

MEETINGS OF OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 15 DECEMBER 1982

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A reconstruction of a mound vegetation in the iron age with use of botanical macrorests

W. FERGUSON (*Internationaal Instituut voor Luchtkartering en Aardkunde (ITC), Boulevard 1945, 7500 AA Enschede*)

Utilization of vegetation data for a land evaluation for grazing in the Escremadura, Spain

A land evaluation according to the FAO procedure (BEEK) for grazing of both livestock (cattle, sheep, goats) and wild life (red deer, wild boar) is carried out in an area around the confluence of the River Tajo and Tietar, Province of Caceres, Spain.

The land evaluation became necessary because of recent changes in the agricultural system in the area. The traditional agriculture includes multiple land use for pig and sheep transhumance, cereal cultivation and charcoal production (*Quercus ilex* and *Quercus suber*, mast production, charcoal and cork). Due to swine fever and socio-economic factors the originally balanced system is changing into cattle and sheep ranching. Some ranches with red deer and wild boar are introduced. (SPIERS, DAMEN et al., FERGUSON, internal reports ITC). In the first stage a land-unit map is prepared with the help of aerial photography, according to I.T.C. procedures (Zonneveld). The land unit map includes information on geomorphology vegetation, soils, and land use. With a field survey the floristic, geological soil and land use content is given to each land unit (mapping unit).

The land unit map data are transferred into so-called land qualities. The following land qualities are considered:

- (1) Forage production of the vegetation: (a) herbs and stubble, (b) shrubs, (c) trees (browse and acorn)
- (2) Nutritive value of the vegetation
- (3) Resistance to degradation of the vegetation
- (4) Erosion hazard due to grazing
- (5) Availability of drinking water
- (6) Availability of shelter and cover
- (7) Accessibility for animals
- (8) Occurrence of disease.

Relative ratings in classes of 1 to 3/5 are given to each land quality and per animal type. e.g. Accessibility, which is determined by slope class and vegetation structure gets three classes: accessible, marginally accessible, not accessible. (Vegetation and other data are used simultaneously.) Potential and actual land utilization types (L.U.T.) for the area are defined and specified in terms of land quality ratings. Subsequently a suitability class for each LUT is given for each land unit per season. A year-round suitability depends on the limiting season and/or limiting land quality.

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The ecological amplitude of grassland species in The Netherlands

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Macro-patterns and micro-patterns in grassland vegetation and their interaction with sheep-grazing

In 1972 an eleven ha area of heathland, woodland and pasture was fenced in for a sheep-grazing experiment. The initial uniform pasture vegetation developed into various vegetation types due to the occupancy pattern of the sheep. This pattern was determined from the amount and dispersion of voided dung, and proved to be constant (BAKKER et al. 1983). The objective of the present research is to describe the annual feeding pattern in various vegetation types.

Table 1. Vegetation types, their area, consumption, production (including initial standing crop) and consumption/production ratio.

Vegetation type	Area % of total (11 ha)	Consumption ton dw.ha ⁻¹ .yr ⁻¹	Production (incl. initial standing crop) ton dw.ha ⁻¹ .yr ⁻¹	C/P
<i>Leontodon autumnalis</i>	4	1.75	3.49 (0.70)	0.50
<i>Agrostis stolonifera</i>	1			0.30
<i>Holcus lanatus</i>	37	1.33	5.06 (1.98)	0.26
<i>Molinia caerulea</i>	13			0.25
grassy heathland	10			0.10
woodland	11			0.10
<i>Agrostis tenuis</i>	12	0.35	5.90 (2.95)	0.06
<i>Erica tetralix</i>	2			0.05
<i>Juncus effusus</i>	9			0.05
<i>Carex nigra</i>	1			0.05

The three studied vegetation types were *Leontodon autumnalis*/*Trifolium repens*/*Cynosurus cristatus*, *Holcus lanatus*/*Anthoxanthum odoratum*/*Agrostis tenuis* and *Agrostis tenuis*. Production (including initial standing crop) and consumption were measured from June 1981 to July 1982. The results are revealed in table 1. Taking into account the needs of the sheep and the available production of these three vegetation types, two periods of forage-shortage in the pasture can be discerned. Firstly, in July the sheep feed substantially in the *Molinia caerulea* vegetation. Secondly, in the autumn little feeding was observed mainly in the heathland vegetation. The measured production/consumption ratio in the above vegetation types and the estimated ratios in the other vegetation types are listed in table 1. It is striking that just a small area is intensively defoliated and large areas are influenced moderately or hardly at all.

In the pasture a micro-pattern developed consisting of an interlocking network of very short, apparently regularly and heavily grazed areas of the *Leontodon*-type, and remaining patches with a higher canopy of the *Holcus*-type under apparently far less intensive grazing. The spots differ in abundance of species, accumulated litter and amount of crude protein, probably influencing the feeding pattern of the sheep. This micro-pattern was constant in successive years ($P < 0.005$), and therefore could enhance differentiation of the vegetation due to various conditions for establishment of new species.

BAKKER, J. P., S. DE BIE, J. H. DALLINGA, P. TJADEN & Y. DE VRIES (1983): Sheep-grazing as a management tool for heathland conservation and regeneration in The Netherlands. *J. Appl. Ecol.* 20: 541–560.

A. ENGELN, also on behalf of F. BONGERS (*Geobotanie, Botanisch Laboratorium, Katholieke Universiteit, Toernooiveld, 6525 ED Nijmegen*)

Vegetation structure and ecology of the Caatinga (Amazonia) in southern Venezuela

Will be published in:

BONGERS, F. J. J. M., A. M. J. G. ENGELN & H. KLINGE. Phytomass structure of natural plant communities on podosols in southern Venezuela. 2. Bana woodland. *Vegetatio*.

L. M. FLIERVOET (*Geobotanie, Botanisch Laboratorium, Katholieke Universiteit, Toernooiveld, 6525 ED Nijmegen*)

Canopy structure of dry grasslands

Will be published in:

FLIERVOET, L. M. & J. P. M. VAN DE VEN (1984): Leaf characteristics of grassland in a microgradient of temperature and moisture conditions. *Phytocoenologia* 12.

MEETING OF THE SECTION OF VEGETATION RESEARCH ON 3 MARCH 1983

W. EVERS and H. GORISSEN (*Geobotanie, Botanisch Laboratorium, Katholieke Universiteit, Toernooiveld, 6525 ED Nijmegen*)

The development of the vegetation of the "Bunderbos c.a." during the past twenty years

The forest reserve "Bunderbos c.a." is situated in the South of Limburg (The Netherlands) on the west-exposed steep of the Maas valley.

This State-owned wood complex represents, by Netherlands standards, a unique combination of various vegetation types, which have been mapped in 1982.

The lecture deals with some aspects of the vegetation development at this place during the past 20 years derived from a comparison of the newly synthesized vegetation map with three former maps made by Dr. W. H. Diemont and some of his collaborators. Both the past and the present phyto-sociological investigations, which are considered, are based on the Braun-Blanquet approach, but nevertheless the vegetation as a whole is viewed in an essentially different manner. Diemont c.s. examined the vegetation on those very places, where the characteristic species composition was fully complete. Moreover the former maps are based on only few (about 55) of such relevés. The legend of the vegetation map prepared in 1982 consists of 22 local types abstracted from a vegetation table containing 180 relevés covering the complete area. The entities distinguished are classified within the *Fago-Quercetum*, the *Stellario-Carpinetum*, the *Pruno-Fraxinetum* and the *Carici remotae-Fraxinetum* with the syntaxonomic system elaborated for The Netherlands by WESTHOFF & DEN HELD (1969).

The main differences between the former and the recent vegetation map, with regard to a subregion of the "Bunderbos c.a." namely "Den Hooghen Bosch", were as far as possible interpreted as varying views of the individual investigators and further as real changes in vegetation.

Where the precise locality of the former vegetation samples was known three trends of development have been observed: (a) a steady state during the last decennia; (b) succession from a phytocen-

ose of the *Carici remotae-Fraxinetum* to one of the *Pruno-Fraxinetum*. It is supposed that the latter can be a consequence of the increasing density of the tree layer, since the earlier forest exploitation as "Mittelwald" has ceased. This change has lead to a shift in the spectrum of species implying that species which require a high light intensity are subdued by species characteristic of more shaded woods.

Another aspect of vegetation change was an obvious process of development in the course of which *Rubus fruticosus coll.* has gained complete dominance. Several causes for this were suggested, e.g. the erosion of soil on the upper part of the slope, radiating from the adjacent table-land. Another cause might be the increasing acidity of rain.

DIEMONT, W. H. (1953): Plan van instandhouding en van verbetering van het Staatsnatuureservaat Bunderbos. Opname 1953. *Intern rapport S.B.B. Consulentschap voor Limburg*.

WESTHOFF, V. & A. J. DEN HELD (1969): *Plantengemeenschappen in Nederland*. Thieme, Zutphen.

M. G. C. SCHOUTEN (*Geobotanie, Botanisch Laboratorium, Katholieke Universiteit, Toernooiveld, 6525 ED Nijmegen*)

Drainage or wild life management: the fight for the last Irish bogs

R. C. LOOYEN (*Vakgroep Plantenecologie, Biologisch Centrum, Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn)*)

Grazing pressure and vegetation dynamics at the Oosterkwelder salt-marsh on Schiermonnikoog

The salt-marsh on Schiermonnikoog, one of the Dutch Frisian Islands, had been freely grazed by young cattle up to 1958 when it was all but abandoned. In 1972 grazing was reintroduced from the end of May until October in a fenced area with a stocking rate of 1.6 animals.ha⁻¹. The objective of the present study is to relate vegetation dynamics and grazing pressure.

Vegetation dynamics were quantified by comparing sequential vegetation maps in 1971, 1976 and 1981 (BAKKER & RUYTER 1981). Trampling and feeding are considered to influence the vegetation. Manuring probably is of minor importance on the regularly inundated salt-marsh. Trampling was measured as the number and dispersion of dung patches, being a good indication of time spent in various vegetation types (ALLERSMA 1977). Feeding was measured as the relation between the grazing capacity (potential cattle number.ha⁻¹, based upon the seasonal production of various vegetation types) and the grazing pressure (actual cattle number.ha⁻¹, based upon the seasonal consumption in various vegetation types).

Table 1. Vegetation types, their area in 1981, changes in area from 1971–1976 and from 1976–1981, grazing capacity, grazing pressure, and presence of young cattle for the period May–October at the salt marsh of Schiermonnikoog.

Vegetation type (dominant species)	Area in 1981 (ha)	Increase in area (ha)		Grazing capacity (cattle.ha ⁻¹)	Grazing pressure (cattle.ha ⁻¹)	Presence (dung patches. ha ⁻¹ .day ⁻¹)
		1971–76	1976–81			
<i>Puccinellia maritima</i>	0.67	0.13	–0.38	3.8	5.4	23
<i>Juncus gerardii</i>	2.71	0.14	1.67	2.2	2.1	19
<i>Artemisia maritima</i>	1.17	–1.02	–0.84	–	–	17
<i>Elytrigia pungens</i>	1.04	–0.92	–1.08	–	–	13
<i>Juncus maritimus</i>	5.86	0.76	0.72	2.9	2.8	17
<i>Festuca rubra</i>	5.54	1.04	0.04	2.7	1.9	18
<i>Armeria maritima</i>	3.60	3.64	–0.04	3.7	3.0	12
<i>Ammophila arenaria</i>	2.94	–2.09	–0.24	3.5	5.2	10

A positive correlation ($p < 0.05$) was found between grazing capacity and grazing pressure, indicating a selection for the amount of forage produced. No correlation was found between grazing pressure and crude protein content, and in vitro digestibility of various vegetation types.

It appears from the results in *table 1* and field observations that the hardly fed *Artemisia maritima* type decreases due to trampling. The decrease of the *Elytrigia pungens* and *Ammophila arenaria* types is due to heavy feeding, whereas the decrease of the *Puccinellia maritima* type might be attributed both to trampling and feeding. These results suggest local overgrazing leading to a decrease of species diversity and community diversity.

ALLERSMA, G. (1977): Begrazing als beheer, aantal en type koeieplakken als indikator van beweidingdruk op kweldervegetaties. *Contactblad voor Oecologen* 13: 44–51.

BAKKER, J. P. & J. C. RUYTER (1981): Effects of five years of grazing on a salt-marsh vegetation. *Vegetatio* 44: 81–100.

J. WIEGERS (*Hugo de Vries Laboratorium, Sarphatistraat 221, 1018 BX Amsterdam*)

Regeneration of the vegetation in a part of the Fochtelooverveen (Fr.) following a fire.

In the first week of May 1980 a fire struck the westernmost part of the nature reserve Fochtelooverveen. On the burnt-over area two permanent plots were marked 90 metres apart to study regrowth of the vegetation. The plots differed in depth of the peat-layer, mean ground water level, and the extent to which the fire penetrated in the litter layer. The plots measured 5×50 m and were transversally dissected by seven shallow parallel trenches.

In June 1982 the vegetation in the plots was mapped on a scale of 1:100. In both plots the vegetation between the trenches was dominated by *Molinia caerulea*. In plot A, the wetter one, the trenches had a vegetation of mainly *Sphagnum cuspidatum* and *Eriophorum angustifolium*. In this plot *Calluna vulgaris* and *Erica tetralix* were rare. In a few places *Eriophorum vaginatum* was present. In plot B, the drier one, *Sphagnum cuspidatum* was rather rare. Young *Calluna vulgaris* and *Erica tetralix* were quite common, even forming close herds in some places.

In both plots three samples from the top-layer of the soil were taken, 2 cm deep and 100 cm^2 in surface. The *Calluna* seeds present in a subsample of each were counted under a dissecting microscope. In plot B a mean of 13.5 seeds per dm^2 was calculated, in plot A a mean number of 1747 seeds per dm^2 .

The conclusion drawn from these observations is that the intensity of the fire at soil level, which differed in both plots, favoured the germination of *Calluna* in plot B to such an extent that its seed bank became almost completely depleted, whereas in plot A the promoting effects of the fire were not enough to overcome the inhibition of its germination, imposed by high ground water levels and low pH.

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The development of Potamogeton vegetation in the Lauwersmeer

The Lauwersmeer is that part of the Lauwerszeepolder which became a lake after the embankment of the Lauwerszee in 1969. The lake itself contained fresh water within $3\frac{1}{2}$ months, but the terrestrial parts of the polder desalinated very slowly; there is still a continuous movement of saline ground water into the lake, running through the littoral. The first reports of submerged macrophytes in the lake date from 1972 and the vegetation increased quickly since then. For both scientific and nature management reasons a survey of the vegetation was made in 1980. A vegetation map was drawn using both a series of aerial false colour photographs and data from an intensive field exploration of the littorals of the lake. Four species were found: *Potamogeton pectinatus* L., *Zannichellia pedunculata* Rchb., *P. crispus* L. and *P. perfoliatus* L., in order of declining abundance. The *P. pectinatus* vegetations were distinguished into five types, *dense*, *disturbed*, *diffuse*, *clones*, *single clone*. Maximum depth on which plants were found growing was 95 cm (*P. pectinatus*); all species had

their leaves in the upper 25 cm layer of water. Due to turbidity of the water, more than 99% of daylight was absorbed in this layer. Local differences in ground water movement and its chloride content also played an important role in the distribution of the vegetation.

Colonisation history was reconstructed mainly through interpretation of the *P. pectinatus* vegetation types. Under certain conditions settling of pioneer plants through germination of seeds succeeded, yet closed vegetations mainly resulted from vegetative extension of the once settled plants. In large water bodies waves cut gaps in the parts of the vegetation growing deepest; in the shallower parts coots (*Fulica atra*) grazed away up to 90% of the biomass of the plants locally. *P. pectinatus* proved to be stronger than *Z. pedunculata*, it normally overgrew the latter. Yet, *Z. pedunculata* recolonized the grazed sites. In autumn Bewick's swans (*Cygnus bewickii*) graze on the tubers of *P. pectinatus*, but only in the densest stands; this caused no perceptible damage to the vegetation.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 18 JANUARY 1984.

H. W. J. VAN DIJK (*Afdeling Milieubiologie, Rijksuniversiteit Leiden, Kaiserstraat 63, 2311 GP Leiden*)

Effects of artificial infiltration of eutrophic water on herbaceous bank vegetations in Dutch coastal dunes

Many coastal dune areas in Holland are infiltrated for public water supply purposes. The effects on the herbaceous vegetations of the banks of infiltration ponds and less influenced seepage pools were studied.

In the case of dune infiltration the quantity of the infiltrated water exceeds the amount of effective precipitation with a factor 10 to 20. In consequence the movement of the ground water is increased considerably. The infiltration water contains unnaturally large amounts of macronutrients. The study was focused on the nutrients orthophosphate, nitrate and potassium in the soil penetrating water.

The infiltration activities appear to increase the occurrence of nitrophilous tall hemicryptophytes in bank vegetations. It is plausible that this is the main cause for the absence of the originally diverse and rare vegetations of phreatophytes in infiltrated dune areas, even at distances over 500 m away from infiltration ponds. At these distance the influence of infiltration activities on the phreatic ground water and the seepage water appeared to be clear simultaneously: the concentrations of potassium and orthophosphate in the upper ground water and in seepage water may be unnaturally high, especially with high ground water movement.

The effects on the vegetation were expressed as the added covers of several ecological species groups. The cover of some of these groups gave high correlations with the ground water movement and with the concentrations of orthophosphate and potassium in the bank infiltrating water. These correlations were positive for the nitrophilous tall hemicryptophytes and negative for the rare and original species. However, a combination of the ground water and these concentrations gave a better explanation for the cover of the ecological species groups than the separate parameters. The combination with the most plausible causal background is the product of the ground water and the orthophosphate concentration in the water. This nutrient supply is called the external phosphate load. This observation only seems to be valid when data of infiltrated dune areas are comprised in the processing of the data set. If the data of non-infiltrated dune areas are processed separately, the variance of the added covers of the ecological groups of nitrophilous tall hemicryptophytes can be explained mainly by the mineralization of the humus in the upper soil, the so-called internal nutrient supply. Especially the internal supplies of phosphate and nitrogen appear to be important in the non-infiltrated dune areas.

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 Brookland forest in north-western Overijssel (The Netherlands)

M. J. WASSEN, R. VAN DIGGELEN, A. P. GROOTJANS and W. WIERSINGA (*Vakgroep Plantenecologie, Biologisch Centrum, Rijksuniversiteit Groningen, Kerklaan 30, 9751 NN Haren (Gn)*)

Changes in trophic gradients in fen meadows, resulting from interferences with the hydrology

In river valleys the occurrence of fen meadow communities is determined by substrate characteristics, ground water levels and residence time of specified ground water types. A change in the species composition following an interference with the hydrology, however, is generally ascribed to changes in ground water levels only. Many sensitivity studies do point to substrate alteration, but without taking into consideration changes in water quality. A slight lowering of the ground water table, however, may eventually lead to large changes in the ionic composition of ground water, especially in enriched soils (BOTH & VAN WIRDUM 1981; GROOTJANS 1980, KEMMERS & JANSEN 1980).

In the State-owned nature reserve 'Mensinghe' in the North-Netherlands the relations were studied between distribution of fen meadow plants, ground water levels and (macro-) ionic composition of ground water (VAN DIGGELEN et al. 1983). Some meadows were slightly affected by adjacent drainage. This was well expressed in the vegetation. Yet most of the reserve still harboured well developed wet meadow communities (*Calthion palustris*, *Caricion curto-nigrae* and *Juncion acutiflori*).

The vegetation was studied in a large grid consisting of squares of 10 × 10 m. Concentrations of macro ions and water levels were measured during the vegetation period in gauges placed at four depths (40, 120, 180 and 260 cm below surface). Electric conductivity measurements were made in the 10–180 cm soil layer.

We found that in the study area the distribution of some 30 fen meadow species were positively correlated with the distribution of water types (mainly typified by Ca^{2+} -, HCO_3^- -concentrations and electric conductivity ($\text{E.C.}_{25^\circ\text{C}}$). Mean highest-, mean lowest ground water levels and soil type did not explain the assessed plant distribution patterns.

Viola palustris and *Hydrocotyle vulgaris* for instance, were restricted to areas influenced by mineral poor infiltration water from lateral subsurface flows. *Caltha palustris*, *Polygonum bistorta* and *Carex acutiformis* were restricted to areas influenced by mineral rich base flow water. The analysis of ground water levels and ionic concentration showed that in the area affected by drainage, the base rich ground water had been replaced by infiltrating rainwater. This effect was most pronounced in autumn.

The distribution pattern of several fen meadow plants appeared to be closely related to these changes in ground water types in the topsoil, while frequently changes in water levels were hardly detectable (see also GROOTJANS & TEN KLOOSTER 1980).

This case study shows that any sensitivity study on changes in ground water regime is incomplete when quality aspects of ground water have been neglected.

BOTH, J. C. & G. VAN WIRDUM (1981): *Waterhuishouding, bodem en vegetatie van enkele gelderse natuurgebieden*. R.I.N. rapport 81/18 (uitg. Prov. Gelderland).

DIGGELEN, R. VAN, M. J. WASSEN & W. WIERSINGA (1983): *Effecten van ingrepen in de hydrologie op natte hooiland vegetaties in beekdalen*. Laaglandbeken projekt, rapport no. 2. SBB/RUG – Dept. of Plant Ecology. (mimeo).

GROOTJANS, A. P. (1980): Distribution of plant communities along rivulets in relation to hydrology and management. In: *Epharmonie*, ed. O. WILMANN & R. TÜXEN. Berichte über die internationalen Symposien der I.I.V. 1979, pp. 143–170.

GROOTJANS, A. P. & W. PH. TEN KLOOSTER (1980): Changes of ground water regime in wet meadows. *Acta Bot. Neerl.* 29: 541–554.

KEMMERS, R. H. & P. C. JANSEN (1980): *De invloed van chemische factoren in grondwater en bodem op enkele vegetatietypen in het C.R.M.-reservaat "Groot Zandbrink"*. I.C.W.-nota 1181, Wageningen.

P. RUSSCHEN and M. DIJKSTRA (*Vakgroep Plantenecologie, Biologisch Centrum, Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn)*)

Grazing, soil salinity, light interception and vegetation dynamics at the Oosterkwelder salt-marsh on Schiermonnikoog

The salt-marsh on Schiermonnikoog, one of the Dutch Frisian Islands, had been freely grazed by young cattle up to 1958 when it was all but abandoned with subsequent litter accumulation. In 1972 grazing was reintroduced in a fenced area with a stocking rate of 1.6 animals.ha⁻¹. Grazing caused not only disappearance of litter but also the transition of mid- and upper salt-marsh communities into lower salt-marsh communities. The objective of the present study is to relate the observed changes to soil salinity and light as a parameter depending on the canopy.

The top-soil salinity (0–5 cm) did not differ between grazed and abandoned salt-marsh areas. Only the uppermost top-soil cm revealed a higher salinity in the grazed salt-marsh, particularly during the dry summer period. Probably the measured soil compaction in the grazed salt-marsh played a role in the evaporation. Moreover, the grazed area showed the most bare soil and the greatest exposure to light.

Greenhouse experiments revealed that lower salt-marsh species produced less dry matter under 50% light interception than upper salt-marsh species. Lower salt-marsh species produced more dry matter above 10 g NaCl.l⁻¹ nutrient solution than upper salt-marsh species.

As a result of the extremely wet spring soil salinities were very low during the germination period. Hence both upper- and lower salt-marsh species germinated under field sowing experiments at the upper- and the lower grazed salt-marsh depending on the soil moisture content. At the sites where little light reached the soil at the ungrazed salt-marsh germination was strongly inhibited, and seedling survival was small. Seedling survival of upper- and lower salt-marsh species was better at the grazed salt-marsh. High summer soil salinities decreased the number of surviving seedlings of upper salt-marsh species.

Thus spreading of lower salt-marsh species probably depend on their higher salt tolerance and on their lower vitality under decreased light intensities as compared to upper salt-marsh species.

BAKKER, J. P. & J. C. RUYTER (1981): Effects of five years of grazing on a salt-marsh vegetation. *Vegetatio* 44: 81–100.

BAKKER, J. P. (1984): The impact of grazing on communities, plant populations and soil conditions on salt marshes. *Vegetatio* (in press).

E. ARNOLDS (*Biologisch Station van de Landbouwhogeschool, Kampsweg 27, 9418 PD Wijster*)
Macrofungi in grasslands

Sixty-four plots of 100–800 m² in grasslands and some heathlands in Drenthe (N.E. Netherlands) were studied with mycocoenological methods in the period 1974–1980 (ARNOLDS 1981, 1982). The plots belong to nine phytocoenological alliances: *Spergulo-Corynephorion*, *Thero-Airion*, *Violion caninae*, *Nardo-Juncion squarrosi*, *Ericion tetralicis*, *Junco-Molinion*, *Calthion palustris*, *Arrhenatherion elatioris* and *Lolio-Potentillion*.

Fundamental problems in mycocoenology are that mycelia can hardly be traced in the soils and, if they are found, they cannot be identified. Microbiological culture techniques are not appropriate to analyse the flora of macrofungi. Therefore the study of mycocoenoses must necessarily be based on the presence of carpophores. Since a distinct periodicity and considerable annual fluctuations occur it is necessary to analyse permanent plots during several years and several times a year.

The results of the present study demonstrate that no general correlation exists between the species numbers of macrofungi and phanerogams per plot. Some vegetation types are poor in both phanerogams and macrofungi (e.g. the *Spergulo-Corynephorion*), others are rich in the two groups (e.g. the *Thero-Airion*) or rich in phanerogams but poor in fungi (e.g. the *Calthion*). Within some alliances no correlation exists at all, e.g. the *Arrhenatherion*.

The average productivity of carpophores (aPC) may be regarded as an expression of the ecological importance of the macrofungi in different environments. No correlations could be found between aPC and the coverage by grasses or mosses, although such correlations have been suggested by

other authors. In my plots a negative correlation was found between aPC and the height of the sward in autumn. This agrees with the results in some experimental plots in Poland (SADOWSKA 1973).

The aPC is low on soils with both a high organic matter content ($> 15\%$, peaty sand to peat) and an extremely low content ($< 1.5\%$), whereas a high aPC is only realized on soils with a rather low humus content ($1.5\text{--}8\%$). In addition the C/N ratio of the organic soil fraction is important: when it is higher than 25 or lower than 15 the aPC is relatively low. In soils with a high C/N ratio decomposition is slow and incomplete, resulting in accumulation of litter or peat formation. In soils with a C/N ratio below 15 the macrofungi are largely superseded by lower fungi and bacteria.

Within mycocoenoses a strong differentiation exists in microhabitat and substrate of the various species. In addition different ways exist for the exploitation of carbon sources, the main strategies being parasitic, symbiotic and saprophytic. On the basis of these criteria a system of ecological groups was proposed for macrofungi in grasslands (ARNOLDS 1981). Besides it is important to make a distinction between fungi living on carbon sources produced inside the community itself ("proper" fungi) and fungi living on carbon sources from outside the community ("alien" fungi), e.g. fungi on blown-in leaves, on excrements of passing animals and mycorrhizal fungi associated with trees outside the plots.

In all plots analyses were carried out of some soil-chemical characteristics, including pH water, total exchangeable bases, extractable phosphate, organic matter content and C/N ratio. On this basis rough conclusions could be drawn on the ranges and optima of grassland fungi concerning these factors. Like phanerogams the macrofungi can be used as indicator organisms.

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H. M. VAN DE STEEG (*Botanisch Laboratorium, Katholieke Universiteit, Toernooiveld, 6525 ED Nijmegen*)

Effects of summer inundation on flora and vegetation of river foreland in the Rhine area

Most of the river foreland along the Rhine tributaries in The Netherlands, used for haymaking and grazing, is protected by small dykes against flooding in summer. This protection in the growing season enables the establishment of flood sensitive plant species and vegetation types. Due to an increased supply of rainwater to the river in recent years, high discharge peaks exceeding the small dykes protecting the grassland in summer occur also in the growing season: the first time in May 1970, and even more frequent in May–June 1978, July 1980 and in May–June 1983. These summer inundations caused extensive changes in flora and vegetation of those formerly protected areas.

The floating species *Stratiotes aloides* and *Hydrocharis morsus-ranae*, which occurred in some former river beds in protected river foreland, have been lifted and transported down-stream. The deeper parts of former river beds are dominated by nymphaeids. Of these *Nuphar lutea* and *Nymphaea alba* could maintain themselves at all locations in the Nijmegen area. On the contrary *Nymphaeoides peltata* disappeared from some of its localities, probably due to exhausting of its reserves in attempting to reach the higher water table again. In the riparian belt flood sensitive species have been eliminated. Formerly well distributed marshland species along former river beds in protected river forelands like *Typha angustifolia*, *Sparganium erectum*, *Equisetum fluviatile* and *Ranunculus lingua* have disappeared or almost so. Other species like *Glyceria maxima* and *Phragmites australis* could more or less maintain themselves, and only *Carex acuta* shows substantial increase. Large strips without vegetation between the nymphaeid belt and the higher riparian belt mark former *Typha angustifolia* populations.

Extensive changes also occurred in the species composition of the grassland in the formerly protected river foreland. Most of the species of the alliance *Arrhenatherion*, which are mostly extremely

sensitive to inundation, have been eliminated. Flood tolerant species of the alliance *Lolio-Potentillion* increased, mostly by vegetative spreading. Now only the flood tolerant species *Alopecurus pratensis* indicates the former extension of *Arrhenatherion* grassland types. The extensive changes in flora and vegetation in the river foreland, formerly not inundated in summer through protection by small dykes, show that the recent summer inundations are very exceptional, and do not belong to the normal hydrological pattern of these river forelands.

MEETING OF THE SECTION FOR THE RELATION BETWEEN PLANTS AND ANIMALS ON 12 NOVEMBER 1983

MARIE-JOSÉE P. J. JENNISKENS (*Hugo de Vries-Laboratorium, Universiteit van Amsterdam
Plantage Middenlaan 2a, 1018 DD Amsterdam*)

Aspects of some reproduction mechanisms in *Taraxacum*

The reproduction of *Taraxacum* has drawn interest from the beginning of this century, when it was shown that (polyploid) dandelions may produce viable achenes without previous pollination.

Although diploids of more or less primitive sections were known from Asia and southern Europe, only recently appreciable numbers of diploid sexual plants of more advanced sections have been recorded from large areas of Europe. It could be demonstrated in the experimental garden that self-incompatible diploids, some hailing from the same and some from geographically separated populations, with approximately the same or with a different morphology, can hybridize, and yield a fully vital and fertile offspring.

Self-compatibility was found in a single diploid plant belonging to the section *Taraxacum* collected in north-western France. Self-pollination in the field had only been known to occur in the sections *Serotina* and *Leptocephala*. Also between diploid plants and triploid pollen donors gene flow resulting in a vital and fertile offspring, mainly with diploid chromosome numbers ($2n = 2x = 16$) appeared to be possible; some filial plants proved to have $2n = 2x + 1 = 17$ chromosomes. The different modes of reproduction of diploid, sexual and triploid agamosperous dandelions may, at least in the experimental garden, have led to differences in some of their flowering characteristics. Flowering and fruiting of a capitulum extends much longer in diploids as compared to triploid plants.

The latter produce fewer pollen grains per floret with a higher percentage sterility and less *Pollen-kitt* in comparison with diploids.

Hybridization between diploids and introgression between diploid and triploid plants, in conjunction with differences in some characteristics of flowering as observed in the experimental garden, may be important for the maintenance of the different ploidy levels in nature.

M. BOS, A. HUIZENGA, B. M. MOELIONO and P. RATERING (*Vakgroep Plantensystematiek, Biologisch Centrum, Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn)*)

An ecological interpretation of the colour change of flowers of *Echium plantagineum* L.

Colour change of the corolla during anthesis occurs in several plant families. It has often been assumed that mutual adaptation of plant and pollinators forms the basis of this phenomenon. In this connection an investigation has been undertaken into the herb *Echium plantagineum* L. The insects *Apis mellifera* and *Bombus agrorum* (Apidae) have been observed as visitors. They exclusively visit the last of the two open flowers.

A morphological analysis of the development of the flower showed that six developmental stages of the flower can be determined. On the basis of these results, the different features of visited and non-visited flowers have been demonstrated, or, to put it differently, the differences between rewarding and non-rewarding flowers. Therefore an analysis was made of pollen production and nectar secretion. A summary of the relevant results of these analyses is given here:

Stage 1–2: U.V. light reflecting spots were detected on the tips of the corolla slips; pollen issue had already started in the buds (stage 1); no nectar was found in either the buds or the almost open flowers (stage 2).

Stage 3–5: Nectar guides were revealed under U.V. light in stages 3 and 4; pollen is available in large quantities in stage 3; most nectar was measured in stages 3 and 4; receptive stigmas were found to be present in stage 4 and 5.

Stage 6: The flowers with their deep blue shrivelled corolla are assigned to stage 6

The last open flowers, which concur with stages 3 and 4, are the most rewarding ones; in contrast to these flowers, the other open flowers which concur with stage 5, are not or little rewarding. For these reasons it is obvious that all pollinators exclusively visited the last open flowers.

In *Echium plantagineum* L., the colour change of the flower serves to signal the location of rewarding and non-rewarding flowers. Plants possessing these positive signals allow pollinators to forage exclusively on flowers containing potential rewards. This results in a high resource/harvesting rate. If visits of pollinators are restricted to viable and receptive flowers, the result will be a pollination efficiency and an increase in fitness in terms of a higher seed set rate. Colour change during anthesis of flowers of *Echium plantagineum* L. can be interpreted as a mutually adaptive feature of ecological significance.

MANJA M. KWAK (*Laboratorium voor Plantenoecologie, Biologisch Centrum, Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn)*)

Bumblebees and flower characteristics

Bumblebees are the main pollinators of the annual, nectar and pollen providing plant species *Rhinanthus minor* L. and *R. serotinus* (Schönh.) Oborny (Scrophulariaceae). These two species occur sympatrically and their flowering times overlap. In the field bumblebees discriminate between them (KWAK 1978). Bumblebees appeared to visit one or two species. If visiting both species, they preferred *R. serotinus*.

Some differences in flower characteristics were tested for their role in the discrimination. In an artificial situation bumblebees were able to discriminate between the scent of *R. minor* and *R. serotinus* corollas.

Nectar characteristics on a cool and a hot day (amount, concentration and sugar ratio) all showed an expected preference for *R. serotinus*. Both species showed a similar trend in nectar secretion during the day with a peak volume at 18.00 hours. The concentration of nectar varied greatly during the day. Although there is a large overlap in values, samples from *R. serotinus* taken at any one time showed higher concentrations than *R. minor* (mean value in *R. minor* is 20% and in *R. serotinus* 30%).

Thin-layer chromatography revealed the three most common nectar sugars in both *Rhinanthus* species: fructose, glucose, and sucrose. In *R. minor* also rhamnose and lactose, and sometimes some unknown sugars were detected. The ratio between the sugars differed: *R. minor* F:G:S = 1:0.4:0.3, and *R. serotinus* F:G:S = 1:0.2:0.9. POUVREAU (1974) investigated the uptake of sugar solutions by bumblebees. His results can be applied to our preference question of *R. minor*-*R. serotinus* nectar. On the basis of three preferences observed by Pouvreau we can conclude that indeed the composition and concentration of *R. serotinus* nectar in addition to the amount will be preferred. Analysis of the plant composition of mixed populations showed a group of pure *R. minor* plants and rather variable *R. serotinus* plants, and introgressive hybridization towards *R. serotinus*.

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N. B. M. BRANTJES (*1e Barendrechtseweg 37, 2991 XE Barendrecht*)

Unidirectional reproductive isolation in *Gymnadenia* spp. (Orchidaceae)

In Val Ferrera, Graubünden, Switzerland, between 1500 and 1700 m alt., measurements of the spur length and other floral characteristics demonstrated the presence of hybrids in several mixed populations of *G. odoratissima* and *G. conopsea*. A combination of three incomplete mechanisms resulted in reproductive isolation, viz., (1) Differences in the visitor spectrum reduced the probability of hybridization. According to literature records and own observations, medium-sized Lepidoptera, such as Noctuidae and a few Zygaenidae, pollinated *G. conopsea*. *G. odoratissima* was visited and pollinated mainly by Zygaenidae and many small-sized Lepidoptera, such as Geometridae, Pterophoridae, Pyralinidae (Crambinae) and by *Rhamphomyia heterochroma* (Diptera, Empididae). (2) The peak time of activity of the visitors differed: in *G. conopsea* the pollinating activity is largest during the evening, whereas *G. odoratissima* received abundant visitors in the early morning and in the late afternoon. The production of fragrances correlated with the visiting activity: nocturnal odour production in *G. conopsea* and a diurnal sweet scent in *G. odoratissima*. (3) The differences in spur length resulted in unidirectional isolation. The spur of *G. odoratissima* (4–5 mm) causes a deposition of the pollinaria on the proboscis at a distance of 5 mm from its tip. In case a visitor carrying such pollinaria in that place might insert the proboscis in the 13–18 mm long spur of a specimen of *G. conopsea*, a pollinarium would become pressed onto the stigma, and effect hybridization. However, pollinaria of *G. conopsea* were found to adhere at a distance of about 15 mm from the tip of the proboscis. If such a visitor would also drink from the short spur of *G. odoratissima*, the pollinaria would remain far away from the stigma. This unidirectional mechanical isolation indicates that the observed hybrids must originate from the cross: *G. conopsea* × *G. odoratissima* and not from the reciprocal one.

Disruptive character displacement resulted in differences in spur length and in the timing of visitor attraction. Both factors might secondarily effect the differences in the visitor spectrum. In *G. odoratissima*, the mechanical barrier protects the specific integrity. The protection is of adaptive advantage because *G. odoratissima* has only a limited distribution and occurs sympatrically with *G. conopsea*. On the other hand, *G. conopsea* has a much wider geographic distribution, and consequently will be without a selective pressure towards isolation mechanisms in most of its range. The existence in locations outside the area of *G. odoratissima* of several records of diurnal visits to *G. conopsea* seems of interest.

The combination of both small-sized Lepidoptera and long-tongued Diptera as substantial pollinators is also found in *Habenaria obtusata* (Orchidaceae) and in *Silene otites* (Caryophyllaceae). Blood-sucking mosquitos (Culicidae) and small, nocturnal moths pollinate both these plant species.

A. D. J. MEEUSE (*Hugo de Vries-laboratorium, Plantage Middenlaan 2a, 1018 DD Amsterdam*)

The significance of insect pollination for the reproduction of *Plantago media*

For full account see:

MEEUSE, A. D. J. (1984): Rate of dependence of *Plantago media* L. on entomophilous reproduction – A preliminary note. *Acta Bot. Neerl.* 33: 129–130.

J. J. PETTINGA (*Consulentschap in Algemene Dienst voor Bijenteelt, Tilburgseweg 32, 5081 NG Hilvarenbeek*)

Guided foraging flights of bees – yes or no?

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON 11 NOVEMBER 1983

R. DUMAS DE VAULX (*Station d'Amélioration des Plantes Maraîchères, INRA, B.P. 94, 84140 Montfavet, France*)

In vitro culture and embryogenesis

Embryogenesis shows a *bipolar* morphogenetic pattern of development with simultaneous differentiation of root and shoot poles following a series of developmental sequences. Embryogenesis is not only zygotic; embryos can be obtained *in vitro* from somatic cells (somatic embryogenesis) or from unfertilized ovules or microspores (gametic embryogenesis).

Somatic embryogenesis can proceed directly from plant tissue or after callus formation. In gametic embryogenesis embryos can be formed directly leading to plants; sometimes abnormal embryogenesis can be observed: proembryos are formed giving plants after regeneration.

Genotype and environmental conditions of the donor plant and the source of the explant greatly influence the embryonic response. Embryogenesis can be induced or stimulated by culture medium constituents (growth regulators) but for gametic embryogenesis also by physical or chemical treatments of anthers and ovules: low (c. 4°C) or high (35–40°C) temperatures, irradiation, centrifugation, hypertonic solutions, &c.

Somatic embryogenesis is generally achieved by induction on an auxin containing medium followed by transfer to an auxin-free medium. The effect of growth regulators is less clear for gametic embryogenesis, but for some plant species 2,4-D is necessary for induction of the first divisions.

The type of sugar and its concentration can also be critical.

The interests but also the limits of these two embryogenic processes were discussed. Somatic embryogenesis is applied to *in vitro* propagation of some species and gametic embryogenesis allows production and utilization of haploids in more and more species.

These two embryogenic procedures are important for both theoretical studies and practical application.

G. VAN DEN ENDE, A. F. CROES, G. W. M. BARENDSE and A. KEMP (*Botanisch Laboratorium, Toernooiveld, 6524 ED Nijmegen*)

The role of glucose on in vitro flower bud formation of *Nicotiana tabacum*

In thin-layer tissue cultures of epidermal strips from flower stalks of *Nicotiana tabacum* direct flower bud formation takes place when the tissues are cultivated on agar containing Murashige & Skoog medium, 10^{-6} M NAA, 10^{-6} M BA and 3% glucose in continuous light. The first flower buds become visible on the sixth day after the onset of culture.

The number of flower buds per tissue increased with increasing glucose concentration from 0.3–1.7%. In lower glucose concentrations no flower buds were formed during an observation period of 21 days.

The role of glucose on flower bud formation was studied by replacing glucose by mannitol (in order to maintain a constant water potential). After a stay of 3, 5, 7, 9, and 12 days on 3% glucose respectively, the tissues were transferred to a medium in which glucose had been replaced by 2.76% mannitol. Seven days on glucose proved to be the minimum requirement for bud formation; the number of flower buds formed increased with a longer stay on glucose.

Withholding glucose for 1–6 days starting either from the first or from the sixth day of culture initially diminishes the number of flower buds formed in comparison with continuous culture on glucose, but this difference disappears with time. Hence, glucose deprivation delays flower bud formation, but the capacity of the tissue to produce flower buds remains unimpaired.

H. M. J. PENNING¹, S. E. DE VRIES² and B. A. UIJTEWAAL³ (¹*Stichting ITAL, Postbus 48, 6700 AA Wageningen*; ²*Biologisch Centrum Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn)*; ³*Instituut voor Plantenveredeling, Landbouwhogeschool, Lawickse Allee 66, 6709 DB Wageningen*))

Somatic cell genetics of potato

Production is in progress of monohaploids by female parthenogenesis (induced by using diploid *Solanum phureja* clones, homozygous for embryo spot, as pollinator) or by anther culture.

Monohaploids are necessary for the production of auxotroph mutants via mutagenesis.

Induction and selection of mutant cell lines is directed to the characters: fermentation of alternative carbon sources, nitrate reductase deficiency, resistance to: antibiotics, lysine + threonine, methionine-sulphoximine.

At present, approximately 30 variant cell lines of the dihaploid HH 260 were selected on media containing mannose or galactose as sole carbon sources.

C. M. COLIJN-HOOYMANS, H. BOOIJ and J. SYBENGA (*Vakgroep Erfelijkheidsleer, Landbouwhogeschool, Gen. Foulkesweg 53, 6703 BM Wageningen*)

Tissue culture and regeneration of rye, wheat and their hybrids

The final aim of our work is the transfer, by microinjection, of individually marked chromosomes or cloning vectors to protoplasts or to single cells of rye and wheat. For this purpose rapidly growing suspension cultures and dividing protoplasts must be available with the ability to regenerate into plants. Therefore the attention is focused on the growth of callus- and suspension cultures, and on the isolation of protoplasts and the regeneration of them into plants.

Callus tissue can be obtained from different types of explants such as immature embryos, leaf bases and rachises. The developmental stage of this starting material plays an important role in the successful induction of callus production. Therefore the specific developmental stages were determined giving rise to 100% callus formation. From two of the initiated callus tissues rapidly growing suspension cultures were established consisting of small aggregates of cytoplasmic cells growing rapidly in microdrop culture. After introduction of a selection marker (by mutagenic treatment) these suspension cultures are used as nurse tissue for the growth of protoplasts or single cells. Protoplasts could be isolated not only from callus- and suspension cultures but also directly from leaf bases. However, up to now these protoplasts do not divide.

Plant regeneration is obtained from callus cultures initiated from immature embryos. Root formation increases with the age of the culture. Nevertheless, some cultures continue to produce plants after more than seven months of subculturing in the case of rye and after four months in the case of wheat. The regenerated plants thus obtained did not show any phenotypic variation. The chromosome abnormalities of 25 regenerated plants were studied. Both in meiosis and mitosis these plants do not show any cytogenetic variation compared with the original plants. This indicates that after seven and four months of subculturing, respectively, no somaclonal variation was shown by these plants.

N. OVERBEEKE, M. A. HARING and A. KOOL (*Vakgroep Genetica, Biologisch Laboratorium, Vrije Universiteit, Postbus 7161, 1007 MC Amsterdam*)

Autonomously replicating sequences from *Petunia hybrida* chloroplast DNA

The aim of our research is to develop methods for the manipulation of cytoplasmic genetic information. Next to methods permitting the transfer of entire cytoplasm (cybridization) construction of specific plant cell organelle vectors will be of great importance. Vectors suited for this purpose must have both a marker permitting selection and a sequence facilitating stable maintenance. With respect to the latter property the chloroplast DNA replication origin will be a likely candidate.

To identify the chloroplast DNA replication origin *Petunia hybrida* chloroplast sequences have been isolated capable of autonomous replication in *Saccharomyces cerevisiae*.

For the isolation of these so-called Autonomously Replicating Sequences (ARS's) the approach was used described by STINCHCOMB et al. (1980). The vector YIp5, carrying the yeast *ura 3* gene, but unable to replicate in *S. cerevisiae* (STRUHL et al. 1979) was linearized and ligated with *P. hybrida* cp-DNA fragments; yeast *ura* cells were subsequently transformed with this preparation. A number of yeast transformants were picked up which all showed a phenotype typical for cells harbouring ARS-plasmids. For a further detailed analysis the plasmids were transferred from yeast to *Escherichia coli*. Physical maps were constructed and the chloroplast character of the inserts was verified by hybridization with ³²P-labelled chloroplast DNA. Furthermore the inserts were located on the chloroplast genome. One set of inserts ranging in size from 3.0 to 5.4 Kb was located on a position between 25 and 29 KB on the chloroplast genome (BOVENBERG et al. 1981). These plasmids are stable both in *E. coli* and in *S. cerevisiae* and have an ARS property when reintroduced in yeast. The other group has a very small insert (0.05 Kb) which most likely is the result of a deletion as they are no longer able to transform yeast *ura*⁻ cells to *ura*⁺.

To obtain evidence about the functioning of these sequences as an origin of replication in the chloroplast *in vivo* an organelle-free DNA synthesizing system will be used prepared from isolated chloroplasts (OVERBEEKE et al., in the press). The most direct evidence, however, will be the reintroduction of the ARS into the chloroplast and its subsequent stable maintenance as a result of autonomous replication.

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A. J. KOOL and C. E. JANSEN (*Vakgroep Genetica, Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Characterisation of nitrosoguanidine-induced 6-fluorotryptophan resistance in cell cultures of *Petunia hybrida*

Mutant cell lines of *Petunia hybrida* resistant to 6-fluorotryptophan (6FT) were isolated after mutagenic treatment of cell suspension cultures with nitrosoguanidine (COLIJN et al. 1979). A number of these 6FT resistant cell lines were also able to grow on auxin-free medium (auxin autotrophy). Plants were regenerated from a 6FT-resistant and auxin-autotrophic mutant cell line (AK 5069). One of these plants was back-crossed with a 6FT-sensitive parental plant. Calli initiated from half of the progeny showed the resistant phenotype.

The auxin-autotrophy of the cell lines as well as the tumour growth on the 6FT-resistant plants suggest an increased auxin synthesis in these mutants. 5MT-resistant mutant carrot cell lines have been isolated by WIDHOLM (1977) with an altered anthranilate synthetase which is less sensitive to feed-back inhibition. This lessened control caused a 20-80 fold increase of the level of free tryptophan (a precursor of auxin) which in several mutant lines resulted in auxin-autotrophic growth of the cell lines. A similar mechanism could be responsible for the 6FT-resistance and auxin-autotrophy of the *P. hybrida* mutant cells. The levels of free tryptophan were measured to test this possibility. The amount of free tryptophan in 6FT-resistant and wild type sensitive callus was observed to be more or less the same, ranging between 20-90 nMol/g fresh weight, which is comparable with the amount found in 5 MT-sensitive (wild type) cell lines of carrot.

These results do not exclude the possibility that the 6FT-resistance in *P. hybrida* cell lines is the result of an altered anthranilate synthetase. It is possible that in the 6FT-resistant cell lines the

altered enzyme does not result in an accumulation of free tryptophan because tryptophan is further metabolized. Present research is aimed at gaining direct evidence for an altered anthranilate synthetase.

COLIJN, C. M., A. J. KOOL & H. J. J. NIJKAMP (1979): An effective chemical mutagenesis procedure for *Petunia hybrida* cell suspension cultures. *Theor. Appl. Genet.* **55**: 101–106.

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H. GUDE and L. H. W. VAN DER PLAS (*Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Influence of ACC on ethylene formation and respiration of potato tuber tissue discs

In callus-forming potato tuber tissue discs (grown on B₅ medium containing naphthalene acetic acid and kinetin) a higher respiration was observed than in non-callus-forming tissue (incubated on B₅ medium without growth inducing hormones). Callus-forming discs also showed a much higher ethylene production.

Addition of the ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid) to the medium of non-callus-forming discs caused a concentration-dependent rise in ethylene production. Concomitant with this rise in ethylene production a stimulatory effect of ACC on both tissue respiration and capacities of the mitochondrial electron transport pathways (cytochrome pathway and alternative, CN-resistant, pathway) was found. The participation of alternative respiration in tissue respiration seemed to be stimulated by ACC as well.

L. H. W. VAN DER PLAS and MARIJKE J. WAGNER (*Biologisch Laboratorium, Afd. Plantenfysiologie, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Influence of long term osmotic stress on growth and respiration of callus-forming potato tuber tissue discs

After 2–3 weeks of growth on a modified B₅-medium with 3% sucrose as carbon source callus-forming potato tuber tissue discs were transferred to B₅-medium with or without sucrose with various concentrations of mannitol (ranging from 0 to 0.5 M).

Transfer to a medium with 0.3 or 0.5 M mannitol led to a rapid decrease in fresh weight due to water loss during the first few days. The dry weight increased immediately after the transfer, probably as a consequence of the uptake of mannitol from the medium. The osmolarity of the tissue extract increased; The observed rise in osmolarity of the tissue equalled that of the medium.

When expressed as O₂-uptake per gram of fresh weight, the total respiration increased upon transfer to a medium with mannitol. The capacities of the cytochrome and the alternative pathway did not increase upon mannitol addition. The increased total respiration was due to an increased participation of the alternative pathway in the total *in vivo* respiration. When expressed as O₂-uptake per disc (reflecting the respiration of the same number of cells, as real growth was negligible) the total respiration was the same as before the transfer to the medium with mannitol, while the cytochrome pathway activity decreased. This decrease was compensated by an increased *in vivo* participation of the alternative pathway.

Apparently potato tuber callus can withstand severe osmotic stress for several weeks, probably by taking up the osmoticum. In this tissue the alternative pathway functions as a reserve capacity, activated during osmotic stress treatment.

A. M. HEMRIKA-WAGNER, T. J. A. KNEPPERS, B. A. M. KROON, F. J. M. PENNEKAMP and L. H. W. VAN DER PLAS (*Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Pentose phosphate pathway in callus-forming potato tuber discs at various growth temperatures

Three methods were used to estimate the ratio glycolysis/pentose phosphate pathway in callus-forming potato tuber discs grown at 28°C and 8°C:

1. Malonate sensitive respiration ("glycolysis") was low at the beginning of callus induction but increased after one week at 28°C and three weeks at 8°C. Malonate resistant respiration ("pentose phosphate pathway") increased in both 8°C and 28°C callus, although 8°C callus showed a delayed development compared with 28°C callus. The percentage malonate inhibition was low at the beginning of callus formation, pointing to an important role of the pentose phosphate pathway in this period.
2. Activities of 6-PGDH in 8°C callus were twice as high as in 28°C callus.
3. The pentose phosphate pathway releases $^{14}\text{CO}_2$ from (1- ^{14}C)glucose, while glycolysis releases $^{14}\text{CO}_2$ from both (1- ^{14}C)glucose and (6- ^{14}C)glucose.

Pentose phosphate pathway mediated $^{14}\text{CO}_2$ production was maximal during the first days of callus induction at 28°C and during days 3–10 at 8°C.

From a comparison of the results of method 1 and 3 the conclusion might be drawn that the greater part of the NADPH produced in the pentose phosphate pathway at the start of callus formation is used for synthetic processes and is not resulting in oxygen uptake.

J. B. M. CUSTERS and J. H. W. BERGERVOET (*Instituut voor Veredeling van Tuinbouwgewassen, Postbus 16, 6700 AA Wageningen*)

Selection for micropropagation ability during the breeding of vegetatively propagated ornamentals *Nerine* and *Gloriosa*

Breeders of ornamental crops regularly express the wish that an *in vitro* micropropagation system should be developed for a newly raised cultivar. Mostly nothing is known of the genetic potentiality for micropropagation of such a cultivar.

With *Nerine* and *Gloriosa*, two new florists' crops, among clones from breeding programs a wide variation in micropropagation ability was found. For the clones with a good multiplication ability it was relatively easy to develop also a commercially viable procedure of micropropagation. For the clones with a low multiplication ability this was not possible. In a number of genotypes good micropropagation ability was associated with high ornamental value and good production characters.

In view of these results it seems appropriate in the breeding of vegetatively propagated ornamentals to include as a selection criterion the character good micropropagation ability. It would save a good deal of labour in tissue culture research.

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Transfer of cytoplasmic streptomycin resistance from *Nicotiana tabacum* to *Petunia hybrida* through somatic cell fusion

H. KOSTER (*Rijksinstituut voor Rassenonderzoek van Cultuurgewassen, Postbus 32, 6700 AA Wageningen*)

Light-influenced sprouting in potato: variation, breeding, and its use

J. KOSTER (*Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden*)

The ontogeny of shoots and leaves from epidermis cells of *Nautilocalyx lynchii* (Gesneriaceae)

J. VAN AARTRIJK and **G. J. BLOM-BARNHOORN** (*Laboratorium voor Bloembollenonderzoek, Postbus 85, 2160 AB Lisse*)

A role of ethylene in adventitious shoot formation on lily tissue

E. M. VAN WIJK and **C. KOCKEN** (*Biologisch Centrum, Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn)*)

Adventitious shoot formation on leaf explants of a monohaploid clone of potato

K. SREE RAMULU, **P. DIJKHUIS**, **G. M. M. BREDEMEIJER**, **H. BURG**, **S. ROEST**, **G. S. BOKELMAN**, **CH. H. HÄNISCH TEN CATE** and **L. ENNIK** (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Variation among plants regenerated from protoplasts of a Dutch commercial cultivar of potato (*Solanum tuberosum* cv. Bintje)

S. ROEST and **G. S. BOKELMAN** (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Plant regeneration from protoplasts of different potato genotypes

A. M. M. DE LAAT and **J. BLAAS** (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Isolation, purification and sorting by flow cytometry of metaphase chromosomes of *Haplopappus gracilis*

K. J. PUITE, **L. J. W. GILISSEN** and **A. M. M. DE LAAT** (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

First steps to chromosome transplantation with *Haplopappus gracilis* using flow cytometry

JOINT MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL
AND TISSUE CULTURE AND THE ROYAL NETHERLANDS BOTANICAL
SOCIETY ON THE 9TH OF MARCH, 1984

Lectures

L. LEFFRING (*Proefstation voor de Bloemisterij, Linnaeuslaan 2a, 1431 JV Aalsmeer*)

Genetical and physiological influences on in vitro propagation

C. BROERTJES and C. A. M. LOCK (*Instituut voor de Veredeling van Tuinbouwgewassen, Postbus 16, 6700 AA Wageningen*)

The use of irradiated soil in establishing in vitro adventitious plantlets of *Chrysanthemum morifolium* Ram. cv. parliament

The transfer of test tube cultured plantlets to soil results in variable survival dependent on plant species and cv, quality of the plantlets, and environmental conditions. Micro-organisms in the soil can also adversely affect plantlet survival but this effect can be avoided by the use of steam-sterilized soil.

Because soil treated with steam has certain disadvantages we have examined the possibility of using radiation-sterilized soil. In one experiment only 30% of the chrysanthemum plantlets survived in unirradiated soil (Trio No. 17, mixed with 10% sand), whereas 100% of the plantlets survived in irradiated soil (the dose was 2.5 kGy = 250 krad γ -rays). In a later experiment, using better quality plantlets, the survival was 85% in unirradiated soil and 100% in irradiated soil, either treated with 2.5 or 5 kGy γ -rays. Advantages of irradiated soil are:

- seeds and micro-organisms are inactivated or killed
- the soil cannot be recontaminated until the sealed plastic bags are opened
- the soil can be used immediately after irradiation
- no appreciable adverse effect on the soil quality nor on the plantlets grown in it
- the costs are comparable with those of steam-treated soil.

P. MIEDEMA (*Stichting voor Plantenveredeling, Postbus 117, 6700 AC Wageningen*)

The effects of growth regulators on vitrification in shoot cultures of *Beta vulgaris*

Adventitious shoots derived from flower buds are used for clonal propagation of *Beta vulgaris* (MIEDEMA 1982, 1983). Shoots are initiated on media with 10 $\mu\text{mol/l}$ BAP; after 6–8 weeks the shoots are transferred to media with 1 $\mu\text{mol/l}$ BAP for multiplication. Some genotypes showed abnormal shoot development on the multiplication medium. The leaves became stunted and thick (vitrification) and the axillary buds did not proliferate. It was demonstrated that these abnormalities are due to the long-term exposure to high BAP concentrations during shoot formation. Addition of GA_3 (1 to 10 $\mu\text{mol/l}$ before autoclaving) to the shoot initiation medium increased adventitious bud formation and promoted leaf elongation. Subcultures of those shoots showed a beneficial after-effect of GA_3 : vitrification was considerably reduced and the shoot multiplication was increased by a factor 3 to 4. The physiological backgrounds of the BA-induced malformations and of the counteracting influence of GA_3 are unknown.

MIEDEMA, P. (1982): A tissue culture technique for vegetative propagation and low temperature preservation of *Beta vulgaris*. *Euphytica* 31: 635–643.

MIEDEMA, P. (1983): The effects of various cytokinin treatments on shoot initiation and shoot morphogenesis in *Beta vulgaris*. *Acta Bot. Neerl.* 32: 237.

A. VARGA (*Vakgroep Plantenfysiologie, Arboretumlaan 4, 6703 BD Wageningen*)
 Instability in *Kalanchoë blossfeldiana* cultured in vitro

G. STARITSKY (*Vakgroep Tropische Plantenteelt, Postbus 341, 6700 AH Wageningen*)
 In vitro conservation of tropical crops, with special reference to aroids

Vegetatively propagated crops and crops with recalcitrant seeds are rather common in the tropics. So far germplasm of these crops is preserved in field collections in which the plants are exposed to diseases, pests and other harmful invaders. International exchange of vegetative material from these collections may be the cause of dispersal of diseases and pests. In vitro genebanks may solve many problems. Therefore the IBPGR (International Board for Plant Genetic Resources) of the FAO supports research in this topic.

In vitro gene banks include:

- “in vitro base collections” – for long-term storage and not for distribution. This can only be material stored by cryopreservation. In vitro base collections do not exist at present.
- “in vitro active collections” – material stored for relatively short periods, preferably in slow growth under defined conditions, to provide material for bio-technology, breeding, multiplication, evaluation, indexing and distribution. Upbuilding in vitro active collections exist in Colombia for cassava, in Peru for potato and in Nigeria for sweet potato.

At the Department of Tropical Crops research is concentrated on the storage of germplasm of tropical aroids. Taro (*Colocasia esculenta* L.) can be stored at 9°C in the dark without loss of vitality for at least three years.

In agreement with IBPGR more attention will be paid to research of the genus *Xanthosoma*, comprising:

- isolation of plant material free from microbial contaminants
- determination of genotypical differences in shoot development and shoot multiplication
- investigation of minimal growth conditions, for example, reduced temperatures, osmotic stress and application of growth inhibitors
- investigation of technical aspects about the establishment of in vitro genebanks.

Some in vitro cultured *Xanthosoma* specimen show genotypical differences in shoot development and multiplication. However, these differences do not seem too big to interfere with the establishment of an in vitro genebank.

T. CREEMERS, G. VAN DEN ENDE, A. KEMP, G. BARENDSE and A. CROES
 (*Botanisch Laboratorium, Toernooiveld, 6525 ED Nijmegen*)
 Morphogenetic expression in thin-layer tissues of tobacco in vitro

Thin-layer tissue culture is a useful technique for studying physiological processes which regulate morphogenetic expression. This study deals with bud formation in vitro on tissue explants cut from the floral ramifications of tobacco (*Nicotiana tabacum* L. cv. Samsun). The number and the type of buds were studied in relation to:

- (1) age of the original tissue taken from the mother plant;
- (2) stage of development of the inflorescence.

(1) Thin-layer tissues from flower- or fruitbearing stalks regenerate flower buds only. The number of flower buds formed is highest in explants from flower stalks. Tissues from the nodes of the inflorescence regenerate both floral and vegetative buds. Explants from “young” nodes produce more buds than explants from “older” nodes. In addition the ratio of vegetative to generative buds is higher in “older” nodes compared with that of “younger” nodes.

(2) At a certain stage of inflorescence development explants produce an optimal number of buds. The numbers of buds produced by explants from comparable nodes decrease with subsequent development of the inflorescence, while the ratio of vegetative to generative buds increases.

Thus in conclusion it appears that the morphogenetic expression of thin-layer tissues of tobacco is determined by the developmental stage of the inflorescence as well as by the age of the tissue itself.

D. H. KETEL (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Differentiation and dedifferentiation in *Tagetes* species in relation to secondary metabolism of cultured plant cells

Cell cultures of plants, as required for the biotechnological production of valuable secondary metabolites in bioreactors, are obtained from calli after morphological dedifferentiation of tissue explants. A major set-back in plant cell biotechnology is that dedifferentiation usually entails the loss of secondary metabolites, e.g. by inactivation of enzymes involved in secondary metabolism.

Calli of *Tagetes* species (*T. erecta*, *T. patula* and *T. minuta*) showed remarkable differences, in this respect, when grown under identical conditions. Callus of *T. erecta* rapidly formed roots and shoots and contained a number of hexane-soluble secondary metabolites, some of which corresponded with thiophenes (natural nematocides) in HPLC retention times and UV spectra. *T. minuta* callus did not differentiate and lacked secondary metabolites. Callus of *T. patula* behaved intermediately.

The observed differences in ontogenesis and metabolism between closely related species are an attractive tool to investigate the relationship between secondary metabolism and the process of differentiation.

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The production of secondary metabolites in callus cultures of *Cinchona ledgeriana* Moens

During the last years a part of the research in our laboratories has been aimed at the production of alkaloids in tissue cultures of *Cinchona* species. It was found (MULDER-KRIEGER et al. 1982) that callus cultures of *Cinchona ledgeriana* in addition to alkaloids also contain anthraquinones. In a recent study (WIJNSMA, in press) we reported on the isolation and identification of fifteen anthraquinone aglucones from callus cultures of *Cinchona ledgeriana* Moens. Seven of these anthraquinones have been found previously in other rubiaceous species, eight of them are new natural compounds. All of the anthraquinones found belong to the type which normally occurs in the Rubiaceae. Thus, the study gives additional chemotaxonomical evidence for the relationship between the genus *Cinchona* and other genera of the Rubiaceae. This is, however, only one aspect of our study: this note is one of the first that reports on one cell-line in tissue culture that produces secondary metabolites belonging to two so different classes of compounds as alkaloids and anthraquinones. The co-occurrence of anthraquinones and alkaloids in one cell-line may be a reason for the very low alkaloid content of the callus tissue, since for both biosynthetic routes mevalonic acid acts as a precursor. If this sort of competition for a mutual precursor exists, it may give an opportunity for channelling the biosynthesis in either of the two routes.

MULDER-KRIEGER, TH. R. VERPOORTE, A. DE WATER, M. VAN GESSEL, B. C. J. A. VAN OEVEREN & A. BAERHEIM SVENDSEN (1982): Identification of the alkaloids and anthraquinones in *Cinchona ledgeriana* callus cultures. *Planta Med.* 46: 19–24.

WIJNSMA, R., R. VERPOORTE, TH. MULDER-KRIEGER & A. BAERHEIM SVENDSEN (1984): Anthraquinones in callus cultures of *Cinchona ledgeriana*. *Phytochem.* (in press).

A. C. VAN SWAAIJ, J. WIJBRANDI, H. HUITEMA and W. TIMMERIJ (Vakgroep Genetica, Biologisch Centrum Rijks Universiteit, Kerklaan 30, 9751 NN Haren (Gn))

Hydroxyproline-resistant cell lines of dihaploid potato: isolation and partial characterization

The toxicity of 4-hydroxyproline (Hyp) is possibly due to the fact that it can be incorporated into polypeptides at the same position as proline. This will result in non-functional proteins. Hydroxyproline-resistance (Hyp^r) can be a result of high endogenous proline content.

In many plants proline has been shown to accumulate during periods of dehydration (drought, high salt concentration and cold). Proline accumulation could be involved in the protection against dehydration, though this hypothesis has been questioned. An answer to this question may be offered by proline accumulating mutants.

83 Hyp^r cell lines were obtained by plating a dihaploid potato cell suspension on medium with 5 and 10 mM Hyp (2 and 4 times the lethal concentration, respectively). On both concentrations the frequency of resistant colonies was the same: 1.0×10^{-5} for non-mutagenised cell suspensions and 1.4×10^{-5} and 0.3×10^{-5} for suspensions treated with 10 and 50 μ N N-ethyl-N-nitrosourea (ENU), respectively.

In addition Hyp^r cell lines were indirectly obtained by selecting cells for their capacity to survive short periods of freezing in suspension: 8 "frost-tolerant" cell lines were tested for growth on Hyp and two appeared Hyp^r.

Growth on different concentrations Hyp of 10 Hyp^r lines and their free proline content were determined: 50% growth inhibition occurred in wildtype at a concentration lower than 1 mM and in the Hyp^r lines between 5 and 100 mM. In the Hyp^r lines proline accumulated up to 30 times as compared with wildtype except for one line which had a normal proline content. In this line other factors than proline accumulation could cause Hyp^r.

N. OVERBEEKE (*Vakgroep Moleculaire Genetica, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Methods and possibilities for genetic manipulation of cytoplasmic plant characters

Posters

J. B. M. CUSTERS and J. H. W. BERGERVOET (*Instituut voor de Veredeling van Tuinbouwgewassen, Postbus 16, 6700 AA Wageningen*)

Callus to overcome post-fertilization barriers in interspecific crosses in *Cucumis* and *Lactuca*.

Callus is a source of genetic variation. It might, therefore, be regarded as a new possibility to overcome post-fertilization barriers in interspecific crosses. Certain crosses between *Cucumis sativus* and allied species failed because the embryos cease their development at the globular-shaped stage. Embryo culture could not induce them to continue differentiation. This lack of capacity of progressive differentiation may in part be caused by the difference in basic chromosome number, as $x = 7$ in *C. sativus* and $x = 12$ in the other species. In a callus from these hybrid embryos chromosome elimination might occur, which can yield a more harmonious karyotype. Species crosses in *Lactuca* are frequently hampered by death of the hybrid plants during their vegetative development. In callus from these hybrids mutants might arise, which can develop normally. The poster exhibited our preliminary results with the callus induction and the regeneration from the callus.

N. OVERBEEKE and A. J. KOOL (*Vakgroep Moleculaire Genetica, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Possibilities and methods for the manipulation of cytoplasmic properties of plants

Up till now, essentially all efforts directed towards the development of genetic manipulation systems for plant genetic information have been focussed on nuclear genes. However, a number of very important plant genes are located in the cytoplasmic cell organelles. The DNA of chloroplasts and mitochondria codes for polypeptides involved in essential processes such as respiration, photosynthetic CO₂-fixation and ATP synthesis. Moreover, a number of other commercially interesting properties like cytoplasmic male sterility and resistance to a number of plant pathogens and herbicides are also located on cell organelle DNA.

Somatic cell fusion has been used to combine cytoplasm and the genetic information therein

but the applicability of this technique seems to be limited to combinations between closely related plant species, most probably because of nuclear incompatibility. Recently transfer of cytoplasm between members of the same plant genus have also been accomplished by cybridization. This technique which involves the use of a fusion partner without a nucleus (or with inactivated nuclei) circumvents the problem of nuclear incompatibility and thus in principle permits the transfer of cell organelles between plant species that are less closely related. In our laboratory this method is used to study the transfer of chloroplasts between members of the Solanaceae. A more direct approach for the transfer of desired cytoplasmic properties would be the introduction of specific cell organelles or, even better, specific cell organelle genes.

The latter approach will require the use of a specialized cell organelle vector. As a first step towards the construction of such vectors we have isolated so-called Autonomous Replicating Sequences (ARS) from *Petunia hybrida* that function as a replication origin in yeast.

A. J. KOOL, W. A. BOVENBERG and H. J. J. NIJKAMP (*Vakgroep Moleculaire Genetica, Vrije Universiteit, De Boelelaan 1987, 1081 HV Amsterdam*)

Physical mapping of the chloroplast DNA encoded subunit genes of the ATPase complex of *Petunia hybrida*

The chloroplast DNA of higher plants encodes gene products involved in important plant processes such as photosynthetic CO₂ fixation and ATP-synthesis. A knowledge of the nature and position of chloroplast genes on the chloroplast DNA will be essential for our intended genetic manipulation of specific cytoplasmic plant properties. Our current research is focussed on the identification of chloroplast genes of *Petunia hybrida* and the study of genetic organization and expression of these genes.

Here we describe the mapping of the genes coding for the subunits α , β and ϵ of the CF₁-part and the subunit III of the CF₀-part of the ATPase complex. We observed that *E. coli* minicells harbouring the cloned *P. hybrida* cpDNA SalI S9-fragment synthesize, in addition to the *rbcL* polypeptide, a few more polypeptides: a doublet polypeptide with molecular weight 56 kD, corresponding in size with the beta subunit and a polypeptide of 15 kD, corresponding in size with the epsilon subunit of the ATPase complex.

The identity of these polypeptides as being the beta and epsilon subunit respectively, was confirmed by competition-immunoprecipitation experiments. By analysis of polypeptide synthesis in *E. coli* minicells harbouring BamHI subcloned fragments of the S9 fragment, the position of the coding region of the gene coding for the beta subunit (*atpB* gene) was determined at a distance of about 770 bp from the 5'-end of the coding region from the *rbcL* gene. The gene coding for the epsilon subunit (about 400 bp; *atpE* gene) was located within a region of about 1500 bp next to the *atpB* gene.

The genes encoding the proteolipid subunit (*atpIII* gene) of CF₀ and the alpha subunit (*atpA* gene) of CF₁ were located on the physical map of the petunia cpDNA by hybridization of cpDNA restriction enzyme digests with internal regions of the cloned *atpIII* gene from wheat cpDNA and the cloned *atpA* gene of *Spirodella* cpDNA. The two genes map close to each other within a region of 5.2 kbp on the physical map of the petunia cpDNA, at a distance of about 42 kbp from the *atpB* and *atpE* genes.

M. M. C. TAN, J. DE BRUIN, G. A. M. VAN MARREWIJK, A. J. KOOL and H. J. J. NIJKAMP (*Vakgroep Moleculaire Genetica, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Elimination of nuclei from protoplasts of *Petunia hybrida* using percoll centrifugation

A. GRONEMAN, A. M. M. DE LAAT, J. BLAAS and D. CREBOLDER (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Biotechnological production of biochemicals by means of plant cells in bioreactors

Abstract already published in:

Poster Symposium on Biotechnological Research in The Netherlands, November 22, 1983, Delft, p. 69.

E. M. VAN WIJK, C. H. M. KOCKEN, J. N. DE VRIES and W. J. FEENSTRA (*Vakgroep Genetica, Biologisch Centrum Rijks Universiteit, Kerklaan 30, 9751 NN Haren (Gn)*)

Isolation of mutants in monohaploid potato using adventitious sprout formation and tuber formation in vitro