Acta Bot. Neerl. 39(3), September 1990, p. 305–320

Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 1 DECEMBER 1989

Organization of Cellulose Microfibrils and Cortical Microtubules in the Shoot Apex

M.M.A. Sassen and A.M.C. Wolters-Arts.
Department of Experimental Botany, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

The arrangement of cellulose microfibrils (CMFs) is different in various regions of the shoot apex: anticlinal in tunica cells, random in corpus cells and transverse in cells of the rib meristem (Sakaguchi *et al. Planta* 1988, **175**: 403–411). These data are based on examinations of cryosections of *Vinca major* apices with the polarizing microscope. Careful examination with EM on *Petunia* and *Vinca* apices confirmed the above findings for tunica and rib meristem cells, but a different type of CMF arrangement was found for the corpus cells.

Apices of *Petunia hybrida* and *Vinca major* were fixed with a mixture of *p*-formaldehyde and glutaraldehyde, and embedded in polyethylene glycol. Longitudinal sections 5 µm in thickness were selected, transferred to nickel oyster grids and extracted with hydrogen peroxide and glacial acetic acid for 30 min at 90°C. After rinsing with water the grids were covered with a formvar film, air-dried, shadowcasted with platinum, and studied with TEM.

In corpus cells microfibrils are deposited in lamella-like sheets, each of which consists of parallel-oriented CMFs. Microfibrils of adjacent lamellae are sometimes interwoven, which means that these lamellae are deposited more or less at the same time but in different directions. Depending on the number of divisions and on the direction of division, corpus cell walls consist of varying numbers of lamellae. Near the pit fields, microfibrils in one lamella deviate, which supports the idea of a random orientation of CMFs. The orientation of CMFs in tunica cells is parallel to the newly formed cell wall, i.e. anticlinal. Cell walls of the rib meristem show parallel-oriented microfibrils perpendicular to the direction of extension of these cells.

As the direction of microfibrils is different for each lamella, it is understandable that the average arrangement, as seen with the polarizing microscope, is random. TEM studies, however, show that the newly synthesized CMFs in *Petunia* and *Vinca* corpus cells are deposited in more or less parallel arrays. This contradicts the generally accepted view that meristematic cells have a random texture. Since the authors mentioned above also found a random orientation of microtubules in the corpus cells, our conclusion is that no co-alignment occurs between microfibrils and microtubules in these corpus cells.

Freeze Substitution of Mature *Papaver rhoeas* Pollen

A.C. Van Aelst and M. Cresti[1]. Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands, and [1]Dipartimento di Biologia Ambientale, Universita di Siena, Via P.A. Mattioli 4, 53100 Siena, Italy

Fast freezing in liquid propane was used to create an absolute moment fixation of all cell constituents. Water inside and outside the cells changed into the vitrified solid state. Freeze substitution in acetone or methanol, in which 2% osmium tetroxide and 1% uranyl acetate is dissolved, was carried out in a special freeze substitution device (CS auto, Reichert) at −80°C.

Three fixation procedures were compared. A conventional chemical fixation in water and two freeze substitution procedures (acetone and methanol), resulted in striking differences in electron microscopical images. In freeze-substituted material, structures containing carbohydrates were highly contrasted, while (phospho)lipid-containing structures were electron transparent. The conventional chemical-fixed material showed the opposite: a high contrast of structures with a (phospho)lipid origin and electron transparency of the carbohydrate-containing structures.

Substitution fluids are organic solvents which have to dehydrate the cell cytoplasm and cell wall by dissolving the ice followed by the diffusion of water molecules. In order to fix and create contrasted structures inside the cell, a reaction of (glyco)proteins, (phospho)lipids and carbohydrates with osmium tetroxide and uranyl acetate has to take place. Unfortunately, these chemical fixatives start reacting at about −60°C. This means that during the substitution procedure at −80°C a selective extraction of cell constituents occurs.

It is concluded that chemical fixation and freeze fixation followed by dehydration procedures result in different electron microscopical images which are

additional to each other in the visualization of the ultrastructure of plant cells.

Experimental Manipulation of the Plane of Cell Division by Wounding

C.J. Venverloo. Department of Plant Molecular Biology, University of Leiden, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands

In vacuolated cells a phragmosome (PS) is formed before mitosis. The PS is a thin cytoplasmic sheath that is positioned at the site of the future cell wall. One of the functions of the PS is to guide the cell plate during cytokinesis. The question is whether the plane of cell division becomes fixed before or during the formation of a PS. The epidermis cells of small explants of *Nautilocalyx* leaves divided periclinally, transversely or longitudinally. When the explants were separated into two parts by a longitudinal cut, most cells near the wound divided longitudinally. Cells which already had a non-longitudinal PS before the wound was made showed different reactions: all cells at some distance (100–150 μm) from the wound and half of the cells close to the wound (0–100 μm) divided in accordance with the PS. In some of the cells close to the wound the original PS was replaced by a longitudinal PS and a longitudinal cell wall was formed. In some other cells the original PS was more or less disturbed and an oblique cell wall was formed.

These results show that the plane of cell division can easily be changed in cells without a PS, but that a strong signal is needed to change it in cells with a PS. In the last case a new PS is formed. This suggests that the plane of cell division is being fixed during PS formation, not earlier (a full account of this study has been submitted to *Protoplasma*).

An Ultrastructural Study of the Development of Oil Cells in *Annona muricata*

M.E. Bakker. Rijksherbarium, PO Box 9514, 2300 RA Leiden, The Netherlands

The ultrastructure of developing and mature oil idioblasts in the shoot apex and leaves of *Annona muricata* (Annonaceae) has been studied with light, fluorescence and transmission electron microscopy.

Three developmental stages can be recognized. *Stage 1.* The small cells do not differ from the surrounding cells by their size, but can be recognized by their less electron-dense cytoplasm, plastids lacking thylakoids, and vacuoles devoid of deposits. *Stage 2* resembles stage 1, but in the cells a suberized layer has been deposited against the initial cell wall. In the cells of *stage 3* an additional layer has been deposited against the suberized layer: the inner wall layer. The cells in stage 3 also contain an oil cavity, surrounded by the plasmalemma. It is attached to the cell wall by a cupule, a bell-like protrusion of the inner wall layer. The plastids contain small plastoglobuli, presumably oil. Larger oil droplets appear loosely scattered in the cytoplasm and often situated near the oil cavity, where they apparently pass through the plasmalemma surrounding the oil cavity. Two specific cell organelles, not reported previously for oil cells, occur in this stage: smooth tubular endoplasmic reticulum, appearing as bundles of linearly arranged tubules, and groups of crystalline bodies, which are probably of proteinaceous origin. In the course of stage 3 the thickness of the inner wall layer, the relative volume of the oil cavity, and cell size increase.

Distribution of Non-Articulated Branched Laticifers of *Morus nigra* L. (Moraceae)

R.W. den Outer and W.L.H. van Veenendaal. Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

The origin, development and arrangement of the branched, non-articulated laticiferous system of *Morus nigra* L. (Moraceae) were studied. Embryos, seedlings, leaves, stems and roots of young and mature plants were investigated using light and scanning electron microscopy. There are two laticiferous systems present in the older plant, a primary and a secondary one. The primary laticifers arise from eight initials in the outer periphery of the future procambium near the cotyledonary node of the young heart-shaped embryo. They produce the first-formed transport system of the plant. No additional primary laticifers are formed in other primary tissues. Secondary laticifers of the same type as the primary ones are produced by initials of the vascular cambium in stem and root. Their number is larger and variable. No fusions of laticiferous cells with one another or adjoining parenchyma cells were observed. The extremely elongated, thin-walled, branched laticiferous cells are devoid of pits. They exhibit a combination of intrusive and symplastic growth; penetrations of other cells were not observed. Laticifers are present in all vegetative tissues with the exception of epidermis, and primary and secondary xylem. The close developmental and distributional relationship between laticifers and phloem suggest a possible functional relationship.

Wood Anatomical Research—its Current Status

P. Baas. Rijksherbarium/Hortus Botanicus, PO Box 9514, 2300 RA Leiden, The Netherlands.

An analysis of the plant anatomy literature database, maintained at the Jodrell Laboratory, Royal Botanic Gardens Kew (UK) by Miss Mary Gregory, revealed

that the number of wood anatomical publications per 5-year period had substantially increased between 1965 and 1985 but now shows a slight decline. Systematic wood anatomy and other classical aspects of comparative wood anatomy mirror this overall trend and have profited from a renewed interest with the advent of scanning electron microscopy. Ecological wood anatomy provides one of the causal explanations of wood structural diversification in plant evolution and shows a steep increase in number of publications over the last 25 years. The same applies to functional wood anatomy, although the total number of studies on hydraulic architecture and metabolic and defence functions of wood in trees is still disappointing. Publications on growth rings and on wood structure in relation to pollution also dramatically increased in number. Studies on xylem development declined in number over the last 5-year period, although important new developments can be registered in terms of integrative morphogenetic hypotheses. The number of publications on wood identification fluctuates over the past 25 years; standardization of wood anatomical features and international co-operation in establishing computerized databases are major recent developments.

Studies to explain or predict physical and mechanical properties from wood structure seem to lag behind these increases in publications on botanical aspects of wood anatomical diversity (see also Baas, P. *IAWA Bull. n.s.* 1989, **10:** 333–334).

MEETING OF THE SECTION FOR PLANT SYSTEMATICS AND GEOGRAPHY ON 24 NOVEMBER 1989

Taxonomy and Phytogeography of 11 Genera in Pinaceae

A. Farjon. Institute of Systematic Botany, University of Utrecht, Heidelberglaan 2, PO Box 80.102, Utrecht, The Netherlands

The family Pinaceae is, with *c.* 220 species, the largest of present-day conifers. Several species of the larger three genera dominate the boreo-montane forests of the northern hemisphere. The majority of genera and species, however, must be regarded as more or less rare palaeoendemics with more extensive ranges in Tertiary time. Monographic work on all genera except *Pinus* has led to a revised classification, based on phenetic comparison of 25 characters ranging from chemistry (e.g. immunology) and karyology to anatomy and morphology. The present author accepts 11 genera in Pinaceae, three of which are monotypic. A review of the long (since Tournefort's 'Institutiones', 1694) history of grouping the conifers shows considerable variations of opinion up to the present day. Despite criticisms, Pilger's system (in Engler and Prantl: '*Die natürlichen Pflanzenfamilien*', 2nd edn. 1926) is still widely used in modern textbooks. This system groups nine genera in Pinaceae as follows (revised version, 1954).

Pinoideae with genus *Pinus*
Laricoideae with genera *Cedrus*, *Larix* and *Pseudolarix*
Abietoideae with genera *Abies*, *Keteleeria*, *Picea*, *Pseudotsuga* and *Tsuga*

The dividing characters are leaf dimorphism (*Pinus*) and shoot dimorphism (all genera in Pinoideae and Laricoideae). Close scrutiny of this reveals an entirely clinal differentiation in shoot dimorphism from very high in *Pinus* to virtually absent in *Abies*, *Picea* and *Pseudotsuga*, with newly discovered (*Cathaya*) or recognized (*Nothotsuga*, *Hesperopeuce* of Page, 1988) genera in intermediate positions. Moreover, this classification is in conflict with most other characters evaluated. A new classification is therefore proposed (see also Frankis, *Notes Roy. Bot. Gard. Edinb.* 45 (3), 1989: 527–548).

Pinoideae with genus *Pinus*
Piceoideae with genus *Picea*
Laricoideae with genera *Cathaya*, *Pseudotsuga* and *Larix*
Abietoideae with genera *Cedrus*, *Abies*, *Pseudolarix*, *Keteleeria*, *Nothotsuga* and *Tsuga*

Within Laricoideae, *Pseudotsuga* and *Larix* are closely related, within Abietoideae *Cedrus* and *Abies*, and *Pseudolarix*, *Keteleeria* and *Nothotsuga* form natural groupings. The family as a whole divides in two: an 'abietoid' (Abietoideae) and a 'pinoid' group of genera. Phytogeography generally confirms this modernized classification, which forms the taxonomic framework of a monograph: '*Pinaceae*' (approximately 340 pages, with 118 plates and 123 maps) to be published in 1990 by Koeltz Scientific Books, Koenigstein, FRG.

Systematics and Stomata in Connaraceae

C.C.H. Jongkind. Department of Plant Taxonomy, Gen. Foulkesweg 37, Wageningen, The Netherlands

In Connaraceae the shape of the stomata and the pattern formed by the surrounding epidermis cells

are useful taxonomic characters. This pattern and the shape of the outer stomatal ledge show a large variation within the family. They are, however, often surprisingly constant within each genus. Both characters are rather easy to recognize and this enables us to use them in combination with other leaf characters, to identify sterile samples down to the genus and in many instances even to the species level. Patterns of neighbouring cells also reflect affinities between taxa.

The most common and probably most primitive pattern in Connaraceae is that in which the stomata are surrounded by four to seven epidermis cells lacking a distinct orientation. Compared to the other epidermis cells, the size of the cells surrounding the stomata may be smaller (cyclocytic) equal to (anomocytic) or larger (actinocytic). These three types are not sharply delimited and gradual transitions occur between them in Connaraceae, particularly between the first two types. These patterns are typical for all genera of the tribes Connareae (= *Burttia, Connarus, Ellipanthus, Hemandradenia* and *Vismianthus*) and Manoteae (= *Manotes*), and for a small part of the Cnestideae. Of the five genera of the Cnestideae only a minority of the species of *Rourea* show this pattern.

Other patterns found in the Cnestideae, are the anisocytic pattern in *Agelaea, Cnestidium*, and part of *Rourea* and the paracytic pattern in *Cnestis*, part of *Rourea*, and probably also in *Pseudoconnarus*. The stomata in *Pseudoconnasus* are difficult to see because of the strongly papillate epidermis. In the anisocytic pattern there are three epidermis cells surrounding the stomata of which one is smaller than the others, these cells lack a distinct orientation. In the paracytic pattern on both sides of the stoma there is one epidermis cell parallel to the guard cells. In many accessions of *Agelaea* one can observe the helicocytic pattern and the anisocytic together in one and the same leaf.

The fourth tribe Jollydoreae (= *Jollydora*) is characterized by a bicyclic pattern.

The shape of the outer stomatal ledge varies independently from the pattern of neighbouring cells but is again usually constant within a genus. (A full account on stomatal features and other leaf anatomic characters is given in Jongkind, C.C.H. 1989. Leaf epidermis and stomatal pattern. In: F.J. Breteler (ed.): *The Connaraceae a Taxonomic Study with Emphasis on Africa.* Agric. Univ. Wageningen Papers 1989, **6**: 21–39.)

Vegetative characters of taxonomic value in other parts of the plant proved to be available in this family as well, e.g. the colour and viscosity of the exudate and several features of the wood, such as the presence of interxylary phloem or the development of furrowed branches. It is regrettable that one is usually unable to make use of such practical characters as they are not known for all species. Collectors should pay more attention to them, report them on herbarium labels and preferably add representative wood samples.

Clonal Diversity in some Microspecies of *Taraxacum* Section *Palustria* (Lindb. fil.) Dahlst from Czechoslovakia and The Netherlands

J. Battjes, S.B.J. Menken, Y. de Jong and H.J.M. den Nijs. Hugo de Vries Laboratory, Kruislaan 318, 1098 SM Amsterdam, The Netherlands

The genus *Taraxacum* forms a polyploid complex in Europe. Diploids are mainly restricted to the southern parts of the widespread distribution area, whereas agamospermic polyploids are found throughout. Among the polyploid microspecies, taxa with either obligate or facultative agamospermy are known. Isozyme electrophoresis was used to determine clonal diversity in microspecies from the polyploid section *Palustria* in The Netherlands and Czechoslovakia (clonal diversity $Hg = 1 - \sum(f_i^2)$, where f_i is the frequency of the ith clone at a given locality).

The species were analysed for eight variable gene loci (*6Pgdh-1, 6Pgdh-2, Mdh, Adh-2, Shdh, Lap-1, Lap-2 and Gpi-2*). In The Netherlands, samples of *T. maritimum* (at five localities), *T. gelricum*) (one locality), and *T. hollandicum* (three localities) were examined. Mean clonal diversity per locality was $0{\cdot}083 \pm 0{\cdot}146$ for *T. maritimum*, and 0 (i.e. uniclonality) for both *T. gelricum* and *T. hollandicum*. Clonal diversity for these species is low in comparison with other clonal plant species (average over several studies: 0·62). Moreover, the preponderant clonal pattern for *T. maritimum* (85% of the 298 screened plants) was identical with the single one found in *T. gelricum*. The single *T. hollandicum* clone differed at most loci from the *T. maritimum* and *T. gelricum* genotypes.

In Bohemia, of 232 *T. hollandicum* individuals sampled at seven localities all except three showed the same clonal pattern as *T. hollandicum* from The Netherlands. The three aberrant individuals differed at one locus from the typical clone. Mean clonal diversity for *T. hollandicum* in Bohemia was $0{\cdot}018 \pm 0{\cdot}048$. To investigate whether *T. hollandicum* from The Netherlands and Bohemia really represent the same clone, small samples from both regions were screened for 11 additional variable loci and indeed showed uniclonality.

In contrast with *T. maritimum, T. gelricum* and *T. hollandicum*, the microspecies *T. vindobonense* had a high clonal diversity ($0{\cdot}897 \pm 0{\cdot}044$) at four localities in Slovakia. Progeny from one individual of this species consisted of two clones. Possible causes of the observed amount of clonal diversity and the variability in one offspring family in *T. vindobonense* are

facultative agamospermy (i.e. sexual reproduction) and autosegregation.

Two Methods in Historical Biogeography Applied to the Genus *Guioa* (Sapindaceae)

P.C. van Welzen. Rijksherbarium, PO Box 9514, 2300 RA Leiden, The Netherlands

Brooks' Parsimony Analysis (BPA; Wiley, E.O., *Ann. Rev. Ecol. Syst.* 1988, **19:** 513–542) and (Component Compatibility CC; Zandee, M. and Roos, M.C., *Cladistics* 1987, **3:** 305–332) were used to analyse the historical biogeography of the New Guinea species of *Guioa* (Welzen, P.C. van, *Leiden Bot. Ser.* 1989, **12**) and the cicada genus *Diceropyga* (Duffels, J.P., *Monogr. Ned. Entom. Ver.* 1977, **8:** 1–227). Both methods normally resulted in similar areagrams except for New Guinea, where BPA found a more parsimoneous areagram than CC did (resp. 87 and 101 steps). Methodologically, the more parsimonious areagram is always selected as the best hypothesis of geological events; in this case the BPA areagram. However, the geographical sequence in the BPA results was curious, e.g. the South Moluccas together with the Solomon Islands were postulated to have been one area, while the geographical sequence in the CC results was logical. Moreover, several areas and groups of areas in the BPA analysis were based on the absence of species, while all groupings in the CC areagram were based on the presence of species. Due to the computer method, BPA (Wagner analysis: computer programs PAUP and HENNIG[86]) can characterize areas on the basis of absence of species, while this is impossible with the CC method (Group compatibility: computer program CAFCA). Methodologically it is incorrect to postulate a relationship between areas on the basis of absence of species, this may only be done on the basis of presence of species. In conclusion: Component Compatibility is superior to Brooks' Parsimony Analysis in historical biogeography.

By comparing different groups with each other both methods also serve as independent tests of the phylogenies of those groups.

Distribution and Speciation in *Microseris* (Compositae)

K. Bachmann. Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands

The 16 species of *Microseris* (x = 9 chromosomes) occur in western North America with the exception of the allotetraploid perennial *M. scapigera* of Australia and New Zealand and the diploid annual *M. pygmaea* of Chile. The species have recently been assigned to three monophyletic groups on the basis of their chloroplast genomes: (1) the annual *M. lindleyi* alone, (2) all diploid perennials, (3) all other annuals plus *M. scapigera* (Wallace, R.S. & Jansen R.K., *Am. J. Bot.* 1988, **75:** 214). This confirms the hypothesis that the parents of *M. scapigera* are the perennial *M. borealis* (on morphological grounds) and a North American annual, which has now been identified as the female parent of the original hybrid. The unlikely chance speciation by hybridization, genome duplication and successful long-distance dispersal across the Pacific must have occurred and will next be studied at the level of nuclear DNA. We are doing this already with the hypothesized origin of *M. pygmaea* after chance dispersal of a single achene from California to Chile. Using a poly-GATA fingerprint probe (Traut, W., *Genetics* 1987, **115:** 493–498) we studied the molecular phylogeny of the Chilean populations and map mutations involved in species differentiation from the founder individual.

MEETING OF THE SOCIETY FOR PHYTOPATHOLOGY ON 11 JANUARY 1990

Effect of Genes for Partial Resistance to Leaf Rust in Barley on the Infection Attempts by Inappropriate Rust Fungi

R.E. Niks and R.G. Dekens. Department of Plant Breeding (IvP), Agricultural University, Wageningen, The Netherlands

Experiments were carried out to investigate whether genes for partial resistance of barley to leaf rust (*Puccinia hordei*) play a role in the non-host resistance of barley to inappropriate rust fungi.

A series of 22 barley lines ranging from extremely susceptible to highly resistant to leaf rust were inoculated with leaf rust of barley (LRB), leaf rust of wheat (LRW) (*Puccinia recondita* f.sp. *tritici*) and leaf rust of wall barley (LWB) (*P. hordei murini*). The proportion of early colony abortion and the size of established colonies were determined microscopically. The results suggest a low (rank correlation 0·30–0·38) but significant correlation between these parameters for LRB (partial resistance) with those for LRW and LWB (non-host resistance). Lines with a high level of partial

resistance to LRB were all highly resistant to LRW and LWB. Lines with a low level of partial resistance to LRB ranged from highly resistant to relatively susceptible to LRW and LWB. It is concluded that not all genes for non-host resistance in barley play also a role in partial resistance to leaf rust.

A Computer Simulation Model as a Tool to Develop a Testing Method for Resistance to Whitefly (*Trialeurodes vaporariorum* Westw.) in Tomato

W.A. Van Giessen and C. Mollema. Centre for Plant Breeding Research CPO, PO Box 16, 6700 AA Wageningen, The Netherlands

The lack of a reliable and easy-to-use testing method for resistance to whitefly in tomato has so far delayed the release of resistant lines. A new method was developed using leaf cages, in which four whitefly parameters per plant are measured: oviposition rate (OR), adult survival (AS), pre-adult survival (PS) and developmental period (DP). These parameters were then fed into a computer simulation model (WHITEFLY). This model describes the life cycle of whitefly in cohorts of 1 day. The different parameters dictate the speed with which this cycle is gone through and hence the (hypothetical) whitefly population growth rate. This simulated growth rate (r_s) is a measure for the level of resistance of a certain host-plant genotype. For the ultimate testing method, the best suitable parameter has to be selected, partly based on a sensitivity analysis with WHITEFLY (and partly on practical considerations). In this way it is possible to determine the relative contribution of each parameter to the growth rate of a population. Further exploration of the model is in progress.

Exchanges of Domains Within the 1a Protein From Two Bromoviruses and Their Effect on Viral RNA Replication

B.J.M. Verduin[1], P. Kroner, P.L. Traynor, and P. Ahlquist. Institute for Molecular Virology, University of Wisconsin, 1525 Linden Drive, Madison, WI 53706, USA, and [1]present address: Department of Virology, Wageningen Agricultural University, Binnenhaven 11, 6703 AW Wageningen, The Netherlands

Brome mosaic virus (BMV) and cowpea chlorotic mottle virus (CCMV) are two bromoviruses: positive single-stranded RNA viruses with similar tripartite genomes and particle morphology but different nucleotide sequences. They have different host ranges and RNA-1 of neither BMV nor CCMV is replicated by the heterologous virus. For both viruses, cDNA clones are available which produce infectious RNA transcripts. In this study domains of the 1a protein, a putative RNA polymerase, were exchanged between BMV and CCMV to locate amino-acid sequences which specify replication or host range determinants.

Oligonucleotide-directed mutagenesis was used to construct an *Eco*RV site in the plasmid containing the complete CCMV-RNA-1 sequence. This mutation is located in the middle of the 1a protein, a region containing amino-acid sequences which are conserved for both BMV and CCMV. The mutation is translationally silent. Using this site, plasmids were constructed in which the 5'-half of BMV was linked to the 3'-half of CCMV and vice versa. The respective plasmids were called PBC1DV3 and PCB1DV4 (BC1 & CB1).

The two plasmids were used to make modified transcripts of RNA-1. These transcripts were tested for their coding capability in a cell-free *in-vitro* translation system. Both directed synthesis of proteins of the expected molecular mass. Their full functioning in the replication of the virus was also tested in barley (*Hordeum vulgare* var. Morex) protoplasts in the presence of transcripts of RNA-2 and RNA-3, both from BMV and CCMV (three-component system). The two constructs were not able to supply the genetic information for the synthesis of detectable amounts of positive-sense viral RNA.

Finally, their function in three-component systems was tested in *Chenopodium hybridum* (local lesion host for both BMV and CCMV), barley (systemic host for BMV) and *Vigna sinensis* (Torner) Savi cv. Queen Anne Blackeye (systemic host for CCMV). Neither of the constructs induced symptoms.

In conclusion, exchanges of 5'- and 3'-halves of RNA-1 between two bromoviruses did not allow viral replication in plants and protoplasts. Further tests for replicability of the hybrid molecules in heterologous and homologous combinations, for synthesis of 1a protein and for synthesis of minus-sense viral RNA will be needed to elucidate the function of the 1a protein and its domains.

Genetic Studies of Virulence Inheritance of Potato Cyst Nematodes, *Globodera rostochiensis*, for the H_1 Resistance Gene in *Solanum tuberosum* ssp. *Andigena* CPC 1673

R. Janssen[1], J. Bakker and F.J. Gommers. Department of Nematology, Agricultural University, Binnenhaven 10, 6709 PD, Wageningen, The Netherlands, and [1]present address: Research Institute for Plant Protection, PO Box 6700 GW, Wageningen, The Netherlands

To estimate as precisely as possible intra-specific variations in virulence in populations of *G. rostochiensis*

and *G. pallida*, a method was developed to rear cysts on roots of sprouts of potato in Petri dishes. Frequencies of virulent phenotype values obtained with this method were more accurate than estimates on the basis of P_f/P_i-assessments determined in pot experiments.

A method was found to circumvent the diapause of potato cyst nematodes by avoiding desiccation of the cysts. Larvae were artificially hatched by cutting cysts into halves and subsequent incubation in potato root diffusate. These treatments had no influence on viability and fecundity. With the artificial hatching procedure it is possible to produce five to six generations a year in Petri dishes and three to five generations in pots.

Virulent and avirulent lines of *G. rostochiensis* for the H_1 resistance gene were selected after controlled single matings in Petri dishes. With these lines the inheritance of virulence was determined for the H_1 resistance gene after crossing the lines in Petri dishes. The 3:1 segregation in avirulent and virulent larvae of the F_2 generation, obtained by selfing the F_1, showed that virulence to the H_1 gene is controlled by a single major gene recessive to avirulence (allele A). The virulence percentages of the F_1 generations agreed with this finding. Reciprocal crosses showed no evidence of sex-linked inheritance of virulence.

The virulent (aa) and avirulent (AA) line were also used to analyse the effect of heterozygosity for virulence on the development of potato cyst nematodes. The genetic constitution of avirulent larvae (AA and Aa) of *G. rostochiensis* had no substantial influence on the development into males.

The Influence of Soil Temperature on the Rate of Root Development and Root Rot in Tulips Caused by *Pythium* spp.

R.J.M. De Greeff and T.L.J. Duineveld. Bulb Research Centre, PO Box 85, 2160 AB, Lisse, The Netherlands

Tulip bulbs for commercial flower production are cold-treated to imitate the winter period. Due to this cold treatment the period in which the tulips develop is shortened to 60 days or less depending on the cold treatment used. When tulips are cold-treated serious root rot problems occur, caused by *Pythium* spp., whereas under natural conditions root rot is almost negligible. Root rot is coupled with a decrease in root fresh weight and fresh weight of sprouts.

In our experiments, different rates of root development were attained by creating soil temperatures of 9°C, 12°C and 15°C, while the temperature of the air was kept at 17°C. Cultivar Gander was used in these experiments.

At 9°C the initial increase in root length and root fresh weight was significantly slower compared to that at 12°C and 15°C. No difference was found between development of root length and root fresh weight at soil temperatures of 12°C and 15°C. Development of sprouts at 9°C was slower than that at 12°C and 15°C during the whole period and plants reached a harvestable stage 1 week later.

When soil cooling was used, the development of root rot decreased significantly with decreasing temperatures. The negative effect of artificial inoculation on fresh weight of sprouts was significantly more pronounced at a soil temperature of 15°C.

Possible explanations for this reduction of root rot will be discussed.

Thermophilic Fungi in Mushroom Compost

G. Straatsma and L.J.L.D. Van Griensven. Mushroom Experimental Station, PO Box 6042, 5960 AA Horst, The Netherlands

Compost for cultivation of *Agaricus bisporus* is almost completely colonized with *Scytalidium thermophilum*. Growth of mushroom mycelium on sterilized compost is strongly stimulated by pre-incubating the compost with this thermophilic fungus. A positive relation exists between the density of *S. thermophilum* in experimental composts and ultimate mushroom yield. *S. thermophilum* is probably already present in wheat straw (Straatsma *et al. J. Gen. Microbiol.* 1989, **135**: 751–759).

Straw and agar media grown with *S. thermophilum* are used as *in-vitro* models to elucidate essential composting processes. Growth rates of mushroom mycelium on these substrates are almost as high as on normal compost. The results provide further evidence for the key role of *S. thermophilum* in mushroom cultivation.

Some additional data on wheat straw as the source of *S. thermophilum* are presented.

Effect of the Phytotoxin of *Verticillium dahliae* on Several Developmental Stages of Tomato

C. Glas, H.E. Miltenburg, B. Roy-Biswas, H.J.J. Nijkamp and J. Hille. Department of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Verticillium albo-atrum and *V. dahliae* are fungi pathogenic to tomato and other crop plants such as potato, cucumber and strawberry. Symptoms of the disease on tomato are: wilting, browning of the lower leaves and in severe cases death of the plant. Infection by *Verticillium* can cause a crop reduction of up to 70%.

Resistance against *V. albo-atrum* is conferred by the single dominant gene *Ve*; from *V. dahliae* two races are known, *Ve* also confers resistance against race 1 but

against race 2 no dominant resistance genes are known.

A fungal toxin is involved in *Verticillium* wilt development; this toxin can be isolated from culture fluid and is non-host specific, i.e. when injected in leaves, this toxin affects both resistant (Ve Ve) and susceptible (ve ve) plants to the same degree. The effect of the toxin on several development stages is investigated and a comparison is made between the effects on tomato (*Verticillium*-resistant and *Verticillium*-sensitive *Lycopersicon esculentum* cv. Craigella) as hosts and tobacco (*Nicotiana tabacum*) as non-host for the fungus.

The influence of the toxin was tested on the following developmental levels: germination of seeds; (detached) leaves; shoot regeneration on leaves *in vitro*; callus growth on leaves *in vitro*; shoot regeneration on callus *in vitro*; growth of minicalli and protoplasts *in vitro*. The toxin causes necrosis on leaves of both the susceptible and resistant tomato cultivars but has no effect on leaves of tobacco.

When isolated protoplasts of tomato were grown in media containing the toxin, the percentage of surviving protoplasts was affected at low concentrations in both resistant and susceptible tomato cultivars. Protoplasts isolated from tobacco showed a low sensitivity to the toxin. When the toxin was added to tomato or tobacco protoplasts after 12 days of growth without toxin, the harmful effect was much smaller. On the developmental levels of tomato and tobacco, no significant effect was noticed.

The *Verticillium* phytotoxin is toxic to tomato leaves and protoplasts; however, on other developmental levels the effect is much smaller or absent. Tobacco cells are far less sensitive to the toxin, implying that resistance to the *Verticillium* toxin, in principle, is possible. The *Verticillium* toxin plays an important role in pathogenesis, it also has a clear effect on tomato protoplasts but not on tobacco protoplasts; therefore it can be a potential selective agent for *in-vitro* selection at the cellular level for disease resistance in tomato cells.

Interaction Between *Fusarium oxysporum* f. sp. *lycopersici* and Callus of Susceptible and Resistant Tomato Lines

B.A.M. Kroon and D.M. Elgersma. Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, The Netherlands

Callus cultures of near isogenic lines of the tomato cultivars Moneymaker and Craigella, resistant or susceptible to race 1 or 2 of *Fusarium oxysporum* f. sp. *lycopersici* were initiated. Sterile hypocotyl pieces were placed on MS medium supplemented with naphthylacetic acid and benzylaminopurine. Callus cultures were subcultured every 3 weeks and 1 week before inoculation. Callus pieces (±1 cm diameter) were inoculated with *F. oxysporum* f. sp. *lycopersici*, race 1 or 2, using either drops of a conidial suspension or pieces of a mycelial mat grown in Petri dishes on potato dextrose agar. No differences in colonization between resistant and susceptible lines were found after inoculation of callus pieces with agar blocks with mycelium of race 1 or 2. Inoculation with a conidial suspension of race 1 of *F. oxysporum* f. sp. *lycopersici*, on the other hand, resulted in a different colonization of resistant and susceptible callus. The growth was restricted on callus derived from resistant plants, whereas callus derived from susceptible plants was totally overgrown by the fungus. The accumulation of the phytoalexin rishitin was estimated after inoculation of the callus lines with *F. oxysporum* f. sp. *lycopersici* race 1 and 2. Two and 3 days after inoculation with race 1, significantly more rishitin accumulated in the resistant line than in the susceptible line. This difference in rishitin concentration between resistant and susceptible lines was not observed after inoculation with race 2. The accumulation of rishitin in plants, after inoculation with race 1, is comparable with the accumulation in callus (Elgersma, D.M. & Liem, J.I. *Physiol. Mol. Plant Pathol.* 1989, **34**: 545–555). Because of the similar results with callus cultures and with whole plants and because of the relative simplicity of callus cultures compared with plants, callus cultures of tomato provide a useful model for investigating the interaction between the plant and *F. oxysporum* f. sp. *lycopersici*.

Pyrolysis Mass Spectrometric Analysis of Carnation Xylem Infected with *Fusarium oxysporum* f. sp. *dianthi*: Lignin Modifications and Phytoalexins in Diseased Xylem of the Cultivars 'Novada' and 'Lena'

J.J. Boon[1], J. Pureveen[1], B. Brandt-De Boer[1], R.P. Baayen[2] and G. J. Niemann[3]. [1]FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, [2]Research Institute for Plant Protection, PO Box 9060, 6700 GW, Wageningen, and [3]Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, The Netherlands

Minute samples of xylem taken from healthy and *Fusarium oxysporum* f. sp. *dianthi*-infected cultivars of carnation were investigated by pyrolysis low voltage (15 eV) mass spectrometry using a JEOL DX-303 double-focussing mass spectrometer equipped with a platinum filament in-source pyrolysis probe. Cylindrical samples (diameter 50 μm) with a length of about 1 mm taken from larger xylem samples with the aid of a microscope were homogenized in 20 μl of water

using an all-glass mini-mortar. A 5 µl aliquot of the suspension was used for the PYMS analysis (mass range 20–750 a.m.u., scan cycle 1 s, temp gradient 20°C/s up to 800°C).

Healthy xylem of both cultivars showed PYMS spectra with markers for cellulose and hemicelluloses, and a mixed guaiacyl–syringyl lignin indicated by mass peaks indicative of monomeric and dimeric pyrolysis products. Microscopically visible infected, brown-coloured xylem of the cultivar 'Lena' (susceptible) and 'Novada' (resistant) showed entirely different PYMS spectra. The PYMS of diseased 'Lena' xylem was marked by a change in the polysacharide composition and by a very drastic change in lignin pyrolysis product pattern, in which practically all syringyl constituents (monomeric and dimeric) were absent). This is evidence for a major change in cell wall composition and architecture due to invasion by the fungus.

The PYMS of infected 'Novada', however, showed an overall decrease in relative intensity of the lignin pyrolysis products but no selective changes in composition. The 'Novada' spectrum also showed m/z 239 and 287 being molecular ions indicative of the phytoalexins dianthalexin and methoxydianthramide S. Comparative studies on green xylem immediately adjacent to the brown gum-occluded vessels showed comparatively low amounts of the phytoalexins, thus pointing to the extremely localized occurrence of these fungistatic compounds. Desorption low voltage EI MS of a methanol extract of about 0·2 mm³ of infected Novada xylem confirmed these data and showed evidence for hydroxyanthranilic acid (m/z 153), dianthalexin (m/z 239), hydroxydianthamide B (m/z 257) and the methoxydianthramides B (m/z 271) and S (m/z 287). Evidently, 'Novada' is able to generate a strong and very localized fungistatic response to infection, thus avoiding major changes in its cell wall composition.

Inheritance of Resistance in Carnation to Races 1 and 2 of *Fusarium oxysporum* f. sp. *dianthi*

R.P. Baayen[1,2] and L.D. Sparnaaij[3]. [1]Willie Commelin Scholten Phytopathological Laboratory, Baarn, [2]Research Institute for Plant Protection, and [3]Institute for Horticultural Plant Breeding, Wageningen, The Netherlands

The inheritance of resistance in carnation to races 1 and 2 of *Fusarium oxysporum* f. sp. *dianthi* was investigated. The cultivars Novada (resistant to both races), Elsy (susceptible to race 1), Lena (susceptible to race 2) and Sam's Pride (susceptible to both races) were selfed and intercrossed. When 3 months old, the seedlings were inoculated via the roots or the stems. Twenty plants per cross, method and race were tested in 10 replicates. Wilting was indexed weekly along a 0–5 scale up to 13 weeks after inoculation.

Analyses of variance were performed on the percentages of diseased plants and on the numbers of plants in categories 0, 1–3, and 4–5. Method × genotype interactions were not detected, from which follows that root and stem inoculation resulted in the same relative resistance levels. Indications for extravascular (root) resistance were thus not found. The capacity to localize the pathogen at the infection site appeared an important resistance component. Resistant cultivars were also characterized by longer latent periods and lower wilting rates. These observations apply to race 1 as well as race 2.

The GCA variances for resistance were highly significant ($P<0{\cdot}001$) with both races. A significant ($P<0{\cdot}05$) SCA variance was only found for resistance to race 2 at 7, but no longer at 13 weeks after inoculation and could be attributed to inbreeding depression. These data show that resistance to both of the races is mainly inherited in an additive way. This was expected for race 2 against which resistance is partial and polygenic. Resistance to race 1 had previously not appeared partial and was assumed to be monogenic. Additivity of resistance to race 1 might be of the intralocus type, however, as the numbers of diseased plants were found to fit both monogenic and polygenic models.

Protection of Gerbera Flowers Against Infection of *Botrytis cinerea* with Anti-Cutinase Monoclonal Antibodies

J. Salinas. Willie Commelin Scholten, Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, The Netherlands

Infection caused by *Botrytis cinerea* has become an important problem in several crops in The Netherlands. Spotting or rotting of ray florets or gerbera flowers caused by the fungus can appear during the growth of the flowers in the greenhouses, but occur most frequently during storage or transport of cut flowers.

This type of symptom can be reproduced in the laboratory by inoculation of flowers with dry conidia of *B. cinerea*. In this case humidity is the only limiting factor. On flowers incubated at 60–70% r.h. the conidia remain ungerminated and no symptoms can be observed. When flowers are incubated at 100% r.h. the conidia produce a short germtube and the infection occurs by direct penetration through the intact cuticle.

The mechanism by which pathogens breach the cuticle has been debated for almost a century. Penetration was long thought to be mechanical, but

the involvement of cutinolytic enzymes secreted by the invading pathogens has often been postulated.

If cutinolytic enzymes are needed for penetration of the fungus through the intact cuticle, specific inhibition of cutinase activity (chemical inhibitors or monospecific antibodies) can protect plants with intact cuticles against infection. To test the second possibility, monoclonal antibodies were raised against purified cutinase of *B. cinerea*. Treatment of inoculated gerbera flowers with the antibodies, completely protected the flowers against the infection of *B. cinerea*.

Induced Resistance in Carnation After Bacterization with *Pseudomonas* sp. Strain WCS417r

R. Van Peer and B. Schippers. Willie Commelin Scholten, Phytopathological Laboratory, State University of Utrecht, Baarn, The Netherlands

The involvement of other mechanisms besides competition for iron in the biological control of *Fusarium oxysporum* f. sp. *dianthi* (*Fod*) in carnation by *Pseudomonas* sp. strain WCS417r, was investigated. Competition between both micro-organisms was excluded experimentally, by a spatial separation of WCS417r and *Fod* by bacterization of the root and inoculation in the stem with microconidia of *Fod*. Numbers of diseased carnation plants were significantly reduced when the roots were bacterized 1 week prior to stem inoculation with *Fod*. *Pseudomonas* sp. strain WCS417r could easily be isolated from the roots but never from the stem tissue. Therefore, disease control cannot be due to competition between *Fod* and WCS417r. Strain WCS417r is apparently able to induce resistance in carnation to *Fod*. Along with induced resistance, an accumulation of phytoalexins was found in the stem segments. No such accumulation was found when plants were bacterized only. It is concluded that signals, provided by WCS417r at the root, induce stabilization in the stem of defence responses to *Fusarium* and of the synthesis and accumulation of phytoalexins. Similar results on disease suppression were obtained when purified lipopolysaccharides (LPS) of WCS417r were applied to the root instead of viable cells. Apparently, LPS of this strain is the inducing factor. Whether the LPS also induces phytoalexin accumulation is currently being investigated.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 4 OCTOBER 1989

The Rate of Succession in Different Forest Types

H. Koop. Research Institute for Nature Management, PO Box 46, NL-3956ZR Leersum, The Netherlands

The rate of forest succession is most substantionally influenced by development of the tree and shrub layer. Therefore, study of growth and stature by analysis of tree rings provides a powerful method for estimating of the rate of forest succession. This is demonstrated in four forest associations in European forest reserves, e.g. a Fraxino–Ulmetum (Ile de Rhinau, France), a Tilio–Carpinetum (Bialowieza, Poland) and a Melico–Fagetum and a Fago–Quercetum (Fontainebleau, France). The tree model developed by the author (Koop, H. *Forest Dynamics, SILVI-STAR: A comprehensive Monitoring System*. 1989, Springer Verlag, Heidelberg) was used for depiction of the complex set of data on spatially irregularly arranged tree growth and stature. For the depiction of an individual tree, its dimensions at any given time, such as crown surface, height of the top and the crown base, have been derived from the reconstructed diameter-increment by regression equations.

At nutrient-poor sites such as the Fago–Quercetum, tree generation changes take the longest time (*c.* 300 years). A clear cut of 1372 triggered an even-aged first tree generation. During the period 1651–1693 this tree generation collapsed and gave way to the oldest beech tree generation to be found today. Due to canopy closure of this second generation trees a regeneration gap can be recognized in the period 1693–1851. The third generation trees established themselves almost continuously from 1851 until now.

On nutrient-rich sites such as the Fraxino–Ulmetum a generation change from an even-aged pioneer Salix–Alnus forest to a mixed species well structured ash–elm forest could be recorded within 150 years.

In the Tilio–Carpinetum *Picea abies* trees pierced from an undergrowth position through a closed *Carpinus betulus* canopy and gap replacements by *Populus tremula* and *Tilia cordata* were found in a fine-grained mosaic spread in time. Here the reconstructed forest dynamics can be considered as cyclic within a steady state mosaic.

Effects of Former Land Use on Flora and Vegetation of Deciduous Woodland in Flanders (Belgium)

M. Hermy. Institute of Nature Conservation, Kiewitdreef 3, 3500 Hasselt, and State University of Gent, Laboratory for Plant Morphology, Systematics and Ecology, K.L.-Ledeganckstraat 35, 9000 Gent, Belgium

From 1977 to 1983, 640 relevés were collected from 183 woodlands on a variety of soils in Flanders. In order to assess the effects of former land use on the composition and richness of woodlands, the plots were divided in two categories: plots from ancient woodlands (pré-1800) and recent woodlands (post-1800; <200 years in use as woodland).

A large number of woodland species were more or less confined to ancient (e.g. *Hyacinthoides non-scripta, Lamium galeobdolon, Convallaria majalis, Maianthemum bifolium, Oxalis acetosella*) or recent woodland (e.g. *Urtica dioica, Galium aparine*). However, regional differences were observed (e.g. *Primula elatior*, an ancient woodland species in the northern part, was more frequent in recent woodlands in the southern part).

The mean number of plant species per plot was not significantly different between ancient and recent woodlands. However, the number of true woodland species was significantly larger in ancient than in recent woodlands. Former land use did not affect the mean species richness per plot, but strongly influenced the species composition and abundancy.

The phytosociological classification yielded 35 woodland communities from Alnion- to Quercion-communities. The proportion per phytocoenon in plots from ancient woodland was positively correlated with the number of true woodland species. A positive correlation was also found with the percentages of topochores and a negative correlation with percentages of anemochores. Although many woodland communities from recent woodlands originated before 1880, they still are clearly distinct from the communities in ancient woodlands. In terms of woodland species they are not fully developed. Therefore, the complete renewing of woodland communities is a matter of centuries, since many woodland species have a very limited colonization capacity.

The Effect of Coppicing on Species Composition and Synsystematics

S. van der Werf. Research Institute for Nature Management, PO Box 46, NL-3956 ZR Leersum, The Netherlands

In The Netherlands and adjacent countries the relationship between high forest and coppice was studied in six association types of pre-holocene soils, dominated by *Fagus sylvatica*, and *Quercus* spp. with *Betula* spp. or *Carpinus betulus*, respectively.

From documents and place names it was concluded that in under-cutting and coppicing regimes *Fagus*-forests most probably shift gradually to *Quercus*-forests and finally to *Betula*-dominated forests. In coppice, the light-indication, according to Ellenberg (*Scripta Geobotanica* 1974, **9**: 1–79) increases considerably with nutrient poorness of the site. Moisture seems hardly involved, but pH and N indication are divergent: decreasing in types on acidic soil, increasing in types of alkaline soil. For the latter, erosion is held responsible.

Although in general species composition is somewhat changed due to coppicing and cutting, the ecological preferences of species from coppice do not deviate from those of uncoppiced forest.

The Sörensen index for floristic composition (Sørensen, *Biol. skr. K. Danske Vidensk. Selsk.*, 1948, **5**: 1–34) shows a mean of 68% correlation, which is of the same order as variants within one association. With regard to ecological features, correlation is even higher. Coppiced forest types can therefore be considered as management variants, not deserving an association status.

Fagus, and other climax trees decreased in occurrence and rejuvenation, but most other trees and shrubs increased in these characteristics. The mean total number of species in 1208 relevés increased from 22·0 to 24·4. Decreasing species include common species and character species; increasing species include shrubs, wood-edge species of heathland or limestone grassland, and nitrophilous species.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 24 JANUARY 1990

Effect of Earthworms on the Soil Seed Bank Dynamics: An Experimental Approach

K.G.A. Huijsmans and J.H. Willems. Department of Plant Ecology and Evolutionary Biology, University of Utrecht, Lange Nieuwstraat 106, NL 3512 PN Utrecht, The Netherlands

Vertical movement of the seeds into the soil is a necessary process in establishing a soil seed bank. The greater part of this seed bank can be found at a depth of at least a few centimeters beneath the soil surface. Regeneration of plants out of the buried seeds can only be successful if the seeds are brought on or near to the surface. From a functional point of view, vertical movement of seeds in both upward and downward direction is very important. Earthworms are supposed to be an important transport factor in this process.

Experiments have been carried out to test the effect of earthworms on vertical movement of seeds in the soil. A number of cylindrical polyethelene tubes of 65-cm height and 20-cm diameter have been filled with calcareous soil. At two different depths (−2 and −32 cm) 5–10 000 seeds of a chalk grassland species have been placed. All tested species have been proven to be eaten by earthworms and to survive the pass through the intestine of these animals. In half of the number of cylinders three earthworms (*Lumbricus terrestris*) were placed, resembling the worm density in chalk grassland.

Six weeks after the start of the experiment the seeds in the cylinders containing earthworms were moved vertically in both directions. This was in contrast with the results in the control cylinders, in which the seeds were found in the original soil layer.

The maximum movement of seeds amounted to approximately 10 cm, both upwards as well as downwards.

A large number of seeds buried at a depth of 2 cm had been transported upward and were encountered in the worm casts on the soil surface.

There was some evidence that seeds of the several species had been moved differently, e.g. seeds of *Carex flacca* tend to move upward rather than downward. However, the much smaller seeds of *Origanum vulgare* were found below the original position. This has been recorded at both depths.

The preliminary results of the experiments mentioned above clearly show the effect of earthworms in the vertical seed transport in the soil. The results will be compared with detailed field studies in order to gain more insight in the role of earthworms in the maintenance of the species-rich chalk grassland ecosystem.

Vegetation Development After Habitat Creation in the Experimental Garden of the RIN at Leersum, The Netherlands

G. Londo. Research Institute for Nature Management, PO Box 46, NL-3956 ZR, Leersum, The Netherlands

In 1972 the experimental garden of the Research Institute for Nature Management was laid out. The main purposes were to gain more knowledge about
—the creation of new habitats for plant species and vegetation important for nature conservation; and
—the behaviour of plant species in space and time.

The habitats were made artificially by supplying various types of soil from different areas in The Netherlands: sand poor in lime, sand rich in lime, loam, clay and limestone, among others. Uniform habitats were made as well as gradients, i.e. gradual transition zones between two adjacent soil types. The management regimes were mowing once or twice a year depending on the vegetation productivity.

The developing grasslands varied strongly in species composition and species number. This number is low in vegetation on sand poor in lime and nutrients and on the formerly manured and cultivated garden soil. However, the grasslands on limestone and on sand and clay rich in lime were very rich in species. Gradient habitats contain more species than uniform habitats. Gradients made by adding an infertile soil layer on a fertile, i.e. formerly cultivated, soil were not expressed in the actual vegetation entailing a homogeneous highly productive grassland. The high fertility suppressed variation in soil characteristics. On the contrary, the same gradients on sand poor in nutrients and lime are expressed fairly distinctly in vegetation gradients with many species.

A consideration in the development of attractive grasslands, rich in flowers, is that of creating a varied habitat with gradients on a soil relatively poor in nutrients avoiding the use of fertilizers.

The Interaction Between Nutrient Availability and the Species Composition of Heathland Vegetation

R. Aerts. Department of Plant Ecology and Evolutionary Biology, University of Utrecht, Lange Nieuwstraat 106, 3512 PN Utrecht, The Netherlands

The interaction between nutrient availability and the species composition of the vegetation was studied in Dutch wet heathland stands dominated by *Erica tetralix* and *Molinia caerulea*, and in dry heathland

stands dominated by *Calluna vulgaris* and *Molinia*. Biomass production (including root production) of both evergreens was about 700 g m^{-2} $year^{-1}$, whereas total productivity of both *Molinia* stands was three times as high. In all three species total productivity equalled litter production. Both nitrogen and phosphorus loss per gram litter production (including root turnover) were equal for all species. However, due to the much higher litter production in *Molinia* stands, soil nutrient input due to litter production exceeded that in the *Erica*- and *Calluna*-dominated stands threefold.

Competitive relationships between *Erica* and *Molinia* and between *Calluna* and *Molinia* were studied along an experimental gradient of nutrient availability, ranging from 0 to 20 g N m^{-2} $year^{-1}$. *Erica* outcompeted *Molinia* when N availability ranged from 0 to 10 g N m^{-2} $year^{-1}$, but lost in competition with *Molinia* at higher levels of N availability. *Calluna*, however, outcompeted *Molinia* over the entire range of N availability. It is shown that the vertical canopy structure of both evergreens determines to a large extent their high competitive ability with respect to *Molinia*. The widespread replacement of *Calluna* by *Molinia* in Dutch heathlands is the combined result in increased levels of nutrient availability and damage of the *Calluna* canopy, e.g. by heather beetle (*Lochmaea suturalis*) attacks or by severe frost spells.

The results suggests a positive feedback between nutrient availability and dominance of *Molinia* in Dutch heathlands. Therefore, management of these heathlands should primarily be directed towards reduction of nutrient availability, e.g. by reduction of external inputs, or by sod-cutting, burning (in *Calluna* vegetation) or grazing by large herbivores.

Half a Century of Landscape Development in the Amsterdamse Waterleidingduinen

L.H.W.T. Geelen. Municipal Waterworks of Amsterdam, Vogelenzangseweg 21, 2114 BA Vogelenzang, The Netherlands

To develop a scheme for adequate dune management, insight is required into changes in landscape, vegetation and hydrology. Unfortunately the Municipal Waterworks of Amsterdam do not have historical field data concerning the 'Amsterdam Waterwork Dunes'. The question was whether changes in landscape and vegetation could be traced by aerial photographs.

In 1985 a pilot study started in the 'Haasvelderduinen', a part of the dune reserve, managed by the Municipal Waterworks of Amsterdam. The transitions in vegetation structures were analysed by means of areal photographs from 1938, 1958, 1968, 1979 and 1985. Field studies were carried out to check the later analyses.

In the 1930s and 1940s, the vegetation structure was mainly open (dry dune grasslands). Since the midfifties this dominating open vegetation has been increasingly encroached by variable patterns of open low shrub. During the last 10 years the diversity in vegetation structures increased by the local development of open low shrub into more dense and/or taller shrub vegetation. During the last 50 years the coverage of the tree layer increased proportionally by the development of small deciduous woodlands and the planting of pine forests.

A great number of factors affect vegetation structure development: (meso)climate, relief, hydrology, soil, fauna and man. In this study hydrology has been of special importance. Drinking water catchment has led to a significant lowering of the groundwater table during the first half of this century. Later on it raised again as a result of changed hydrological management. Grazing has also been of great influence, especially the termination of cattle and sheep grazing in 1920. Moreover, the sudden decrease of rabbit grazing, as a result of a myxomatosis outburst in 1954, has favoured the growth of woody species.

Probably the present day management of 'doing nothing' has to be changed to prevent further loss of the diversity of vegetation structures. Maybe cattle grazing or the development of blowouts can be used as management tools to retard succession and re-introduce the characteristic young dune phases.

Hydrology and Vegetation Development in the 'Kwade Hoek'

C. Braat and I. van Zanten[1]. Department of Nature Conservation, Wageningen Agricultural University, Ritzema Bosweg 32A, 6703 AZ Wageningen, and [1]Delta Institute for Hydrobiological Research, Vierstraat 28, 4401 ED Yerseke, The Netherlands

The Kwade Koek is a coastal area (350 ha) situated west of the Haringvliet in the estuary of the SW Netherlands. It is formed by growth of the shore since the late 19th century. The area consists of four ranges of dunes, alternated with dune valleys. The eastern part is formed by saltings and mudflats, which shade off into a sandbank. The area can be inundated with seawater because it is situated outside the dike; it is bounded in the south by a seawall formed by dunes. The Kwade Hoek is rich in natural gradients, caused by sea-water inundation. Going from east to west, the salinity of soil and groundwater, and the amounts of nutrients and silt, decrease. Part of the salting is being grazed by cows.

Since the Haringvliet was closed (1970), the inundation frequency of the Kwade Hoek has increased, because the water level went up (30 cm). Because of less mixture with fresh river water, the salinity of the inundation was also increased. As a consequence of

this the salinity of the groundwater in the Kwade Hoek increased. This resulted in a strong spreading of the communities of the haloserie (N. Joanknecht & L. Meuleman, In: *De Levende Natuur* 1980, **82:** 89–98).

In 1977–1979, the seawall was raised. We studied the effects of this measure on the hydrology and the vegetation of the Kwade Hoek. The main results are the following. The enlarged dune-complex caused an increase in the amount of fresh seepage water in the southern part of the Kwade Hoek. This resulted in an increase of the groundwater level and a decrease in the salinity in this part of the area. In the western part of the area, which is not being grazed, *Phragmites australis* has partly driven out *Scirpus cariciformis* and the community of *Mentha aquatica* and *Hydrocotyle vulgaris*. In the eastern part, which is being grazed, vegetation belonging to Galio–Koelerion and Saginion have replaced Loto–Trifolion and floodmark communities (Atriplici–Cirsietum arvensis).

Possible causes for these vegetation changes are:
—a change in the inundation pattern, due to the formation of sills elsewhere in the area. This may result in an increase of the inundation in the eastern part and a decrease in the western part of the area;
—an increase in the amount of fresh seepage water, which is beneficial to the growth of reed; and
—the closing of the Haringvliet still has an effect on the vegetation development. In 1977 the vegetation was still not in equilibrium with the environment.

Ranunculus hederaceus as an Indicator for Changes in Land Use in The Netherlands

H. Beukema, K.J. Noorman and R. van Diggelen. Department of Plant Ecology, State University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

In The Netherlands *Ranunculus hederaceus* is a rare species that occurs in flowing water. A literature survey showed a large ecological tolerance of this species, which seems to be contradictory to its rareness. A preliminary study correlated the distribution of the species to groundwater systems (Diggelen, van, R. & Klooker, J. *Gorteria* 1990, **6:** 29–38). Their results seemed to indicate that it was confined to zones where a constant seepage of relatively young and mineral-poor groundwater took place. It seemed to avoid seepage areas with mineralized groundwater.

Three *R. hederaceus* stands in the province of Drenthe (Gieten, Oudemolen and Deurze) were selected for further investigation of the hydrological characteristics. Indeed the species were restricted to seepage areas where the upwelling groundwater was characterized by a fairly low mineral content. Quality and quantity of the water were very stable throughout the growing season.

To investigate whether these circumstances were representative for The Netherlands, some 35 surface water samples were taken from different *R. hederaceus* stands throughout the country. The samples showed wide ranges in mineral contents. Response analysis showed significant correlations ($P < 0.05$) between the degree of cover and mineral contents of the water samples only for bicarbonate and phosphate. When the contents of the water were multiplied with stream velocity (i.e. yielding a measure for mineral supply per time unit) significant relations ($P < 0.05$) were found not only for bicarbonate and phosphate, but also for sulphate and nitrate. From these results it was concluded that the species requires a stable and high supply of nutrients, while stream velocity of the water can compensate for the lower nutrient levels found.

To stress the importance of *R. hederaceus* as an indicator species, the situation near Oudemolen can serve as an example. Previous research showed that this area was nourished by seepage of mineral-rich, nutrient-poor groundwater, *R. hederaceus* was not at all expected at this site, but in fact the species was found there in 1971 and has increased ever since. Detailed investigations showed that artificial drainage was responsible for the diminishing of the deep groundwater flow, giving room for a nutrient rich lateral flow from adjoining agricultural grounds. Hence the increase of a rare plant species like *R. hederaceus* is not always indicating the well-being of a nature reserve.

Vegetation Succession and Hydrology in De Bollemaat, De Wieden

W.J. Molenaar, R. van Diggelen and A.M. Kooijman[1]. State University of Groningen, Department of Plant Ecology, PO Box 14, 9750 AA Haren and [1]State University of Utrecht, Department of Plant Ecology, Lange Nieuwstraat 106, 3512 PN Utrecht, The Netherlands

Vegetation succession in floating fens in relation to hydrological conditions was studied in 'De Bollemaat', a former peat-digging area, situated in the nature reserve 'De Wieden' (NW-Overyssel, The Netherlands). An old vegetation map revealed that in 1956 a mesotrophic and calciphilous fen community (Scorpidio–Caricetum diandrae) was present over large areas. A recent survey (1989) showed that only small patches had remained. An analysis of the vegetation maps revealed that the calciphilous Scorpidio–Caricetum diandrae was almost entirely replaced by more acid communities (Pallavicinio–Sphagnetum and Sphagnetum palustri–papillosi) or woodland (Carici elongatae–Alnetum). The Scorpidio–Caricetum diandrae was restricted to areas where Ca-rich water was present in the root zone. Hydrological investigations showed that these alkaline conditions were not caused by seepage of Ca-rich

groundwater. In fact the entire study area appeared to be an infiltration area. The observed zonation in water quality could best be explained by the pattern of infiltrating surface water originating from the neighbouring polder areas, where the excess of (Ca-rich) groundwater is pumped directly into the surface water system.

Measurements of the conductivity revealed that infiltration of surface water from the main water channels was only noticeable within a range of 20 m. In more isolated sites the Ca release from the cation exchange complex is thought to be an important factor determining the distribution of water types and associated plant communities.

Under the present hydrological conditions the calciphilous Scorpidio–Caricetum diandrae represents a relatively short phase in the vegetation succession. It is concluded that attempts to restore this community should be aimed at the use of surface water instead of isolating the area from it.

MEETING OF THE SECTION OF THE RELATIONSHIP BETWEEN PLANTS AND ANIMALS ON 3 JUNE 1989

Seed Dispersal of Three Nymphaeid Macrophytes (*Nymphaea Alba* L., *Nuphar Lutea* (L.) Sm. and *Nymphoides Peltata* (Gmel.) O. Kuntze)

A.J.M. Smits. Laboratory of Aquatic Ecology, Catholic University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

The various stages from flower bud to seed release of *Nymphaea alba* L., *Nuphar lutea* (L.) Sm and *Nymphoides peltata* (Gmel.) O. Kuntze have been described by Van der Velde and Van der Heijden (*Aquat. Bot.* 1980, **10:** 261–294) and Van der Velde (*Acta Bot. Neerl.* 1986, **35:** 111–113). However, information concerning the dispersal of the diaspores of these plants was scarce and incomplete. Recently, several aspects of the hydrochorous, endozoochorous and epizoochorous dispersal of these nymphaeid macrophytes were examined. These were: (1) buoyancy of seeds or carpels; (2) survival of seeds after passage through the digestive system of two aquatic birds and one fish species; and (3) desiccation tolerance of the seeds.

Buoyancy of *N. lutea* carpels was found to be poor compared to *N. alba* and in particular *N. peltata* seeds.

Mallard duck (*Anas platyrhynchos* L.) and coot (*Fulica atra* L.) digested all seeds of *N. alba, N. lutea* and *N. peltata* completely, in contrast to seeds of *Potamogeton natans* L., *P. obtusifolius* Mert. et Koch and *P. pectinatus* L. Similar results were obtained when the seeds were fed to common carp (*Cyprinus carpio* L.). Because of the complete digestion of the seeds of the nymphaeid waterplants by coot, duck and carp, endozoochory will not contribute to a successful dispersal.

Unlike *N. peltata* seeds, the seeds of *N. alba* and *N. lutea* were killed by desiccation. In view of these properties and the morphology of the seeds it can be concluded that epizoochory may be an important factor in the seed dispersal between isolated water bodies of *N. peltata*, hardly of importance in that of *N. alba* and *N. lutea*. Within one water body hydrochory seems to be the main dispersal mode of the three nympaeid species studied.

Angiospermy, Gymnospermy and Pollination

A.D.J. Meeuse. Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands

Angiosperms differ from gymnosperms in that the endostome (orifice of the integument) is not exposed, which means that the role of the pollination droplet as a pollen-receiving device was replaced in angiosperms by the stigmatic surface. As the droplet was also involved in early entomophily, the insect visitors had to be lured in some other way if biotic pollination had to be maintained. Several escape routes were possible, but the change-over to angiospermy cannot have been an abrupt happening (otherwise the reproduction would not have been continuous). Conceivably, an intermediate phase may have existed, especially in cases where alternatives (nectaries, deceit, pollen as reward) did not develop. What one would expect is an exudate from the tip of the carpel or style in those cases where the stylar canal is not closed at anthesis. This condition is found in *Nelumbo* and apparently in some species of *Drimys*; the exuded droplet is consumed by pollinators and pollen is absorbed by it. In *Nelumbo* the stylar canal is incompletely closed by hairs at its distal end, so that one may speculate that the origin of the sticky stigmatic fluid and the papillae in angiosperms may have originated from a transitional condition still found in the primitive genus *Nelumbo*.

Researchers are advised to look for exuded droplets on carpel tips in primitive families and in taxa where there is a connection between ovule and ovary apex through tegumentary protrusions.

Flower Choice by Bumblebees: Cause and Consequences for the Plant

M.M. Kwak and O. Jennersten[1]. Laboratory of Plant Ecology, Biological Centre, PO Box 14, 9700 AA Haren, The Netherlands and [1] Department of Zoology, Uppsala University, Box 561, S-751 22 Uppsala, Sweden

Bumblebees foraging on two sympatrically, simultaneously flowering plant species, *Melampyrum pratense* (Scrophulariaceae) and *Viscaria vulgaris* (Caryophyllaceae) were studied during the flowering season of *Viscaria* in Sweden. Both species shared the main visitor, queens of *Bombus hortorum*, which collected nectar on both species but pollen particularly on *Melampyrum*. The pattern of visitation changed over the season; bumblebees preferred *Viscaria* early but changed to *Melampyrum* later in the season (1986): in 1988 they preferred *Melampyrum* from the beginning onwards, but early *Viscaria* flowers still received about 5·5 visits per flower per day.

Preference for *Melampyrum* flowers can be explained by the higher sugar content of *Melampyrum* nectar (40%; *Viscaria* 25%) and the possibility of collecting both pollen and nectar from the same flower. Energy intake per minute, a combination of volume, concentration and visitation speed was more profitable in *Viscaria*. Nectar composition did not offer an explanation for the preference for *Melampyrum*.

Seed production in *Viscaria* was affected, especially late in the season, by the switch of bumblebees from *Viscaria* to *Melampyrum*, resulting in a low visitation and the change of improper pollen transfer.

Seed production in *Melampyrum* was also lower later in the season but this was mainly a result of pollinator satiation because *Melampyrum* became very abundant with time.

Although *Viscaria* flowers produced large amounts of nectar (4 µl per flower) especially later in the season, other bumblebee species did not start to visit *Viscaria*.