

# Meetings of the Royal Botanical Society of The Netherlands

## MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 21 NOVEMBER 1991

### Twelve years of Chalk Grassland Management in the Belgian Part of the Sint-Pietersberg (prov. Liège, Belgium)

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The authors describe the results of 12 years of mowing and grazing management carried out on some chalk grassland reserves on the Belgian part of the 'St-Pietersberg', which is situated south of the Dutch town of Maastricht. The effects of the different management regimes were investigated by means of permanent plots which were recorded each year.

On the 'Thier de Lanaye' grassland, mowing and removing the litter in September proved to be a good management regime. It resulted in a higher number of species per plot than mowing without removing the litter, abandoning or burning this grassland area.

Another grassland called the 'Brachypodium-slope' came into existence about 25 years ago. When grazing with 'mergellandschapen', a local sheep race, was started in 1985 the grassland was very poor in species and dominated by the grass *Brachypodium pinnatum*. Now the number of species in the plot has considerably increased and the vegetation presents a diversified structure.

By 1978 the 'Heyoul 1' grassland was invaded by trees, shrubs and lots of coarse herbs. After cutting both the trees and the shrubs, the grassland was mown each year in August or in September. It is now very rich in species and it contains species belonging to the Mesobrometum, Brachypodio-Sieglingietum and Arrhenatheretum. From 1986 onwards parts of the grassland have been grazed by sheep. This improved the structure of the vegetation and it resulted in a slightly higher number of plant species; the other parts are still being mown.

On the 'Tiendeberg' at Kanne, the vegetation belongs to the Brachypodio-Sieglingietum which is a local association of the Violion caninae. Through the early 1980s this grassland was used as a non-permanent pasture by a local farmer. From 1988 onwards grazing with 'mergellandschapen' sheep was practised. After 3 years of grazing during summer and autumn, some of the species flowering in September, such as *Succisa pratensis*, show a slight decrease.

In conclusion, we can say that the differences between mown and grazed chalk grasslands are not

very important although in the grazed plots species numbers tend to be a bit higher.

### Epiphytes and Epiphytic Vegetation in Flanders, Belgium

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Until recently, corticolous cryptogams were rarely or only fragmentary studied in Flanders, not in the least because climatic, historical and anthropogenic factors are unfavourable for a luxuriant epiphyte flora. Since the last century, a large number of lichens and mosses disappeared from the area.

When comparing more recent floristic data on cryptogamic epiphytes (1960–63) with contemporary data we observe a further general and rather spectacular decline in number of species (Central Limburg and Dender area) or a severe decrease in species frequency (industrial area of Ghent). In spite of a general decrease in SO<sub>2</sub>-pollution since the 1960s, an increase in pollution-sensitive epiphytes has so far failed to occur. The epiphyte flora of the provinces East- and West-Flanders is clearly impoverished between Ghent and Antwerp, north-east of Lille (France), around Ruien (power plant), Courtrai, Bruges, Ostend, Aalst and Dendermonde. The Westhoek (in the extreme west of Flanders) is richest in species (generally > 20 spp./site).

Data on epiphytic vegetations in Flanders are even rarer. At the University of Ghent we have been studying these vegetations since 1982, phytosociologically as well as ecologically. We classified them into five 'main vegetation types', enclosing nine 'types' and 16 'variants'. Syntaxonically these show affinity to the Desmococcetum olivacei, Lecanoretum conizaeoidis, Chaenothecetum ferrugineae, Parmelion caperatae, Parmelion acetabulae, Xanthorion parietinae, Lecanorion carpineae, Diploicion canescentis and Tortulion laevipilae.

The more important factors in differentiating vegetation appear to be the climate (moisture regime in particular), exposure of the phorophyte, level of SO<sub>2</sub>-immission, vicinity of topdressing areas (NH<sub>3</sub>), and acidity, conductivity, NH<sub>4</sub><sup>+</sup>-, SO<sub>4</sub><sup>2-</sup>-, (K<sup>+</sup> and PO<sub>4</sub><sup>3-</sup>) content of the bark. Water capacity Na<sup>+</sup>-, Ca<sup>2+</sup>-, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>-content of the bark seem to have less differentiating importance.

Some phytocoena mentioned in literature were not observed anymore, i.e. the *Lecanoretum subfuscae*, *Ramalinetum lacerae* and *Tortuletum latifoliae*; the *Ramalinetum fastigiatae* and *Parmelietum revolutae* are endangered.

### Dispersal Experiments in the Vegetation of Road Verges

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Dispersal experiments are part of a study on the vegetation of road verges in Flanders. Inventarization and classification are the first part of the study. The second part is experimental and deals with management and dispersal strategies.

Literature (Grime, Hodgson & Hunt 1988: *Comparative Plant Ecology*. Unwin Hyman, London) suggests that building up a soil seedbank is probably the most important dispersal strategy in road verges. An experiment in which seeds were buried, and after 1 year allowed to germinate in a heated greenhouse, suggests the existence of a persistent seedbank for many species about which there is no known literature. Also the size of the seedbank often does not confirm the literature data, probably indicating that the size of the soil seedbank is determined by local factors. Differences in germination of 20 to 40% are found between different populations of the same species.

A seedbank analysis under ruderal vegetations in road verges reveals another species spectrum than the above-ground vegetation. More surprising is the fact that the seedbank spectrum is quite different from the species composition in an experimentally *in situ* bared soil. The species spectrum of the seedbank has about 30% more species than the above-ground ruderal vegetation. The seedbank consists of about 40% annual species and 60% perennials. The flora of experimentally-bared soil contains most 'rare species'. The number of rare species is intermediate in the seedbank flora germinated in the greenhouse and lowest in the ruderal vegetation at the sampling site. The number of seedlings resulting from germination in the greenhouse varies from  $\pm 25\ 000$  to  $670\ 000\ m^{-2}$ .

### Population Dynamics of the Long-Leaved Sundew (*Drosera intermedia* Hayne) in Different Heathland Habitats

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Eight populations of long-leaved sundew (*Drosera intermedia* Hayne) ramets were studied in three hydrologically and phytosociologically differing heathland habitats over a 4-year period: a pool edge, an old path through the wet heath and a seepage area

(State Nature Reserve 'De Kalmthoutse Heide', N-Belgium).

At the extreme sites, with high levels of water-table fluctuations (pool edge and dry path), both survival and recruitment were low, resulting in small populations. The pool-edge populations went extinct after 3 years. At the less extreme sites (wet paths and seepage area), populations were stable, but ramet and genet turnover was higher at the path sites than in the seepage area. Demographic variation between populations was explained by differences in adult survival and sexual recruitment (asexual recruitment did not differ clearly) which, in turn, were related to specific biotic and abiotic factors. Likely adult mortality causes were summer drought (all habitats), winter frost (path habitat), cover with a crust of iron bacteria after heavy summer rainfall (seepage area) and shading by *Sphagnum* and algae (pool edge). Low seedling recruitment was caused by a low germination success (inundation at the pool edge, possibly shading by *Sphagnum* in the seepage area) and seedling survival (seepage area, dry path).

### Hydrology and Ecology of a Calcareous Fen: the Buitengoor near Mol, Belgium

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The Buitengoor is a headwater valley fen in the Campine region of Belgium. This region is known for its sandy substrate and acid soils. However, in the Buitengoor, low growing herbaceous-rich fen vegetation of the alliance *Caricion davallianae* is present. The natural landscape cannot generate the base-rich soil environment that is necessary for this vegetation type. In a groundwater chemistry study, it was shown that a small irrigation canal recharges the groundwater with calcareous water just upstream of the fen. This establishes local base-rich conditions in the fen. The fen vegetation is very diverse, but a large-scale gradient from rich to poor fen was detected with canonical correspondence analysis. The localized, artificial input of base-rich water reverses the normal transition from poor to rich fen. In the study area, the rich fen is situated upstream from the poor fen. Consequently, the irrigation water is responsible for the presence of the rich fen vegetation and all intermediate types.

The internal management of the site is aimed at regulation of the natural succession. In fen margins, a closed canopy of *Molinia caerulea* and *Myrica gale* replaces the low growing fen vegetation. For a decade, these dominant plants have been removed locally during different years. In all cases, a low growing, species-rich fen vegetation established after a few years. The external management is directed towards regulation of the hydrological relations. Both water-table disturbance and eutrophication are possible threats for the Buitengoor.

## MEETING OF THE SECTION FOR PLANT SYSTEMATICS AND GEOGRAPHY ON 3 DECEMBER 1991

### Problems in the Taxonomy of Wild Relatives of the Potato

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The potato is a crop with an exceptionally large group of wild relatives. A number of these wild species participated in the origin of the crop and/or is used in breeding programs to enhance the genetic diversity of the commercial potato. In his recent treatment of the group Hawkes (Hawkes, G. (1990): *The Potato—Evolution, Biodiversity and Genetic Resources*. Belhaven, London) recognizes 232 species. Seven of these are designated as 'cultivated species'. These species are closely related to the cultivated potato (classified as *S. tuberosum* spp. *tuberosum* and spp. *andigena*). Their treatment as taxa on the species level using Latin binomials has been questioned a.o. by Dodds (in Correll, D.S. (1962): *The Potato and Its Wild Relatives*. Texas Research Foundation, Renner), who proposed an alternative classification in 'groups' within the conglomerate 'species' *S. tuberosum*.

The related wild species are classified in series. Different numbers of series are accepted by various taxonomists, while many species have been placed in different series in the course of time. The circumscription of at least some of these series is unsatisfactory.

Intensive taxonomic work continues to the present day. Since Correll (1962) 183 new taxa have been described, resulting in 530 validly published names. Genebank managers and plant breeders using the present taxonomic system encounter numerous problems in identifying accessions. In many cases the variability of the species has been underestimated and a too narrow species concept has been applied. The genebanks of Braunschweig (Germany) and Sturgeon Bay (U.S.A.) co-operate on research projects to re-investigate hypotheses of species boundaries, hybrid origin of taxa, series affiliation of species and similar problems using numerical taxonomic methods. The distinction of subspecies within *S. microdontum* (van den Berg & Spooner, *Plant Syst. Ecol.* [in press]) and of the very similar species *S. berthaultii* and *S. tarijense* (Spooner & van den Berg, *Taxon* [in press]) is questioned by the results of a numerical analysis. The latter two taxa, now hypothesized to be conspecific, have been classified in different series that even belong to different superseries (Hawkes 1990). Many morphological characters used to delimit species can be shown to vary within populations and large complexes of very similar species need to be reinterpreted in view of these results.

### Variation Patterns in *Beta* Section *Beta*

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The sea beet, *Beta vulgaris* (L.) spp. *maritima* Arcang., is distributed along the Atlantic coasts of Europe and along the Mediterranean littoral. It extends as far east as the Indian subcontinent. Close relatives of the sea beet within section *Beta* are found in the Aegean region (*Beta adanensis* Pamuk.) and on Madeira (*Beta patula* Ait.). Diploid *Beta macrocarpa* Guss. is distributed along the western and eastern part of the Mediterranean coast, while its tetraploid cytotype is restricted to the Canary Islands. *B. patula* is morphologically distinct, but the others are not readily segregated by morphological characters. Glomerule characters were found helpful in separating the taxa *B. adanensis* and *B. macrocarpa* from *B. vulgaris* ssp. *maritima*.

In a field trial at Wageningen, The Netherlands, time of bolting and time of flowering of the species were tested. Flowering in *B. macrocarpa* and *B. adanensis* started on average 52 days after sowing, which is about 1 month earlier than in Mediterranean *B. vulgaris* ssp. *maritima* populations. *B. macrocarpa* (invariably) and *B. adanensis* (partly) behaved as annuals, while the other taxa predominantly perennated. Genetic variation for earliness of bolting and earliness of flowering was demonstrated in *B. vulgaris* ssp. *maritima* accessions derived from northern and southern latitudes.

Seventy-six accessions of representatives of section *Beta* were surveyed for isozyme variability. A total of 11 isozymes were studied. Genetic variability coefficients were calculated based on nine loci. In *B. vulgaris* ssp. *maritima*, the largest isozyme diversity was met in accessions originating from the Mediterranean Basin. *B. macrocarpa* diverged at a number of loci, notably *Lap1*, *Acp1*, *Pgm2* and *Px2*. The species *B. patula* and *B. adanensis* express allozymes commonly found in *B. vulgaris* ssp. *maritima* accessions, but they could be characterized by the expression of specific allozymes at high frequencies. The distribution of one particular allozyme (*Acp1-2*) suggested a relationship of *B. patula* with Atlantic *B. vulgaris* ssp. *maritima* populations. The co-dominant expression of 'ssp. *maritima*' allozymes and characteristic '*B. macrocarpa*' allozymes in the tetraploid cytotype of *B. macrocarpa* supports an allotetraploid origin of this taxon. Interspecific differences in levels of heterozygosity could be related to the breeding system. The Mediterranean (self-compatible) species expressed lower levels of heterozygosity than (self-

incompatible) populations of *B. vulgaris* ssp. *maritima*, indicating increased autogamy in the Mediterranean species.

Several factors are thought to have been of importance in the speciation process in section *Beta*: loss of self-incompatibility, a tendency towards autogamy, geographical isolation and a shift in flowering period among some of the sympatric taxa.

### Biogeography of the Eastern Cape Province in Relation to the Taxonomy and Distribution of Mesembryanthemoideae (Aizoaceae)

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This subject is related to the study of the Aizoaceae, which were originally restricted almost entirely to southern Africa. The taxonomy of this plant family has been studied intensively in the Institut für Allgemeine Botanik in Hamburg since 1960. Until now, monographs of only a minority of the c. 2500 species were published, mainly those belonging to the sub-families Mesembryanthemoideae and Ruschioideae.

At our Department, a small group of the Ruschioideae, the *Stomatium* group, is being investigated. Without a proper field-study, it proved impossible to complete monographs of genera of this group. Data on ecology, distribution and plant association of these genera are unknown. It is difficult to interpret herbarium material of these highly succulent plants. Recently, a field study of the eastern Cape province was undertaken. The area, which has been neglected by botanists until recently is a distribution centre of the Aizoaceae. Two new locations with populations of the very rare endangered genus *Orthopterum* have been discovered. An *Orthopterum* species which is likely to be new was found at one of these localities. The second population needs further investigation. A field study of populations of *Faucaria* proved that there exists a large variation in taxonomically important characters of the epidermis and leaves. It turned out that only five of the 33 described species of *Faucaria* could be identified as valid species and retained.

### The Variable Taxon: Coding for Polytypism within a Cladistic Analysis

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The presence of more than one character-state within a single taxon is a comparatively frequently encountered phenomenon, especially within plants. A simple

example of three taxa *X*, *Y* and *Z* which possess the character states *a*, *a+b* and *b* respectively is used to illustrate the problem. State 'a' represents the plesiomorphy. The most parsimonious explanation for the occurrence of polytypism is to presume that taxa possessing only the apomorphous state (*Z*), have evolved from that part of the polytypic taxon (*Y*) which already acquired this apomorphy. In this way only one evolutionary step is necessary. Two different treatments for polytypism in a cladistic character matrix are usually given. The first option is: 'create a third character-state to accommodate the polytypic state (*a+b*)'. The second option (which has the same effect as binary coding) is: 'create a distinct character for the occurrence of each state ('a' present yes/no, 'b' present yes/no)'. The first option fails to recognize the synapomorphy of the polytypic taxon with other taxa possessing the apomorphous state, unless the character is ordered with the polytypic situation as the intermediate state. This can however only be applied to simple cases of polytypism. The second option does recognize synapomorphis and can be applied to more complex cases.

However, both in the first option (ordered variant) as well as in the second one an unintended weighing factor of 2 has been introduced. Two steps are now needed to change from state 'a' to 'b', while theoretically, the most parsimonious solution requires only a single step. This also applies to more complex cases. Consequently, characters in which polytypism occur, which are handled according to either one of the given solutions, should be given a weight of 0.5. Alternatively, all other characters are to be given a weight of 2.

This conclusion also implies that one should pay more attention to the concept of homology. Not only should all character states within a character be homologous, but moreover *all* homologous states should be accommodated within one character or else unintended *a priori* weighing is introduced.

### On the Malesian Myristicaceae

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While revising the genus *Myristica* as a part of the treatment of the family Myristicaceae for *Flora Malesiana*, it appeared that both the treatment of Sinclair, J. (1968: *Gard. Bull. Sing.* 23: 1-540) and that of Foreman (Foreman, (1978): In Womersley, S. (ed.): *Handbooks of the Flora of Papua New Guinea* 1: 175-215) give rise to problems. The division of *Myristica* into two sections on the basis of the type of inflorescence breaks down in E. Malesia.

A comparative study of the inflorescence in all c. 20 genera of the family revealed the existence of a basic

type of inflorescence, of which the architecture is the same as the typical mode of vegetative branching encountered within the family. This basic type is an axillary compound, with a smooth common peduncle and with the basal two lateral branches opposite while those higher up are alternate. The opposite lateral branches appear to be homologous with the generally dormant buds present in the axils of the two transversally placed minute prophylls which can be found within the vegetative axillary buds of all Myristicaceae. This basic type of inflorescence occurs in about half of the genera of the family. The remaining genera have an inflorescence which is composed of several of these basic elements. This type can be recognized by the common peduncle which is always provided with the scars of prophylls. The inflorescence is very variable (from a large panicle-like to a sessile brachyblast type) but the presence or absence of scars of prophylls towards the base of the main peduncle is an essential and easily observed criterion.

The single inflorescence type is characteristic for the Malesian genera *Knema* and *Myristica* while *Endocomia*, *Horsfieldia* and *Gymnacranthera* possess the compound type.

The inflorescences of the two sections within the genus *Myristica* (viz. section *Myristica* and section *Fatua*) both belong to the single type, that of section *Fatua* being a strongly condensed form. Intermediate forms were encountered.

The better insight in the basic structure of the inflorescence, as well as other newly discovered characters on the lower leaf surface (non-traumatic wart-like blackish dots and a papillate epidermis) will hopefully lead to a satisfactory treatment of the family in Flora Malesiana.

### Phylogenetic Relations and Historical Biogeography of *Fordia* (Including *Imbralyx*) (Papilionaceae: Millettiae)

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The phylogenetic and biogeographical relations of two Malesian papilionid genera were studied. *Fordia* spp. Hemsley is characterized by raminascent to supra-axillary pseudopanicles and two-ovulated ovaries and *Imbralyx* Geesink shows typical spindle-shaped flowerbuds and imbricate calyces. A macro-morphological data matrix was scored from available descriptions and cladistically analysed with HENNIG86 under 'mhennig' and 'bb\*' (Farris, J.S. (1988): *HENNIG86 reference*, [version 1.5], New York). *Millettia pulchra* was selected as a suitable outgroup. In the resulting three trees, *Imbralyx* appeared to be derived from *Fordia* spp., leaving *Fordia* spp. as a paraphyletic group.

Leaf anatomical characters, especially the mesophyll structure and the presence of papillae confirmed this relationship. Analysis of morphological and anatomical data also resulted in a topologically similar tree (length 167 and CIU 0.46) after automatic iterative character weighting. Consequently, *Fordia* spp. and *Imbralyx* are united under *Fordia*.

This cladogram and the distributions (Kwangtung, Yunnan, Malacca, Borneo and Sumatra) were used for a biogeographic analysis with CAFCA and assumption O (Zandee, M. (1988): *User Manual for CAFCA/PC* [version 1.9.6], Leiden). Two areagrams were found (length 36, CI 0.971). A general areagram could not yet be constructed due to absence of compatible and cladistically revised genera.

The areagrams start with a separation of Borneo, followed by Kwangtung and either Yunnan or Sumatra. This pattern may have been caused when the warming climate resulted in sea-level rise and a sequential isolation of the Sunda Islands. Earlier, the areas were connected during Pleistocene ice ages with low sea-levels and savannas on the dry Sundra-shelf. Homoplasious events may be the result of conflicting patterns of two or three different ice ages, but more cladistic analyses are needed to recover the biogeographic history of the Malay Archipelago.

### *Lepidopetalum* Blume (Sapindaceae): Taxonomy, Phylogeny and Historical Biogeography

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The genus *Lepidopetalum* Blume (Sapindaceae) contains six species, of which one, *L. fructoglabrum* is new. Typical for the genus are the domatia (hair tufts), the petals (small, triangular with much larger, usually united scales) and the fruits (flat, usually two-locular, sarcotesta present).

The differences among the species are very slight. Important specific characters are the pilosity of the upper part of the pedicel, the shape of the disc, the type of hairs inside the fruit and the type of sarcotesta. Four species are found on New Guinea, one on the Nicobar Islands and Sumatra and one on the Philippines. Specimens from Java, Sulawesi, Moluccas and the Lesser Sunda Islands could not be identified due to the lack of mature fruits.

A phylogeny of *Lepidopetalum* was hypothesized with the genus *Arytera* as outgroup and HENNIG86 (Farris, J.S. (1988): *Hennig86 reference*, [version 1.5], New York) as computer program. Three cladograms were found of which the one with the highest redundancy quotient (via computer program CAFCA; Zandee, M. (1988): *A user manual for Cafca/PC* [version 1.9.6], Leiden) was selected as the accepted phylogeny. This cladogram showed the successive origin of *L.*

*xylocarpum*, *L. subdichotomum*, *L. fructoglabrum*, *L. micans*, *L. montanum* and *L. perrottetii*.

A historical biogeographic analysis of all cladograms with the component compatibility method (Zandee, M. & Roos, M.C. (1987): *Cladistics* 3: 305–332) and Brooks' Parsimony Analysis (Wiley, E.O. (1988): *Syst. Zool.* 37: 271–290) resulted in four areagrams of which the one produced by the accepted

phylogeny proved to be the best. This areagram showed two, possibly three, vicariance events and three dispersal events, of which two resulted in speciation. The first speciation and vicariance event in *Lepidopetalum* occurred 15 million years ago, with the collision between N. New Guinea and the rest of New Guinea. This suggests that the genus as a whole is relatively young.

## MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 25 OCTOBER 1991

### A Survey of the Fossil Wood Record—Evolutionary and Ecological Implications

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Literature on over 1200 Cretaceous and Tertiary fossil woods has been abstracted for 91 different anatomical features in order to test the Baileyan hypothesis on xylem evolution and to assess whether the fossil wood record can be used to trace climatic changes of the past.

The Baileyan transformation series for vessel elements, parenchyma distribution and ray composition are strongly supported by the fossil record, but the tempo of specialization has been different for various features. For instance, major vessel specializations such as the replacement of scalariform by simple perforations and the scalariform to opposite by alternate intervessel pits already occur in almost 'modern' frequencies in the early Tertiary (Paleocene, Eocene), while ray and parenchyma specialization proceeded gradually from the Cretaceous up to the late Tertiary. One of the few results not predicted by the Baileyan hypothesis is the peak incidence of septate fibres in Cretaceous woods.

Some specialized features, at present associated with seasonal climates, such as ring-porosity, vessels distributed in tangential bands, and helical vessel wall thickenings did not arise until the early to mid-Tertiary. Many modern ecological trends can be retraced in the fossil record by comparing the Northern (Laurasian) fossil woods with Southern ones of mainly tropical, Gondwanan provenance. However, for the Cretaceous different wood anatomical parameters give conflicting inferences for climatic reconstruction, if modern ecological trends are applied. This is presumably because of phylogenetic constraints, i.e., the generally low level of xylem specialization in the Cretaceous precluded the existence of such trends. Growth ring expression

indicates tropical conditions throughout the Cretaceous. The pattern of changes in incidence of growth rings and other ecologically sensitive wood anatomical features is suggestive of a gradual increase of seasonality in the Northern hemisphere during the Tertiary. There is no wood anatomical evidence to support a severe climatic deterioration towards the end of the Eocene.

For a full account see Wheeler, E.A. & Baas, P. (1991): *IWA Bull.* 12: 275–332.

### Structural Aspects of Particle Gun and Micro-Injection Techniques on Immature Maize Embryos

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The goal of this project is to develop a micro-injection method for the transformation of maize. Particle gun-mediated transfer of DNA was applied to test plasmids containing either the glucuronidase (GUS) reporter gene or the leaf colour (Lc) reporter gene. Both plasmids were constructed for high expression in maize callus. After bombardment with the GUS construct, calli were further cultured and permitted to regenerate plantlets. Sections were made from regenerated leaves with blue veins indicating the presence of the enzyme glucuronidase. Examined light microscopically, these sections showed blue crystals in all living cells. The Lc gene which starts anthocyanin production resulting in red stained cells was also expressed in calli. Injections were performed on embryos 9 days after pollination. At this stage the apical meristem is still exposed for injection and embryos can regenerate into a mature plant from this time onward. Before injection the embryo was immobilized in a thin layer of 0.8% (w/v) agarose. For

mapping the fate of the injected cells, gold particles were injected as a marker. After the injection, embryos were immediately fixed in formaldehyde and glutaraldehyde and embedded in epon. Thin sections were made on a microtome, stained with toluidine blue and examined by light microscopy. If injuries caused by micro-injection were spotted, ultra-thin sections were made which were examined without post-staining in the electron microscope. Post-staining was omitted in order to prevent interference of precipitates with the detection of injected gold particles. In this way it was possible to evaluate the damage to the individual cells of the meristem. Several meristematic cells contained gold particles. Based upon their structure, some of these cells seem to be vital at the moment of fixation. Current research is extended to analyse injected embryos after varying times of culture.

### **Histology and Pyrolysis Mass Spectrometry of Maize Somatic Embryos from Suspension Culture**

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Single somatic embryos of maize (4C1) from suspension culture are blocked in shoot meristem formation (Emons, A.M.C. & Kieft, H. (1991): *Plant Cell Rep.* 10: 485-488). In the culture globular, ovoid and polar structures occur, representing the young embryo axis only. If, however, the somatic embryo is attached to a callus aggregate, a complete somatic embryo with a scutellum is formed. By means of pyrolysis mass spectrometry we started to analyse polysaccharide and (poly)phenol content and diversity of developmental stages of both types of somatic embryos and compared them with zygotic embryos.

Pre-embryonic stages were collected by sieving the suspension cells through sieves with different pore sizes, which resulted in samples containing primarily elongated cells (50 and 125  $\mu\text{m}$  sieve), cell clusters (212  $\mu\text{m}$  sieve) and small globular stage somatic embryos (300  $\mu\text{m}$  sieve). Ovoid and two polar (1.5 and 5 mm long) stages were manually selected.

The root meristem is initiated at ovoid stage and meristematic cells are present at the stem side from the small polar stage onwards; vascular cells are initiated at this stage and vascular tissue connects the meristematic region of the stem with the root meristem at the late polar stage. If attached to callus tissue a starchy scutellum is present as well as a proper stem meristem and leaf primordia.

The different sieve fractions were subjected to pyrolysis mass spectrometry to determine the monomeric and oligomeric composition. All cells contain quite an amount of phenolics. In the elongated cells ferulic acid content is higher than coumaric acid content. In comparison to elongated cells the embryogenic cell clusters contain more pentosans and hexosans derived from hemicelluloses than elongated non-embryogenic cells. In the globular stage, the amount of hexosans increases. At ovoid stage, when in the sections the first cells with vascular thickenings are seen, syringyl lignin is seen in the mass spectrogram and in the later stages syringyl amount remains higher than guaiacyl, which is unexpected for young cells. In comparison, the zygotic embryo contains far less phenolic acids, no lignin and a much higher amount of hexose sugars, presumably starch. The pyrolysis mass spectrogram of the somatic embryos that are attached to callus tissue resembles that of the zygotic embryo more than that of the single embryos.

We conclude that an aberrant phenol metabolism might be involved in the expression of the developmental block in the single somatic embryos from suspension culture.

### **Exocytosis and Endocytosis in Tobacco Pollen Tubes**

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Pollen tubes of *Nicotiana tabacum* L. were grown *in vitro* for 3-4 h on agar (2%) supplied with sucrose (10%) and boric acid (0.01%). Structure and distribution of organelles involved in exo- or endocytosis were studied on serial sections of cryo-fixed and freeze-substituted pollen tubes. In the extreme tip, numerous secretory vesicles accumulate, but fusions with the cell membrane are seldomly seen. Golgi bodies accumulate in the region between 15-25  $\mu\text{m}$  behind the tip. Coated pits at the plasma membrane accumulate in the region 10-20  $\mu\text{m}$  behind the tip. The secretory vesicles in the extreme tip cannot originate from the vesicles surrounding the Golgi bodies, but seem to derive from the trans-Golgi-network (TGN) that is almost invariably associated with the Golgi bodies. Vesicle clusters that derive from the TGN seem to be the precursors for the secretory vesicles. Like in animal cells the TGN might be the target site for endocytotic vesicles. Thus, as coated pits accumulate behind the tip, it may be hypothesized that the accumulation of Golgi bodies may relate to endocytosis instead to exocytosis. An intact endoexocytotic cycle of membrane proteins from the growing tip may be a prerequisite for tip growth.

## MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON 23 JANUARY 1992

**Biological control of *Botrytis* Leaf Blight of Onions by Suppression of Sporulation**

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Onion leaf blight caused by *Botrytis squamosa* can lead to severe economic damage. Onion leaves are infected by conidia during the whole summer, if weather conditions are appropriate. Sporulation cycles of *Botrytis* are frequently observed on dead leaf tips, when water content of the dead leaf tissue is sufficient during dew or rain periods. Therefore, in practice, fungicides have to be sprayed weekly during July and August.

To develop a biological control agent, two strategies can be followed. (1) Antagonists can be applied onto the green leaves in order to prevent *Botrytis* infections. The short interaction periods of a few hours during germination of conidia of *Botrytis* makes this strategy less reasonable, also because high population densities of antagonists have to be established continuously during the whole growing season. (2) Saprophytic antagonists can also be applied on the dead leaf parts to reduce sporulation of *Botrytis*. Once the antagonist is established in this substrate, interaction periods between antagonist and the saprophytically growing mycelium of *Botrytis* are relatively long. Our research was aimed at the selection and application of saprophytic antagonists which can suppress sporulation of *Botrytis*.

In a first field experiment in 1991, the effect of an antagonist was simulated by artificial removal of dead onion-leaf parts. When approximately 40% of the debris was removed during the growing season, the epidemic of *Botrytis* was significantly delayed. The content of *Botrytis* conidia in the air above the crop was reduced up to 66% compared to the control. In late August, 1.1 spots cm<sup>-2</sup> leaf area were counted in the control and 0.6 spots cm<sup>-2</sup> after removal of dead leaves. These results indicate that most infections are caused by conidia produced within the crop. Thus, the suppression of sporulation by antagonists will have a direct effect on *Botrytis* epidemics.

A bioassay with dead onion leaves, pre-inoculated with *Botrytis*, was developed to select efficient antagonists. Strains of *Gliocladium* spp. and *Trichoderma* spp. suppressed sporulation of *Botrytis* almost completely. The adaptation of antagonists to the reverse environmental conditions in dead leaves, e.g. the daily alternation of wet and dry periods, will be tested.

**Ethylene Influences Development of Acquired Resistance in Hypersensitively Reacting Tobacco**

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Local infection of tobacco with tobacco mosaic virus induces a systemic acquired resistance, expressed as a reduction in size of the necrotic lesions developing upon challenge inoculation. This induced resistance is stronger in leaves above than in those below the primary inoculated leaf and is associated with an increase in the activity of the ethylene-forming enzyme (EFE). After inoculation of a single leaf on a plant, EFE activity increases rapidly in distance leaves, even before ethylene production in the inoculated leaf is increased concomitantly with the development of necrotic lesions. Subsequently, EFE activity in the non-infected leaves decreases but remains elevated when acquired resistance develops within 3 days after the primary inoculation. Treating the primary inoculated leaf with the ethylene synthesis inhibitor AVG immediately after inoculation largely abolished the increase in ethylene production in the inoculated leaf and reduced the extent of acquired resistance developing in distant leaves. As AVG did not inhibit local lesion formation and slightly reduced lesion size in the inoculated leaves, the increase in lesion size observed upon challenge inoculation of other leaves points to an interference of AVG with the development of acquired resistance. Apparently, the increase in ethylene production in primary inoculated leaves induces or liberates a factor stimulating the acquisition of induced resistance in other plant parts. Besides, local treatment with AVG had pronounced effects on the morphology of developing leaves.

**Involvement of Agglutinins in Adherence to Roots and Colonization of Roots by Fluorescent *Pseudomonads***

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The involvement of root agglutinins in adherence to roots and in root colonization by fluorescent pseudomonads was studied for several crops, using different and distinct *Pseudomonas* isolates from these crops. Three approaches were used: (a) several isolates, showing different agglutination reactions, were compared with respect to their adherence to roots and root colonization,

(b) adherence and root colonization of agglutination negative mutants of *Pseudomonas putida* 'Corvallis' was studied in comparison to the wild-type strain and (c) the effect of addition of agglutinins on the adherence to roots was studied with selected isolates.

By comparing different and distinct fluorescent *Pseudomonas* isolates, no agglutination dependent adherence and root colonization could be demonstrated. This suggests that the agglutination phenotype does not play a dominant role in determining adherence and root colonization. Agglutinins, however, can play a role in the root colonization process of individual isolates, as agglutination-negative mutants of strain *P. putida* 'Corvallis' were significantly reduced in their adherence to roots and root colonization in comparison to the wild-type strain. Moreover, only isolates that were able to agglutinate were significantly influenced in their adherence to roots by addition of root agglutinins. However, this effect could vary depending on the isolate tested and the concentration of agglutinins added. The latter results may explain why adherence to roots and root colonization by the different *Pseudomonas* isolates tested did not positively correlate with their agglutination reactions.

It is concluded that agglutinins are involved in adherence to roots and in root colonization by fluorescent pseudomonads. However, their effect in these processes may differ for different strains and their role therefore cannot be generalized.

### **Influence of Mutations in Genes Involved in Siderophore Biosynthesis or Regulation of Siderophore Production on the Antagonistic Effect of *Pseudomonas* Strain 7NSK2 Against *Pythium* spp.**

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*Pseudomonas aeruginosa* 7NSK2 is a plant growth-beneficial bacterium which can significantly increase the dry weight of plants grown under unfavourable conditions. Under iron-limiting conditions and in the presence of certain heavy metals, *P. aeruginosa* 7NSK2 produces pyoverdinin, a yellow-green fluorescent siderophore, and pyochelin, a thiazoline-derivate. Via transposon mutagenesis (Tn5) mutants impaired in the biosynthesis (pyoverdinin deficient or pyoverdinin and pyochelin deficient) or regulation of siderophore production were obtained. Two regulatory mutants were isolated. Mutant M2 was unable to produce siderophores under iron-limiting conditions, but siderophore production was induced in the presence of  $Zn^{2+}$  or  $Cd^{2+}$ . Mutant SSS still produced siderophores under iron-stress, but did not react to heavy metals anymore. In-vitro antibiosis of the various mutants against a plant pathogenic *Pythium*

sp. was tested on KB medium with and without added  $Fe^{3+}$ . The wild-type strain 7NSK2, the SSS mutant and a pyoverdinin-deficient mutant inhibited the growth of *Pythium* on KB plates, while the double siderophore mutant and the regulatory mutant M2 had no effect. No antibiosis was observed on iron-amended plates. In a filter paper assay with *Pythium* sp., supernatant of a 7NSK2 culture grown in KB medium, but not the washed bacterial cells, protected tomato seedlings against *Pythium* infection. In non-sterile pot soil, artificially infested with various amounts of *Pythium* oospores, the wild-type strain 7NSK2 (soil-inoculated) was able to increase the number of healthy tomato seedlings with 30–50% in comparison with the *Pythium*-infested control. The biosynthetic siderophore mutants had no protective effect at all. The regulatory mutants SSS and M2 slightly increased the number of healthy plants (10–30%). Applying the bacteria closer to the infection site by means of seed coating may improve the protective capacities of the antagonists.

### **Molecular Analysis of the Avirulence Gene *avr9* of the Fungal Tomato Pathogen *Cladosporium fulvum***

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Different races of *Cladosporium fulvum* have arisen as a result of adaptation of the fungus to resistance genes present in cultivated tomato. These new races have overcome their initial avirulence by evading recognition by the plant. In this way several fungal races have overcome the resistance gene of the cultivar Cf9 of tomato by a deletion of the avirulence gene *avr9* encoding the corresponding race-specific elicitor.

Genomic clones carrying the *avr9* gene were isolated from a genomic library ( $\lambda$ EMBL3) of race 5 of *C. fulvum* using a cDNA probe encoding the race-specific elicitor. Sequence analysis revealed a 59 bp intron, a possible TATA-box and several repeats in the promotor- and terminator-region.

Stable transformants were obtained after co-transformation of the *avr9* gene with pAN7-1 (Hygromycine resistance) to race 2.4.5.9.11, which is virulent on tomato cultivar Cf9. Cultivar specificity of these transformants was converted phenotypically from virulent to avirulent on tomato cultivar Cf9, indicating that the introduced *avr9* gene has changed the genotype of race 2.4.5.9.11 into race 2.4.5.11.

This is the first report on the cloning of a fungal avirulence gene of which the processed gene product (28 aa.) is the primary inducer of the hypersensitive response (HR) in a resistant cultivar. Many avirulence genes of plant pathogenic bacteria have been cloned until now, but for none of them the gene product itself induces HR.

### Induction of Genes for Proteinase Inhibitors and Other PR-proteins in Tobacco by Virus Infection, Wounding and Systemin

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Pathogenesis-related (PR) proteins are induced in plants reacting hypersensitively to infection by viruses, viroids, fungi and bacteria. Their accumulation is temporally correlated with the induction of a broad resistance against subsequent infection, both in the infected as well as in the uninfected parts of the plant (systemic acquired resistance). We have characterized the genes encoding tobacco PR-proteins and have studied their expression upon various forms of stress. These studies have shown that PR-proteins can be divided into homologous sets of extracellular proteins, which in tobacco are all acidic and intracellular isoforms which are relatively basic. Among the PR-proteins are chitinases and glucanases, with a potential antifungal function and osmotins, which have recently been found to possess antifungal activity.

Wounding of tomato leaf, such as caused by feeding insects, triggers the synthesis of specific proteinase inhibitors (PI), which are capable of specifically suppressing the insects digestive enzymes, thereby preventing further attack. Like the basic PR-proteins PIs accumulate intracellularly in the vacuoles. In the present study we have isolated cDNA probes for the tobacco homologue of the tomato PI-1 and used that to follow the induction of the tobacco PI-1 genes by various stress conditions. Furthermore, we used exogenously applied tomato systemin, which functions as a peptide hormone triggering the systemic induction of PI genes in tomato, to investigate its potential to induce tobacco PR-protein genes. Thus it appeared that basic PR-proteins and proteinase inhibitors, which are both involved in the active protection of the plant against different forms of attack from the environment, are co-ordinately expressed.

### Carnation Phytoalexins, Facts and Artefacts

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On fungal infection, carnations (*Dianthus caryophyllus* L.) accumulate hydroxydianthalexin B (HDxB, 2-phenyl-7-hydroxy-1,3-benzoxazin-4H-one, formerly called dianthalexin) and a series of dianthramide phytoalexins (Ponchet, M. *et al.* (1988): *Phytochemistry* 27, 725-730). Further analysis of isolated HPLC peak fractions of cv Pallas carnation

stems infected with *Fusarium oxysporum* f.sp. *dianthi* race 2 yielded a number of new 2-aryl-1,3-benzoxazin-4H-ones like hydroxydianthalexin S and methoxydianthalexin S and dianthramides like methoxydianthramide A and its methyl ester. A structurally related compound, the *meta*-hydroxyanilide of benzoic acid (HANB), formerly considered to be a pyrolysis degradation product of the dianthramide HDB (Niemann, G.J. *et al.* (1990): *Neth. J. Plant Pathol.* 96, 133-153), appeared to be a natural carnation phytoalexin. The presence of another phytoalexin, structurally not related to the dianthramides, was indicated.

The relative distribution of the phytoalexins localized by pyrolysis-mass spectrometry in the xylem appeared to differ from that found by HPLC in acetone extracts (Niemann, G.J. *et al.* (1991): *Phytochemistry* 30, 3923-3927). *In situ*, in the xylem, there is a predominance of 2-aryl-1,3-benzoxazin-4H-ones over the more fungitoxic *N*-aroylanthranilates. At least part of the dianthramides in the extracts has to be ascribed to artefacts apparently due to hydrolysis of benzoxazinones during isolation.

The occurrence of artefacts and the relatively high fungitoxicity of the dianthramide MDS, HANB and the unidentified phytoalexin comparative to that of HDxB and HDB, may lead to a re-evaluation of the background of existing correlations with plant resistance.

### Monoclonal Antibodies for Detection of *Botrytis cinerea* on Gerbera Flowers

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The fungus *Botrytis cinerea* has become one of the most important threats for the production and export of cut flowers in the Netherlands. Spores of *B. cinerea* spread easily through the air and can infect flowers through their ray florets. However, for their germination they need water, for instance in the form of condensation.

Condensed water is easily formed on the ray florets during transport of the flowers after the harvest when they are frequently moved from a relative warm environment to the cold and vice versa. A reliable screening method would be of great value for the in-process control of *B. cinerea*, i.e. to check for the presence of the fungus in the process from growth to sale of the flowers.

Therefore, we are developing an immunoassay based on monoclonal antibodies (MAs) against conidia of *B. cinerea*. Mice were immunized with whole conidia, membrane fragments of conidia or their extracellular proteins. From hybridoma fusion experiments two monoclonal antibodies were obtained which may be of use for an immunoassay. MABC 1 is genus specific as it also reacts with conidia

from *B. squamosa* and *B. aclada*, but it does not react with about 15 other fungi which are frequently found in the atmosphere of greenhouses where cut flowers are grown. MABC 2 is *B. cinerea* specific. However, this MA does not react equally well with all isolates of *B. cinerea*. In immunofluorescence, the number of fluorescent conidia ranged from 40 to 100% dependent on the isolate used.

MABC 1 and MABC 2 as well as some recently obtained new MAs are presently characterized in more detail in order to evaluate their value for routine diagnostic purposes. The routine immunoassay will be used either before the flowers are cut or at the flower auction before they are sold.

### The Cause of Replant Disease in Asparagus

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Replant disease in asparagus is well-known in every region of the world where asparagus is grown. The cause of this disease in The Netherlands is not sufficiently known. The causal factor is very persistent: yield reductions occur even on fields where production was stopped 15–25 years ago.

Investigations into the cause were focused on the role of root residues as a source of autotoxins and on that of soil-borne fungal pathogens.

Root residues decompose extremely slowly in soil: 10 years after asparagus production was stopped, the residues still amounts to 400–1100 kg ha<sup>-1</sup> (dry wt). Extracts of roots of living plants and of 10-year-old root residues were toxic to young plants. Autoclaving these extracts did not annihilate toxicity. Sterilized roots added to soil in high amounts (15 g l<sup>-1</sup>) inhibited growth and non-sterilized roots were even more inhibitory. Extracts of asparagus roots had a selective effect on growth of soil fungi.

An analysis of fungi present in diseased tissue of roots and stem bases revealed that *Fusarium oxysporum* was by far the most frequently isolated species. In pot experiments under greenhouse conditions this fungus caused considerable damage. Replant disease could be reproduced in pot experiments. After a growth period of 3 months, plants grown in asparagus soil had lower root weights and lower numbers of (healthy) feeder roots than plants grown in fresh soil. The inhibitory factor could be eliminated by heat treatment of soil at 60°C. This suggests that a microbial agent is involved and that direct growth inhibition by autotoxins is of minor or no importance in soil. Results of all pot experiments showed a close correlation between a reduction of root weight and the presence of lesions caused by *F. oxysporum*. It was concluded that *F. oxysporum* f.sp. *asparagi* plays the major role in replant disease of asparagus in The Netherlands.

### Stimulation of Nematode Hatching by Herbicides in an Integrated Plant Protection System

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After application of the thiocarbamate herbicides diallate and cycloate, an increased infestation of sugar beet by beet cyst nematodes (*Heterodera schachtii*) can often be observed. However, a synergistic effect in the control of these nematodes was found when systemic nematicides, such as aldicarb were used together with the thiocarbamates. This can be explained by an increased permeability of the lipid membrane of the nematode's egg-shell, caused by the thiocarbamate herbicides. This leads to a higher penetration of aldicarb and its toxic metabolites into the egg and also to an increased hatching of the larvae, bringing them into a life stage, more sensitive to nematicides. It is possible that there is also an indirect effect of thiocarbamates on hatching and mobility of the nematodes towards the host plant, namely via an altered exudation of root diffusates by the host plant, exposed to thiocarbamate herbicides.

### Specific Interactions Between Trichodorid Nematodes and Tobacco Rattle Virus

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Tobacco rattle virus (TRV) is transmitted in nature by *Paratrichodorus* and *Trichodorus* nematode species. The virus causes diseases in potatoes (spraing, stem mottle), ornamental bulbous crops (notched leaf, malaria, rattle) and a variety of other crops when grown on light to medium light soils which are the preferred habitats of the vector nematodes. Results from bait-tests using individual trichodorid nematodes showed that specific associations between vector species and antigenic properties of TRV-isolates exist. For example, *P. pachydermus* from a variety of locations, from three different countries all transmitted TRV-isolates of the PRN-serotype, whereas other vector species transmitted TRV-isolates representing different serotypes. Furthermore, virus-free *P. pachydermus* from Scotland acquired and transmitted PRN-serotype isolates originally transmitted by individual *P. pachydermus* but failed to do so when allowed access to TRV-isolates belonging to other serotypes.

Virus-free *P. pachydermus* from The Netherlands acquired and transmitted TRV from Scotland, the virus-free *P. pachydermus* from Scotland acquired and

transmitted TRV from The Netherlands indicating that transmissibility is not dependent on geographical origin of vector or virus. However, both virus-free populations failed to acquire and transmit TRV-strain PLB, which had been isolated from infected potato tuber and which was serologically indistinguishable from isolates transmitted by *P. pachydermus*. These results suggest that the virus coat protein plays an important role in determining vector specificity and transmissibility and that minor differences in the coat protein may strongly affect transmissibility.

### Resistance of Starch Potato Cultivars to the potato Cyst Nematode *Globodera pallida* in the Northeastern part of The Netherlands

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Starch potatoes have been grown intensively in the northeastern part of The Netherlands. From fields in this area, soil was sampled that contained the potato cyst nematode *Globodera pallida*. Differences in virulence of 392 *G. pallida* populations to a set of seven cultivars of starch potatoes were investigated, using closed beakers. For each combination of population and cultivar the square root of the relative reproduction was determined. The relative reproduction was defined as the ratio of the number of newly-formed cysts that were visible through the transparent walls of the beakers and the number of new visible cysts on the susceptible cultivar Mentor.

By means of cluster analysis, several virulence groups of *G. pallida* were distinguished. On the more resistant varieties, the relative reproduction of the most virulent group was about 11 times as high as of the least virulent group. The ranking of the cultivars in order of resistance was rather consistent for the different virulence groups. However, the arrangement of the virulence groups in order of virulence was less consistent for the different cultivars.

Differences in vertical resistance of the cultivars, as revealed by principle component analysis, could well be explained from their descent from distinct sources of resistance to *G. pallida*.

## MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 28 JANUARY 1992

### Macrophytes in Dutch Streams: Victims of Transboundary Water Pollution?

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Transboundary rivers and streams carry with them amounts of dissolved and particulate material into

### Pathogenicity, Vegetative Compatibility and Restriction-Fragment Length Polymorphism in *Fusarium oxysporum* f.sp. *gladioli*

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Isolates of *Fusarium oxysporum* collected from diseased gladioli and other Iridaceae were examined for pathogenicity, vegetative compatibility and DNA restriction-fragment length polymorphism (RFLP) to investigate the genetic variation in the population. Pathogenicity of isolates was tested on the two differential cultivars Peter Pears and Nymph. Isolates could tentatively be divided into two races. The isolates pathogenic to both gladiolus cultivars were designated race 1, while isolates only pathogenic to 'Nymph' were named race 2.

All self-compatible isolates of *Fusarium oxysporum* f.sp. *gladioli* belonged to one of three distinct vegetative compatibility groups (VCGs) 0340, 0341 or 0342, and were incompatible with isolates not pathogenic to gladiolus. Isolates of race 1 were restricted to VCG 0340 while isolates of race 2 were present in all three VCGs (Roebroek, E.J.A. and J.J. Mes (1992): *Neth. J. Plant Path.* 90: 57-64.). HindIII digested total DNA of *Fusarium* isolates were hybridized with a HRP-labelled DNA-fragment from *Fusarium oxysporum* f.sp. *dianthi* (Manicom, B.Q. *et al.* 1987, *Phytopathology* 77: 669-672) and autoradiographed. After cluster analysis of the fragment patterns three distinct groups could be identified which fully corresponded with VCGs.

Isolates from iris, freesia and crocus were pathogenic to at least one of the two gladiolus cultivars and could be assigned to one of the three VCGs/RFLP-groups. This confirms the hypothesis that the host range of the forma specialis *gladioli* includes more genera of the Iridaceae. We have identified two races. Both races should be taken into account in resistance breeding programs, but as long as no genotypes are found that are susceptible to race 2 and not to race 1, it will be sufficient to use isolates of race 1 to screen for resistance to *Fusarium* in gladiolus. Most of all, we have developed methods to further characterize *Fusarium oxysporum* f.sp. *gladioli* isolates.

The Netherlands. The aquatic vegetation of these streams has been decreasing over the last 20 years. Sewage effluent, consisting of dissolved nutrients and metals and of organic particulate material, is probably one of the main causes for this decrease.

In many transboundary streams, a change in vegetation composition is observed. Comparing the current situation with c. 20 years ago, a tendency

is seen towards more highly productive species, generally not occurring in flowing water (e.g. *Elodea nuttallii*, *Glyceria maxima*, *Potamogeton crispus*, *P. pectinatus*, *P. trichoides*). Simultaneously, many species disappeared from several streams (e.g. *Apium nodiflorum*, *Elodea canadensis*, *Ranunculus peltatus*).

The inorganic nutrient status of the water is not likely to be an important factor in this process. Transplantation experiments with *R. peltatus* showed that this species could not survive in a sewage-polluted stream, with a moderate nutrient concentration and a large load of organic particulate matter.

Sediment transplantation experiments among a polluted and a non-polluted stream with *R. peltatus* and *R. natans* showed that the polluted stream inhibited plant growth, so that turbidity is probably the main inhibiting factor. The polluted-stream sediment stimulated plant growth. Laboratory experiments with *R. peltatus* and *R. natans* showed that when plants were grown on a sewage-polluted stream sediment, the percentage survival was much lower compared with a clean sediment. However, the stream sediment supplied more nutrients. This was a positive factor in plant growth.

Sewage effluent, causing turbidity and organic sediment layers, can be considered a serious pollutant in our streams, with detrimental impacts on the stream vegetation.

### Choosing the Right Place for the Introduction of Plant Species by Using Vegetation Composition

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Introduction of plant species is a management technique that might be used for species conservation or conservation of a vegetation type. The aim of introduction is the establishment of a new viable population of a target species (often rare species) in a target area. Carefully choosing the target area is vitally important to the success of any introduction.

Both abiotic and biotic factors control the establishment of target species in a vegetation. The species composition of the vegetation in the target area can be used as an indicator of the controlling environmental factors in that area. The comparison of the target area with the vegetation characteristic from one or more sites with a viable population of a target species gives an indication of the suitability of the target area for the introduction of the target species. The comparison can be made in several ways.

One method is to collect releves with the target species (reference releves) from sites with viable populations. Homogeneous groups of releves and

synoptic tables can be established by clustering and/or ordination techniques. Releves of the vegetation without the target species, made in the target area, can be compared to those synoptic tables. Such a comparison should take into account both the mean abundance and the percentage of releves in which the individual species were found.

A second method is to combine the reference releves with the releves from the target area and analyse all releves together with indirect ordination or other multivariate methods. This method gives the relative position of the target-area releves to the reference releve scores.

The methods need calibration before they can be used in the field for judging sites, because otherwise they only give relative measures of the suitability of the target area. Calibration can be done by actually introducing the target species to several target sites of several numerical distances to the reference releves.

There are some restrictions to the use of the methods using vegetation composition only. First, the methods cannot be used in situations where rapid succession takes place, unless detailed information is available on species composition during succession. Because of changes in environmental conditions, vegetation composition may reflect conditions of the past. Secondly, for a similar reason, a poor accessibility of the target area for other species than the target species may also distort the judgement on suitability. In both cases other target-site characteristics like abiotic factors should be taken into account before making any judgement. Finally, the choice of the reference releves can be difficult if there are no or very few proper reference sites left. In that case one could sometimes resort to releves made in the past.

### Anthropogenic Vegetation Changes during the Alleröd Period (Late Glacial) in the South-East of The Netherlands

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In the south of The Netherlands, late palaeolithic Federmesser settlements are known from cover-sand ridges surrounded by wet depressions. These hunter/gatherers were highly dependent on their natural environment and lived off all kinds of (non-migrative) animals, seeds and fruits. Due to their way of life, it is presumed that their impact on the vegetation was very small.

Pollen analysis on different cores along a transect allows a reconstruction of vegetation, in space as well as in time. Near Milheeze (Noord-Brabant, The Netherlands) a transect was made from a cover-sand ridge (with archaeologically proved Palaeolithic occupation), into a former adjacent lake. On the ridge,

a disturbed (Late Alleröd) Usselo soil was found. Four cores were analysed, one from the cover-sand ridge, two from the lake margins and one from the lake centre. In this centre the regional vegetation will be reflected, while the ridge and margin cores will show more (extra) local developments.

One lake-shore diagram shows four, strong minima of *Pinus* pollen during the second half of the Alleröd period. Pollen influx values indicate that during that time only a few pine trees were standing on the cover-sand ridge. Charcoal concentrations are independent of these minima. Also the duration of the *Pinus*-minimum periods (c. 25 year) indicate that natural fires and regeneration have not been responsible for this feature. The cyclicity and different depths of these minima are probably the result of the repeated human occupation of the cover-sand ridge, also known from archaeological investigations.

This leads to the conclusion that, during the Alleröd, groups of Late Palaeolithic hunters/gatherers near the present Milheeze have probably been responsible for fine scaled local vegetation changes. In particular they will have influenced the number of pine trees standing in their immediate surroundings.

### Palynology of the Post-neolithic Period in the Rheinische Loessborden (Germany), in Relation to the Archeological Record

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In the Early Bronze Age, the natural forest of the loess region was dominated by lime (*Tilia cordata*). Elm declined at about 4000 B.P., related to the neolithic practice of feeding cattle with branches and leaves. Lime gradually declined in the late Bronze Age during the so-called 'Urnenfelderkultur' period (3100–2700 B.P.), while oak pollen is more abundant. Beech immigrates in the region. In the Bronze Age, palynological evidence of crop growing is rather scarce.

In the Iron Age (from 2700 B.P.), a major shift in cattle farming took place, leading to the establishment of species-rich hayfields and meadows in the valley floors. Alder declines, grasses and sedges become dominant in the local pollen rain. The high values for pine pollen in this period can be interpreted as an extra-regional pollen influx, indicating the strong degradation of the forests in the region. Cereal pollen occurs frequently.

In the Roman period non-arboreal pollen declines markedly, the grassland vegetations in the valleys disappear, the forest regenerates and hornbeam immigrates, leading to establishment of the *Stellario-Carpinetum*. We know from the archeological record

(numerous *villae rusticae*), that the area was a major source of agricultural products. The pollen record can only be explained by the assumption that the Roman colonizer designated the entire loess region as a primary source of cereals. The cereal species grown in the Roman period (as in previous times), are cleistogamic and pollen is only released by threshing and winnowing. This makes it very difficult to estimate the importance of cereal growing from the pollen record. In the same time, the cattle needed for ploughing, harvesting and dung production, was held in close vicinity of the village. The use of the remaining forest was strictly organized. Walnut and sweet chestnut were introduced.

In the early medieval times the valley floors were once more transformed into grassland; rye, an allogamic cereal with excellent pollen dispersion became an important crop and cornflower pollen occurs regularly. Heathland, which already existed in the Iron Age, expands. The area of upland forest gradually decreased.

### Nutrient Limitation during Grassland Succession after Stopping Fertilizer Application

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To examine the nutrient limitation during grassland succession, we determined nutrient concentrations in the dominant plant species and performed a fertilization experiment, in four hay fields situated in the nature reserve 'Stroomdallandschap Drentsche A' (The Netherlands). In this area, fertilizer application was stopped and mowing continued in order to restore former species-rich communities. This yields a successional sequence from relatively nutrient-rich (field A) to relatively nutrient-poor fields (field D). Field A (1 year unfertilized) was dominated by *Agrostis stolonifera*, field B (5 years unfertilized) by *Holcus lanatus*, field C (19 years unfertilized) by *Anthoxanthum odoratum*, and field D (45-years unfertilized) by *Juncus acutiflorus*. The experiments were set up in April 1990 and were harvested in July 1990 and 1991. When comparing the different fields, from A to D, we initially found a change in biomass allocation from shoot to fine roots while finally rhizomes became more important. Furthermore, species richness increased. For the determination of the changes in internal nutrient concentrations, we used species which appeared in more than one field and compared within each species the concentrations of nitrogen, phosphate and potassium. We thereby assumed that a decreasing concentration in the plant was an indication of increasing limitation by a specific nutrient. These results showed, from field A to B, increased

limitation of all nutrients. Subsequently from field B to C, we found a serious increase in potassium limitation, while finally in field D all nutrients became equally limiting. The fertilizer application experiment showed clear differences between species in response to fertilization. This experiment revealed that nutrient limitation changed from field A to field D, from initially just nitrogen limitation to both nitrogen and potassium limitation, while finally all three nutrients became limiting. This result was consistent with the results from the internal nutrient concentrations.

### Water Quality and Vegetation Gradients in a Dune Slack Complex

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The decline in calciphilous plant species of the Kapenglop, a dune slack on the Wadden island of Schiermonnikoog is well documented. They disappear from the slopes, but survive at the foot of small sand dunes (Grootjans, A.P. *et al.* (1991): *J. Veg. Sci.* 2: 545–554). The mineral topsoil of the dune-slack complex turned out to be completely decalcified to a depth of 2 m. The influence of sand blowing from dunes with a high lime content is not found to be significant. Only a change in a local hydrological system could explain the dramatic changes in the Kapenglop area (Grootjans, A.P. *et al.* (1992): *Proc. Coastal Dune Congr* 1989).

In the present hydrological study, the pattern of disappearance of calciphilous species is explained by the following hydrological mechanism. During the wet season, infiltration water accumulates under the small sand dunes in the slack. A decline in water level causes a reduction in evapotranspiration on the top of the dune. In the lower parts evapotranspiration continues and the infiltration water is quickly replaced by calcium-rich groundwater. As a result, the lower parts of the small sand dune become a suitable habitat for calciphilous species. In former days, the Kapenglop area was more influenced by seepage. High water levels were prolonged and less reduction in evapo-

transpiration occurred on the top of the dunes. A larger part of the slopes was influenced by calcium-rich groundwater and this provided more habitats for rare species.

### Growth, Morphology and Leaf Characteristics after Simulated Herbivory in Saplings of three Subtropical Evergreens

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Growth, morphology and leaf characteristics were studied after simulated late-seasonal defoliation in second-year saplings of three Chinese subtropical evergreen trees: *Castanopsis fargesii*, *Elaeocarpus japonicus* (both late-successional) and *Pinus massoniana* (early-successional). On 1 October, the leaves of experimental plants were clipped by 0, 50, 75 and 90% of their area, respectively. Newly expanded leaves were clipped by the same percentages until 6 November. On 20 May after the leaf flush, the plants were harvested. In *Castanopsis* exact compensation in terms of plant biomass was shown after removal of 50 and 75% of the leaf tissue (as compared to unclipped plants), and very slight undercompensation after 90% defoliation. After increasing levels of defoliation, new leaf growth was increasingly favoured at the expense of root growth. The morphology of the above-ground parts changed after defoliation: with increasing defoliation level more new leaves were formed per unit of stem and branch length, the average size of these new leaves decreasing and their per-area organic nitrogen increased. *Castanopsis* and *Pinus* both compensated for 50% leaf area removal, whereas *Elaeocarpus* did not. The fast regrowth in *Castanopsis* and *Pinus* corresponded with their larger relative investments of biomass in leaves than *Elaeocarpus*. Their favourable response to herbivory may partly explain why *Pinus* and *Castanopsis* dominate in their respective phases of succession and *Elaeocarpus* does not.