years, there was a good correlation between increment reduction and nationally recorded insect pests over the last four decades.

Both in Douglas fir and pendunculate oak no clear correlation was found between the relative amount of sapwood and tree vitality or ring width pattern.

EVOLUTIONARY TRENDS IN DROSERACEAE BASED ON THE SEED STRUCTURE

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Ovule and seed development in the four genera of Droseraceae are described. The ovule primordia are dizonate. The integuments are dermal in origin. The ovules of *Dionaea, Aldrovanda* and *Drosera* are characterized by strongly enlarged cells of the nucellar epidermis. This feature is lacking in the more primitive *Drosophyllum*. The basic seed structure with endotegmic sclerotized or pigment layer and crushed remaining outer tegmic layers is essentially the same. The seed coat structure is variable in the four genera. *Drosophyllum* has the most complex seed coat with an endotestal crystal layer, *Dionaea* and *Aldrovanda* are less complex and are exotestal and exo/endotestal respectively and *Drosera* has a simplified testa, which represents the most derived condition. Although the seed dimensions are different, the operculate seeds with starchy endosperm and small embryo have a similar construction. From the seed anatomical point of view, the four genera are correctly placed in the family Droseraceae.

TECTUM ARCHITECTURE IN POLLEN OF *SAPINDACEAE/ NEPHELIEAE*

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Pollen of the tribe *Nephelieae* of the family Sapindaceae is mostly colporate. Parasyncolporate pollen occurs in the genus *Alectryon*. Usually there are three apertures. Exine stratification is distinct. At least with electron microscopy, nexine, columellate layer, and tectum are well delimited. More than 400 samples, taken from herbarium material and covering nearly all of the about 76 species within the 12 genera of *Nephelieae*, were acetolysed and studied (SEM) to reveal the architecture of the tectum.

An inventory of the variation of tectum architecture yielded six main ornamental types: psilate, perforate, reticulate, striate, rugulate, and scabrate. Within the scabrate main type three subtypes are recognized: scabrate s.s., finely echinate, and finely striate. Striate ornamentation is common in *Nephelieae*. The reticulate main type is limited to one genus. A few genera have a wide range of ornamental variation.

In many genera intermediate forms were observed. On the basis of the occurrence of two main types and their intermediates within a species, five of the six main types could be connected with each other, rendering five morphological series: striate–rugulate, striate–psilate, striate–perforate, perforate–scabrate, and rugulate–perforate. By postulating the direction of the series, four transformation series (evolutionary trends) could be drafted: striate→rugulate, striate→perforate, rugulate→perforate, and perforate→scabrate. Uniqueness, the occurrence of derived states of other pollen characters, and great diversity are considered indicative for the derived status of characters in tectum architecture. The argument of complexity (more complex states are derived) and the argument of ontogeny (ontogeny reflects phylogeny) do not hold for the most common evolutionary trend in *Nephelieae*, namely striate→perforate. Perforate ornamentation is less complex than striate ornamentation. It is envisaged as a reversal to an ancestral type which preceded the striate type. As in the ontogeny of pollen of *Nephelieae*, perforate ornamentation preceeds striate ornamentation, the occurrence of the perforate type in mature pollen is considered as a neotenous character.

Possibly a rare (though poorly known and difficult to demonstrate) kind of self-pollination, in which staminodes of female flowers produce a small amount of viable pollen which causes fruit set, introduced the neotenous perforate ornamentation into the character set of mature pollen.

FUNCTIONAL ASPECTS OF THE STRUCTURAL DEVELOPMENT IN THE TUBER OF *RAPHANUS SATIVUS* L. VAR. SAXA NOVA

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For a growth analysis of the radish tuber, plants were cultivated on a substrate composed of clay and sand. The tuber mainly consists of the thickened hypocotyl. The longitudinal growth proved to be very irregular. A strong growth in length of the lower half of the hypocotyl is sometimes coupled with a shortening of the upper half and vice versa. The xylem consists of a parenchymatous tissue enclosing only a few slender vascular strands. This xylem parenchyma has a strong growth capacity in all directions.

Sooner or later parenchyma cells die beyond a certain rather constant distance from the vascular strands; they form a dried-out tissue. Afterwards the living vasicentric parenchyma may develop

into a somewhat wider sheath around the vascular strand oppressing adjoining cells beyond, while apotracheal parenchyma cells die and develop emboli. Further growth in the length of the tuber results in the development of large lacunae in the dead tissue. The relation between developing parenchyma tissues and functioning of the vascular strands has been studied. The transpiration of the radish plant and the absorption of an eosin solution were measured. The transpiration proved to be directly proportional to time, but absorption was strongly variable. The colouring of vascular strands in cross sections of the tuber visualizes the pathways of transpiration transport. This transport proved to be performed by a number of functioning vascular strands situated outside the area with dried-out tissue. Anatomical research showed broken vessels in dried-out tissue and parenchyma cells growing into the fractures, blocking the transport. As a result the apotracheal parenchyma cells will die. The walls of the vasicentric parenchyma cells adjacent to the vessels form very thick layers of pectin slime, sometimes penetrating into the vessels. Perhaps this slime functions as a temporary sealing of the leakages. The occurrence of dried-out tissues in the radish tuber turns out to be related to the growth in length of the tuber; after about 1 week of strong longitudinal growth dried-out tissue arises.

MEETING OF THE SECTION FOR THE RELATION BETWEEN PLANTS AND ANIMALS ON 4 JUNE 1988

DISPERSAL SPECTRA AND DIASPORE CHARACTERISTICS OF SIX PEATLAND COMMUNITIES IN THE NETHERLANDS

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Seed dispersal plays an important role in the life cycles of flowering plants. Our knowledge of seed dispersal mechanisms is strongly species-centred. Consequently, very little is known about dispersal strategies at the level of plant communities.

To investigate this subject, six plant communities representing different stages of a hydrosere were investigated in the nature reserve 'De Weerribben' in Northwest Overijssel, The Netherlands, during the spring, summer and autumn of 1986. For each plant community the composition, the life forms, and the dispersal types and diaspore weights of the represented Spermatophyta were determined. The phenology of the species was also studied.

Passing from the first to the last phase in succession, the predominant life forms change from helophytes to hemicryptophytes, and ultimately to phanerophytes. In addition, hydrochory (the most important dispersal method) is first replaced by anemochory, after which, in the last phase, hydrochory and anemochory are of equal importance. In the last two stages zoochory also becomes an important dispersal method.

On average, the zoochorous diaspores were heaviest, followed by the hydrochorous and anemochorous diaspores, respectively. Following the succession, the series shows an increase in variation in diaspore weight with a much higher representation of the higher weight classes. These findings are probably related to the increasing specialization among the diaspores and to the increased need for a nutrient reserve for the seedlings as a consequence of the more shaded conditions for their establishment. From the study on the phenology it appeared that no mature seeds were produced before April. The number of seed-bearing species was highest at the end of July.

THE DISTRIBUTION AND THE SYSTEMATIC RELEVANCE OF CADUCOUS NECTARIES AND PERSISTENT NECTARIES IN THE MAGNOLIOPHYTINA (ANGIOSPERMS)

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The morphological nature of the floral nectaries of the Magnoliophytina was determined, mainly on the basis of the localization of the nectariferous zones. For this purpose, species of a wide range of families were observed by SEM. In accordance with our research strategy, the distribution of the floral nectary types was studied by means of the two-dimensional 'Dahlgrenograms' of the Magnoliatae and the Liliatae (Smets 1986).

Within the persistent nectaries (*nectaria persistentia*) a distinction is made between the axial or receptacular nectaries (*nectaria axialia*), the gynoecial nectaries (*nectaria gynoecialia*) and the gynopleural nectaries (*nectaria gynopleurica*; previously called 'septal' nectaries) (Smets & Cresens 1988). In addition to these characters, a fourth type of persistent nectaries has recently been delineated, namely the gynostegial nectaries (*nectaria gynostegialia*), which are typical of the Asclepioideae (Asclepiadaceae). Within the caducous nectaries (*nectaria caduca*) it is possible to distinguish between two characters, the phyllodial nectaries (*nectaria phyllodialia*) and the androphyllominous nectaries (*nectaria androphyllomina*). In our opinion, neither the caducous nectaries and persistent nectaries, nor their respective characters, should be treated equally since they are associated or homologous with non-homologous floral morphomes. It should be borne in mind that trichomatous nectariferous zones are also typified on the basis of the localization of the nectar-secreting floral parts.

The systematic relevance of the different characters or nectary morphomes is shown by the distribution on the Dahlgrenograms. Indeed, the predictive value of the natural classification system may be increased by assessing the distribution of the nectary types and their character-states.

Smets, E. (1986): *Bull. Jard. Bot. Nat. Belg.* **56:** 51–76.
Smets, E. & Cresens, E. (1988): *Acta Bot. Neerl.* **37:** 121–128.

A COMPETITION FOR POLLINATORS: DIFFERENCES IN ATTRACTIVENESS BETWEEN *ECHIUM VULGARE* L. AND *ECHIUM PLANTAGINEUM* L.

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In the summer of 1987 a study was undertaken to detect competition for pollinators between two *Echium* species and, if this proved to be the case, to relate preferences of the visitors *Bombus agrorum* Fabr., *Apis mellifera* L. (both nectar-gatherers) and Syrphidae (pollen-gatherers) to differences in attractiveness. *E. vulgare* differs from *E. plantagineum* in having a few spike-like to paniculate inflorescences, whereas *E. plantagineum* has branched inflorescences. Due to its more bushy shape and 50% larger flowers, *E. plantagineum* displays more flower-mass.

The experiments were carried out in a random block of 25 plants of about the same size. Of each plant in the block the number of visitors per 3 min was counted. This procedure was repeated 6–9 times. Attractiveness was studied by comparing 16 plants of *E. plantagineum* and nine of *E. vulgare*. The preferences for the number of fresh flowers and the total flower-mass were tested by removing the withering non-rewarding flowers of 13 plants of a total of 25 plants of *E. plantagineum*.

The number of visits of *B. agrorum* significantly increased with the number of fresh flowers. Although *E. vulgare* had significantly fewer flowers than *E. plantagineum*, there was no significant difference in the number of visits per plant. Despite this, the preference of *B. agrorum* for *E. vulgare* was clear: more visits per flower and a higher flower-constancy. This can be explained by the seven times higher nectar content of *E. vulgare* flowers.

Both *A. mellifera* and Syrphidae significantly preferred a larger flower-mass, i.e. *E. plantagineum* to *E. vulgare*. The very rare visits of *A. mellifera* to *E. vulgare*, despite the higher nectar content of its flowers, can further be explained by its flower morphology. Whereas *E. plantagineum* has only two exerted stamens, *E. vulgare* has four to five, which form a barrier for the short-tongued *A. mellifera* to enter the flower but not for the long-tongued *B. agrorum*.

THRIPS AS VISITORS AND POLLINATORS OF *STELLARIA MEDIA* L.

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Like many other common weeds, *Stellaria media* L. has an efficient self-pollination mechanism. Movements of the filaments, in combination with closing movements of the flower as a whole, push the anthers against the stigma at the end of the day.

Several plant species are reported to be thrips-pollinated, especially in situations where other kinds of pollinators are unavailable. *Thrips* species are very frequently present in flowers of *S. media*. During the day, before self-pollination occurs, there seems to be ample opportunity for (cross-) pollination by visiting insects. The possible role of *Thrips* was, therefore, investigated. There was a considerable difference between the behaviour of the male and the female individuals. Unless disturbed, the females almost always stay in a flower once entered, until it closes, whereas males pay only short visits to several flowers. Male visits to flowers occupied by females were of longer duration than visits to unoccupied flowers. This suggests that the insects mate in the flowers. It was estimated that males were three times more numerous than females.

Both sexes of the investigated *Thrips* species are winged and they were seen to fly distances of up to 40 cm, enough to reach neighbouring *S. media* plants. Males and females appeared to carry small amounts of sticky *Stellaria* pollen grains. As females do not switch flowers, only the males can possibly account for some (cross-)pollination.

Although they did visit considerable numbers of flowers, the insects do not seem to contribute much to the pollination of *S. media* due to the small amount of pollen carried. However, in pioneer-situations thrips may well be the only pollen vectors.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 10 MAY 1988

PAST VEGETATION AND REINDEER ON EDGEØYA, SPITSBERGEN, BETWEEN *c.* 8000 AND *c.* 4000 YEARS BEFORE PRESENT

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Two buried peat deposits named Rosenbergdalen 1 and 2 from the valley of Rosenbergdalen on Edgeøya, Spitsbergen, were radiocarbon dated and studied for pollen, spores, and mosses.

Rosenbergdalen, 1 is 70 cm thick and is dated *c.* 7900–6700 BP. The vegetation must have been a Homalothecium nitens tundra. Three phases of this tundra can be distinguished: an initial phase and two later phases separated by a shift in local hydrology. Rosenbergdalen 2 is 2 m thick and is dated *c.* 5000–3800 BP. Three phases in the vegetation history can be distinguished: initially a very wet Calliergon vegetation along a stream and later two phases of a Homalothecium nitens tundra separated by a shift in the local hydrology. A layer of *Sphagnum squarrosum* suggests the presence of a temporary ice lens in the last phase. It is confirmed by the conclusion of Beyens & Chardez (1987), based on the study of testate amoebae that this *Sphagnum* layer represents a relatively dry local vegetation. The past vegetation in Rosenbergdalen 1 was drier, more open, and richer in vascular-plant species than that in Rosenbergdalen 2. The former presence of peat mires in Rosenbergdalen, where they are now absent, and the relatively high influx values of long-distance transported pollen (10–12 grains. cm^{-2}. $year^{-1}$) compared to those of later periods (<5 grains. cm^{-2}. $year^{-1}$; van der Knaap, 1985), suggest that the climate was warmer and that the debris slopes were more densely vegetated than today.

Sub-fossil faecal pellets of reindeer (*Rangifer tarandus* L.) discovered in Rosenbergdalen 2, and fresh reindeer faecal pellets collected by P. Oosterveld in summer in the same area, were studied for pollen and spores. The results were compared with palynological results for peat samples from the same deposits. Sub-fossil faecal pellets were encountered at 12 levels in Rosenbergdalen 2, but were absent from Rosenbergdalen 1. Reindeer therefore probably immigrated into Spitsbergen in the interval between Rosenbergdalen 1 and 2, dated *c.* 6700–5000 BP. Pollen concentrations in recent and sub-fossil faecal pellets are higher than in those in the peat samples. This points to preferential feeding by reindeer on flowers in the summer season. In Rosenbergdalen 2 the presence of several plant species is indicated by pollen that is present in sub-fossil faecal pellets but not in peat samples. Bird-manured vegetation is indicated by pollen in recent faecal pellets but not by pollen in sub-fossil faecal pellets. In contrast, plant species from unmanured tundra are better represented in sub-fossil faecal pellets. It is therefore concluded that the vegetation of the Rosenbergdalen valley changed after the period represented by Rosenbergdalen 2.

Beyens, L. & Chardez, D. (1987): *Polar Res.* **5:** 165–169.
Knaap, W.O. van der (1985): *Arctic Alp. Res.* **17:** 371–387.

JUNCUS BIGLUMIS L.: AN AUTECOLOGICAL STUDY ON EDGEØYA (SPITSBERGEN ARCHIPELAGO)

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The autecology of *Juncus biglumis* L. was studied on the north-west point of the island of Edgeøya (78°N, 22°E) in August 1987. The vegetation in the area is mainly governed by two phenomena: an overall leveling stress situation due to low temperatures, high moisture content of the soil and low availability of nutrients, leading to monotonous large-scale vegetation units and a spatially differentiating situation due to frost action in the active soil layer (including solifluction and congelifluction) leading to a wide range of microgradients in soil moisture content and availability of nutrients with many ecotones as a result.

The occurrence of *J. biglumis*, as of many other species, is apparently associated with cryogenic processes, resulting in structures such as frost boils. In such sites the plants reach an average height of 61 mm (5–150 mm) with roots of 69 mm (6–165 mm). Annual seed production is abundant.

However, only long-established plants are found. Germination requires, among others, open and well insolated soil. Thus it seems that the involved cryogenic processes, leading to frost boils, do not occur every year.

Soil types on Edgeøya are generally poor in exchangeable nutrients (median and maximal values in p.p.m., respectively): phosphate (0·8, 22·5), nitrogen (mainly ammonium; 15, 68) and calcium (151, 293). At the optimal *J. biglumis* sites on the edge of frost boils between the acid moist tundra and the alkalic bare ground, the pH ranges from 5 to 7·5. The mean soil moisture content is 160%. The soil of these sites contains, compared with the surroundings, large exchangeable amounts (median and maximal values in p.p.m., respectively) of nitrogen (25·4, 68), potassium (31, 254), and phosphate (1·1, 11·5). The sulphate content is rather high (80, 195), as is the case with calcium (201, 293) and manganese (1·5, 17·3). The phosphate content is correlated positively with nitrogen ($r = 0{\cdot}52$; $P = 0{\cdot}021$).

The ash content of the plants is 15%. The ash is, compared with other *Juncus* species, relatively rich in calcium (10 mg/g) but relatively poor in nitrogen (7·4 mg/g). In comparison with the surrounding vegetation, it contains higher amounts of phosphate (2·2 mg/g), potassium (4·2 mg/g), and magnesium (2·3 mg/g). The iron content was 4·8, manganese 0·5, and sodium 0·9 mg/g, respectively. The lichen *Leptogium sinuatum*, considered as an important nitrogen accumulator, is rather abundant at *J. biglumis* localities. The lichen has an ash content of 24%, with nitrogen (18 mg/g), phosphate (1·5 mg/g), calcium (8·2 mg/g), magnesium (1·9 mg/g), sodium (0·9 mg/g), potassium (1·4 mg/g), iron (3·9 mg/g), and manganese (0·9 mg/g).