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MEETING OF THE SECTION FOR ALGOLOGY ON 8 DECEMBER 1994

A Molecular Phylogeny of the Diatoms (Bacillariophyta)

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A phylogeny of the diatoms (Bacillariophyta) has been inferred from nuclear-encoded small subunit ribosomal RNA (rRNA) sequence analyses of 30 taxa in 11 orders. Maximum likelihood, weighted maximum parsimony, neighbour-joining, and Log-Det transformation methods each recover two clades, neither of which correspond to the classes of diatoms presently recognized: Class Coscinodiscophyceae (centric diatoms), Class Fragilariophyceae (araphid pennate diatoms) and Class Bacillariphyceae (raphid pennate diatoms). Nor do the clades correspond to the traditionally recognized centric and pennate diatoms. One clade is defined by the centric diatom orders Coscinodiscales, Rhizosoleniales, Corethrales, and Melosirales. The second clade contains the bi- (multi) polar centric diatoms, the centric diatoms with strutted processes, and the pennate diatoms. Tests of alternative phylogenies under maximum likelihood strongly support these divergences. Fossil evidence from the earliest best-preserved diatom deposit (115–110 Ma ago, Ocean Drilling Program (ODP) Leg 113, Site 693, Antarctica) also suggests that these divergences in the diatoms occurred early in their evolution and are correlated taxonomically with the absence (clade one) or presence (clade two) of a central tube in the silica cell wall. Its presence can be traced to the central tube or labiate process in the bipolar centric taxa, to the central tube or strutted process in the order Thalassiosirales and probably to the organelle that evolved into the raphe of the pennate diatoms. Divergence times place the origin of the diatoms around 300 Ma ago (Carboniferous).

The Impact of Atmospheric Deposition on Diatoms and Chemistry of Surface Water in Running Water in The Netherlands

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This paper summarizes the first report on the impact of acid atmospheric deposition on biota of running waters in The Netherlands (H. van Dam, A Mertens & L.M. Janmaat 1993, IBN-DLO rapport 052. Wageningen).

In 1990, 35 samples were taken for analysis of diatoms and chemistry in 16 springs and first and second order groundwater fed soft-water streams in oligotrophic, sandy areas. The residence time of the groundwater in the catchment before discharge into the springs and streams is between 1 and 196 years (median 87 years).

The results of the chemical analysis were compared with those of similar investigations conducted in 1974 and 1981, which were available for a number of sampling stations. At these stations the mean pH in 1990 was 6·4, which was not significantly lower than in 1974 (6·5). The alkalinity decreased significantly from 355 to 251 meq m^{-3} and nitrate increased significantly from 17 to 158 mmol m^{-3} between 1974 and 1990. The sulphate concentration increased from 200 to 229 mmol m^{-3} over the same period.

Diatoms were studied as they are excellent indicators for acidification of surface waters. They are the most important algae in the streams investigated. The most important diatom is *Aulacoseira crenulata*, which was recorded for the first time in The Netherlands and occurs rarely in small, clean running waters in western Europe. Many other rare species were found, but their number did not change significantly between 1974 and 1990.

It appears from canonical correspondence analysis that pH and nitrate are the most important measured environmental variables. Other important variables are the area of cross-section of the stream and the ratio Ca/(Ca+Cl). The diatom assemblages in different headwaters of the same stream, at different distances from the origin in the same headwater and on different substrates at the same station may be very dissimilar.

Between 1974 and 1990 there was a shift in taxonomic composition, which indicated acidification. The 1990 samples were used as a calibration set for inferring pH with diatoms by weighted averaging. The transfer function was used for inferring the pH in 10 comparable samples from 1974. The diatom inferred pH declined significantly from a median value of 6·8 in 1974 to 6·6 in 1990.

The discrepancy between the changes in directly measured and diatom-inferred pH-values is probably due to the methodological problems associated with direct measurement of pH of soft water in the past.

It is recommended that similar investigations be conducted every 5–10 years and that research should begin on the spatial and temporal variation of algal assemblages in relatively clean streams in The Netherlands. Also, the impact of acidification on the macroinvertebrates of these vulnerable ecosystems should be assessed.

The Mainly Tropical Algal Genus *Caulerpa* (Ulvophyceae, Chlorophyta)

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A new monographic revision is needed for the genus *Caulerpa*, from which a non-holocarpic sister genus *Caulerpella* has recently been separated. The most recent monograph of the genus was published in 1898 by Weber-van Bosse (Weber-van Bosse, A. 1898, *Ann. Jard. Buitenzorg* **4**: 243–401). and the interpretation of the status of subspecific variation differs today in many respects from earlier views. Instead of considering many varieties and forms in the genus *Caulerpa*, the designation ecological phenotype (ecad) is now favoured for the different growth forms (Coppejans & Prud'homme van Reine 1992 *Meded. Zitt. K. Acad. overzeese Wet.* **37**: 667–712). Advantages and disadvantages of the use of the ecad entity were discussed, and problems in relation to designation of new species and uniting taxa were reviewed. For the new monographic revision experimental methods such as transplantation culture experiments and molecular approaches should be used to produce a phylogenetic analysis of this interesting green algal genus.

Eutrophication of the Wadden Sea, A Continuing Story?

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Monitoring in the Marsdiep, the westernmost inlet of the Wadden Sea, indicates that lowering of P-discharges by the Rhine have also led to lower P-concentrations in the Marsdiep. Whereas PO_4-P reached winter and summer values of up to 3 μM, with a short spring minimum in between, in the early 1980s, winter and summer maxima were in 1994 only *c.* 1·5 μM and the duration of the spring minimum tends to increase. However, this has still not caused lower phytoplankton primary production, values for 1994 (>400 gC m^{-2}) were even the highest observed so far. Production values for 1993 and 1994 strengthen our earlier conclusion based on data up to 1992 (*Neth. J. Sea Res.* **31**: 147–152): lower P-discharges of the Rhine cannot be responsible for lowering of stocks of suspension-feeding bivalves, brown shrimps and demersal fish in the coastal zone as sometimes suggested, there is enough food produced.

Phytoplankton succession in the Marsdiep starts with a spring bloom of diatoms. This bloom declines when Si becomes depleted; at that moment, P and N are not yet depleted. This enables development of non-diatom algae. With low summer N/P ratios in the Marsdiep since the late 1970s *Phaeocystis*, being a good competitor under N-limitation (Reigman *et al.* 1992, *Mar. Biol.* **112**: 479–484), was very successful, forming long-lasting blooms. The recent lowering of P but not of N causes an increase of the N/P ratio. Under P limitation *Phaeocystis* was found to be a poor competitor (Riegman *et al.* 1992). The recent decreasing trend in height and duration of *Phaeocystis* blooms in the Marsdiep might be related to the lower PO_4-P concentrations observed now.

A Deviating Mechanism for Cell Division in *Microthamnion* (Chlorophyta)

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The ultrastructural details of the processes involved in both vegetative cell division and zoosporogenesis in *Microthamnion* are presented. The mechanisms of division for vegetative cell division and zoosporogenesis are identical, but have never been reported before for green algae. The mechanism starts with the migration of the centriole pair from the polar interphase position along the nuclear surface towards a lateral prophase position between the nucleus and the longitudinal cell wall. The centriole pair duplicates (centriolar complex) and the root templates of the parental centrioles grow out. Two microtubules of the two microtubular rootlets per centriole run parallel to each other at some distance from the centriolar complex and eventually form two four-stranded microtubular bundles running along the cell wall in the equatorial plane of the cell. At metaphase the separated centriole pairs have migrated to positions at approximately 180° away from each other via a sliding mechanism along the newly formed microtubular bundles. A small invagination of plasmalemma at the previous prophase position of the centriolar complex indicates the plane of the coming division. At anaphase the centriole pairs co-migrate with the separating chromosome halves. Thereafter, the two microtubular bundles show a shifted orientation with respect to the long axis of the cell. In this stage the unilateral septum starts to develop in

between the two microtubular bundles. At telophase the daughter nuclei are reformed and the septum development proceeds. After cytokinesis the centrioles either resume their interphase position (vegetative cell division) or start a second division cycle. Eventually, a sporangium containing 4-6-8 biflagellate zoospores is formed.

The mechanism for mitosis and cytokinesis in *Microthamnion* shows characteristics of other green algal groups, i.e. the Pleurastrophyceae/Pleurastrales, with a metacentric spindle and some chlamydomonadalean algae (Chlorophyceae) with the migration of centrioles along microtubular bundles during mitosis. The phenomenon that the centrioles in *Microthamnion* migrate within the equatorial plane is unique.

THE 'WILLIE COMMELIN SCHOLTEN' MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON 19 JANUARY 1995

Expression and Analysis of a Cutinase Gene of *Botrytis cinerea*

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Botrytis cinerea Pers.:Fr. infects soft fruits, flowers and vegetables of many economically important crops. Besides the infection of wounded, senescent or dead tissue, the fungus is also able to penetrate undamaged tissue. It has been postulated that this penetration can be mediated by the enzyme cutinase, although mechanical penetration by appressoria is not excluded.

To investigate the importance of cutinase in the penetration of plant tissue, we aimed at cloning the encoding gene to enable expression studies during penetration. For this purpose the strategy of reversed genetics was followed: purification of the cutinase protein and subsequent cloning of the corresponding gene.

In vitro cultures of *B. cinerea* were induced for cutinase production and the resulting culture filtrate was fractionated by gel filtration and chromatofocusing. This resulted in the purification of a 18 kD protein showing a hydrolysing activity on cutin labelled with the chromogenic group Remazol Brilliant Blue. Partial amino acid sequence analysis identified the 18 kD protein as cutinase.

Oligonucleotide primers were designed based on the amino acid sequences determined and used to amplify the corresponding DNA sequence by RT-PCR. The sequence of the amplified fragment showed homology to known cutinase genes, and the fragment was used to clone the gene from a genomic library of *B. cinerea*.

The expression of the cutinase encoding gene *in vitro* and *in planta* is currently being investigated by Northern blot analysis and RT-PCR.

Composition and Structure of the Cell Wall of *Fusarium oxysporum*

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It is conceivable that in the initial phase of infection, cell wall components of phytopathogenic fungi play a role in the interaction between pathogen and host. To address the question as to the nature and specific role(s) of such components a study was initiated to analyse the cell wall of *Fusarium oxysporum*.

It appeared that (N-acetyl)-glucosamine, glucan, mannose, galactose, uronic acid and proteins are present in each of the three *formae speciales* of *F. oxysporum* tested: *lycopersici, radicis-lycopersici* and *gladioli*. Quantitative differences were not observed. The structure of the hyphal cell wall was studied with ConA-FITC (mannose specific) and WGA-FITC (chitin specific) and revealed a structural difference between the hyphal tip and the subapical wall. In the former, mannose and chitin are accessible for ConA and WGA whereas in the latter these compounds were partly accessible for ConA but not accessible for WGA. Calcofluor White, a small chitin-binding compound, was able to penetrate the whole cell wall, indicating the presence of chitin in the subapical wall. Treatment of the mycelium with NaOH did not alter the shape of the hyphal tube, removed the mannose and made the chitin in the subapical wall accessible for WGA. Our results suggest that chitin is covered at least by a layer of mannoproteins. These proteins could be released from the cell wall by a β-1,3-glucanase, which shows a linkage between β-1,3-glucan and mannoproteins. The presence of a glycan moiety in these proteins was established by PAS staining. Future research will be focused on the mannoproteins that are exposed at the hyphal tip and

may play a role in the interaction between fungus and host.

Structure-function Studies on Race-specific Elicitors of *Cladosporium fulvum*

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The interaction between tomato and the fungal pathogen *Cladosporium fulvum* fits the gene-for-gene model which means that *Cf*-resistance genes in tomato interact with *Avr*-avirulence genes of the fungus (De Wit, P.J.G.M. 1992, *Ann. Rev. Phytopathol.* **30**: 391–418). Recognition of the avirulence gene products AVR4 and AVR9 (race-specific elicitors) by putative receptors in Cf4 and Cf9 tomato genotypes leads to incompatibility characterized by a hypersensitive response (HR=local necrosis). The avirulence genes *avr4* and *avr9* and their encoded gene products have been studied extensively (Joosten, M.H.A.J. *et al.* 1994, *Nature* **367**: 384–387; Van den Ackerveken *et al.* 1992, *Plant J.* **2**: 359–366). Races of *C. fulvum* virulent on Cf9 genotypes lack the matching avirulence gene *avr9*. In contrast, *C. fulvum* races virulent on Cf4 tomato genotypes contain alleles with point mutations in the *Avr4* gene leading to a change in one amino acid residue and at the same time into a non-functional AVR4 elicitor.

The Potato Virus X (PVX) expression system (Chapman *et al.* 1992, *Plant J.* **2**: 549–557) was used to express *Avr4* and *Avr9* in *Nicotiana clevelandii* and in the tomato genotypes Cf4 and Cf9. Infection of Cf4 tomato genotypes with PVX/*Avr4* results in a systemic and constitutive HR leading to death of the whole plant. The same effect was observed when Cf9 tomato genotypes were inoculated with PVX/*Avr9* constructs. This PVX expression system was applied to screen *Avr9*-mutants for altered ability to develop systemic HR.

Based on 2D-NMR studies, a preliminary 3D-structure of wild-type AVR9 was determined. Surface-borne amino acid residues thought to interact with a plant receptor and important for HR-inducing activity were chosen to be exchanged by site-directed mutagenesis. Until now, 12 *avr9* mutant PVX constructs were expressed in Cf9 tomato genotypes. A mutant in which histidine residue 28 was exchanged by leucine (HIS28LEU) showed consistently reduced HR, while a ARG8LYS mutant showed consistently higher HR activity than the wild-type AVR9. Wild-type AVR9 binds specifically to plasma membranes of tomato irrespective of whether they contain Cf9 or not ($K_d \sim 50$ pM). The HIS28LEU mutant AVR9 peptide was purified from intercellular washing fluid of *N. clevelandii* and showed significantly lower affinity to tomato plasma membranes than wild-type AVR9.

Variation in Virulence of the Root Knot Nematodes *Meloidogyne hapla* and *M. chitwoodi* on Potato Cultivars

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The root knot nematodes *Meloidogyne hapla* and *M. chitwoodi* are polyphagous endoparasites that can cause considerable reduction in quality and yield of many important crop plants. Virulence, described as the capacity of the pathogen to cause symptoms in spite of the presence of resistance genes in a host species, was studied in potato, in an experiment with 10 cultivars inoculated with five populations of *M. hapla*, three of *M. chitwoodi* type T (='Rips') and two of *M. chitwoodi* type B (='Baexem'), all from The Netherlands. Reproduction can be considered as a main component to describe virulence and was estimated by three parameters: the number of egg masses produced after one generation per inoculated juvenile, the number of juveniles produced per egg mass and the number of juveniles produced after one generation per inoculated juvenile (Pf/Pi).

Although potato is considered to be a susceptible crop to *Meloidogyne* spp., statistically significant effects were obtained for cultivar, *Meloidogyne* species and population within a species, for the three reproduction parameters except for Pf/Pi between *M. chitwoodi* populations. Significant cultivar × population interaction effects were obtained between *M. hapla* populations for number of egg masses and Pf/Pi, which could indicate the presence of several (a-)virulence genes. One polyploid *M. hapla* population appeared to be relatively avirulent to most of the 10 cultivars. *M. chitwoodi* type B populations were more virulent than *M. chitwoodi* type T, and *M. hapla* populations were significantly less virulent than both types of *M. chitwoodi*.

Ribosomal DNA Restriction Fragment Length Polymorphisms between Isolates of Root-Knot Nematodes

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The plant parasitic root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi* cause serious problems in The Netherlands. Two different types of

M. chitwoodi have been found in The Netherlands. They show different isozyme patterns, they differ morphologically and they have different host ranges. A prerequisite for studying these nematodes is the ability to identify them in field populations and to culture them as pure populations for research purposes.

Comparative analysis of coding and non-coding regions of ribosomal DNA (rDNA) is a popular tool for the specific or subspecific identification of many organisms. rDNA has the advantage of being repetitive and its abundance in the genome makes its visualization relatively easy. Its coding sequences are conserved, whereas there is great variability within the ITS-regions and non-transcribed spacer regions. The more conserved sequences are most useful for classification at higher taxonomic levels (genus to phylum), while the variable sequences are useful at specific and subspecific levels.

In the work present, DNA fragments containing the ITS rDNA were amplified from total DNA from a large amount of geographical widely distributed isolates of *M. hapla, M. chitwoodi*, and from two isolates of *M. javanica* and *M. incognita* by PCR. The amount of DNA present in a single juvenile was already sufficient to amplify these PCR products. The amplified ITS fragments were relatively short as compared to those found in other nematode genera. Digestion of the ITS regions distinguished *M. hapla* and *M. chitwoodi* from each other as well as from *M. incognita* and *M. javanica*. The different types of *M. chitwoodi* could also be separated by ITS RFLPs. Results of this work and ways to apply ITS RFLPs will be discussed.

Characterization of Salivary Proteins from Potato Cyst Nematodes

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.Antibodies are versatile tools to endow plants with new properties by *in situ* inhibition of biological processes. They offer perspectives for molecular resistance by interfering in essential steps in the plant–pathogen interaction. We chose the potato cyst nematode *Globodera rostochiensis* as a model system for plantibody mediated resistance.

For its growth, development, and reproduction *G. rostochiensis* is fully dependent on food supplied by a feeding cell (syncytium). Syncytia are redifferentiated root cells. The redifferentiation process is triggered by saliva proteins of the nematode. Video-enhanced contrast light microscopy demonstrated saliva secretions to be injected via the stylet of the nematode into root cells.

Monoclonal antibodies (MAbs) were raised against salivary proteins of second stage juveniles (J2). On Western blots of preparasitic J2 homogenates, these MAbs reacted with a highly immunodominant epitope present on three protein bands (32, 39 and 49 kD). These subventral gland proteins were consistently expressed throughout the nematode's life cycle. With immunogold electron microscopy the antigens were localized in the endoplasmic reticulum and secretory granules of the subventral oesophageal glands.

We have detected the three proteins in stylet secretions of preparasitic J2 *in vitro* by using indirect immunofluorescence microscopy and Western blot analysis.

Immunoscreening of a large cDNA library ($2{\cdot}6 \times 10^6$ primary recombinants) of preparasitic J2 resulted in the isolation of two clones containing a 900 bp insert. Western blots of these clones demonstrated the production of a (*LacZ* fusion) protein binding to all subventral gland specific MAbs. This fragment will further be used for the isolation and characterization of genes encoding subventral gland proteins.

Control of *Chenopodium album* by Soil Application of Spores of *Ascochyta caulina*

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Chenopodium album L. is an annual plant and considered a troublesome weed in many arable crops. At present, *C. album* is controlled by combinations of cultural, chemical and mechanical control methods. Biological control of *C. album* might be possible by applying a massive dose of fungal inoculum to the weed.

From 1991 till present, control of *C. album* by *Ascochyta caulina* (P. Kast.) v.d. Aa & v. Kest. was studied as a part of the long-term crop protection plan (MJPG) of the Dutch government. *A. caulina* is a facultative, plant pathogenic fungus on *Chenopodium*- and *Atriplex*-species and causes necrotic lesions on leaves and stems of infected plants. Up to now, only the asexual stage of this fungus is observed. Pycnidiospores of *A. caulina* can be produced on artificial media, and are used as inoculum in experiments. In the study presented here, we investigated the effect of soil application of spores of *A. caulina* on disease incidence and mortality of

C. album. The experiments were carried out under controlled conditions. *C. album* was planted in soil in plastic pots. Spores were applied to soil by mixing spore suspensions through the soil or by spraying spore suspensions on the soil. Emerged, diseased and dead plants were counted at different time intervals within one month.

Application of pycnidiospores to the soil resulted in disease development of *C. album*. Infected seedlings had an olive-green colour, or carried necrotic lesions on cotyledons or hypocotyls. They were retarded in growth or died. Disease incidence and plant mortality were affected by numbers of spores applied, soil moisture content and soil type.

Way of spore application and planting depth had no significant effect on disease incidence or plant mortality We concluded that application of spores of *A. caulina* to soil can be a way to control *C. album*.

Effects of Flooding, Soil Fumigation and Composted Organic Household Waste on *Pythium* Root Rot in Bulbous Iris

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Pythium is a soil-borne fungus which causes root rot in several bulb crops. To reduce the use and dependence on fungicides, non-chemical control methods have to be developed. *Pythium* is a fast-growing fungus which is susceptible to competition by other micro-organisms. A light infestation in non-sterilized soil causes moderate root rot, whereas the same infestation in sterilized soil leads to severe disease development. Pot experiments were performed to study the impact of several cultural practices on *Pythium* root rot in bulbous iris (*Iris* L., Dutch Iris group 'White van Vliet') in relation to the condition of the soil microflora. Flooding and soil fumigation are generally applied in ornamental bulb culture to control some diseases and weeds. Flooding, however, does not kill *Pythium*. Flooding of infested field soil resulted in enhanced root rot compared to the non-flooded treatment. A similar effect was found when *Pythium* was introduced after soil fumigation with methylisothiocyanate or dichloropropene. Infestation of fumigated field soil resulted in enhanced root rot compared to infestation of non-fumigated soil. In the absence of other micro-organisms (heat-sterilized soil) flooding and fumigation treatment had no effect on the disease development, indicating the crucial role of the microflora in the adverse effects of flooding and fumigation.

Apparently, *Pythium* benefits from the elimination or disturbance of the soil microflora by sterilization, flooding or fumigation. To restore the natural disease suppression in treated soils the effect of adding composted organic household waste was investigated. In biologically disturbed soils small amounts of compost may effectively serve as inoculum of a broad spectrum of micro-organisms which induce general supressiveness.

The introduction of micro-organisms by adding 1% matured compost (equivalent to the maximal allowed dose for field application) to heat-sterilized soil 1 week prior to infestation with *Pythium* resulted in a reduction of both the pathogen population and the disease development, compared to treatments without compost or with sterilized compost. Further experiments are performed to determine the effects of compost application after flooding and fumigation of field soil.

Possibilities for Biocontrol of *Phytophthora infestans* in Potato with Two Bacterial Antagonists

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In this study we tried to develop a biological method to control *Phytophthora infestans* in potato. The aim was to interfere directly with leaf infection by *P. infestans* through a preventive bacterial spray. Of more than 150 bacteria and 40 yeasts, screened in a detached-leaf test, 11 bacterial isolates gave more than 70% reduction of *P. infestans* severity. Two of these bacteria, *Pseudomonas fluorescens* strain C148 and *Bacillus* strain B39, were chosen for further studies. Both bacterial antagonists were also able to reduce *P. infestans* severity to very low levels (<10%) on whole plants under controlled environmental conditions.

Field trials with B39 and C148 were less successful. A significant delay (5–7 days) of a *P. infestans* epidemic was observed only once, using weekly applications of B39. From population dynamic studies, under controlled environmental conditions, a positive relation between population density on the leaf surface and suppression of *P. infestans* was found for both bacterial antagonists. It appeared that antagonist populations needed for control of *P. infestans* were rarely reached in the field.

The bottleneck for a successful use of these bacterial antagonists under field conditions is their survival on the leaf surface. Both B39 and C148 are unable to reach and maintain population densities needed for control of *P. infestans*. Development of a formulated product of the antagonists may improve their survival and effectiveness. Furthermore, it may be concluded that our approach was not optimal, especially if one considers the very short time

available for the antagonist to interact with the pathogen. It takes the pathogen only 2–4 hours to enter the plant and antagonist populations have to reach sufficiently high densities in that time to prevent infection. Screening of antagonists which act by induced resistance may be a more successful approach for the control of this devastating disease.

Bacterial Determinants Involved in Induction of Systemic Resistance against Fusarium Wilt in Carnation and Radish

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Fluorescent *Pseudomonas* bacteria have been studied extensively for their ability to suppress soil-borne fungal diseases. One of the mechanisms involved appears to be systemically induced resistance. Evidence for the involvement of this mechanism was obtained in carnation and radish infected with *Fusarium oxysporum* f.sp. *dianthi* and f.sp. *raphani*, respectively, upon treatment of roots with selected strains of *Pseudomonas fluorescens*. Induced resistance was demonstrated by spatially separating the inducing bacterium from the challenging fungus, thus avoiding direct interactions between these organisms.

Bacterial determinants involved in the induction of systemic resistance were studied by using specific mutants and/or by applying specific metabolites produced by the strains *in vitro*. Bacterial mutants lacking the O-antigenic side-chain of the lipopolysaccharide (LPS) were ineffective in inducing resistance. Conversely, resistance was induced by applying purified LPS. However, when iron availability in the container medium was lowered, LPS mutants did induce resistance and the level of disease suppression was comparable to that of the wild-type bacteria. Apparently, iron-regulated metabolites of these bacteria can also induce resistance. Upon iron limitation, fluorescent pseudomonads produce their fluorescent siderophore (pseudobactin); some strains also produce salicylic acid. When applied as a purified compound both pseudobactin and salicylic acid induced systemic resistance in radish.

We are currently working on the isolation of further defined mutants lacking pseudobactin production, salicylic acid production, the O-antigenic side chain of the LPS and all possible combinations of these. This will enable us to evaluate the relative importance of each of the different determinants involved, as well as to study whether the same determinants induce resistance in other plant species.

Induced Resistance in Radish: a Matter of Time

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Induced Systemic Resistance (ISR) to subsequent infection commonly develops when a pathogen triggers a hypersensitive response. However, Van Peer and Schippers (1991, *Phytopathology* **81**: 728–734) found that in carnation ISR is induced by non-pathogenic *Pseudomonas fluorescens* WCS417r. Bacterially induced ISR differs from 'classical' induced resistance in that no symptoms are evident before challenge inoculation. Leeman (1995, PhD thesis, Utrecht University) demonstrated similar ISR in radish, using a bioassay in which inducing pseudomonads at the root tip were spatially separated from pathogenic *Fusarium oxysporum* f.sp. *raphani* WCS600 on the base of the roots.

When induced root parts of radish (c.v. Saxa*Nova, moderately resistant to WCS600) were amputated later than 3 days after application of bacteria disease suppression still occurred. This indicates that ISR develops within 3 days and that, when induced, it is maintained in the absence of the inducer.

When disease development is followed in time, there is a distinct difference in infection rates of induced plants versus non-bacterized controls. Once infection has occurred, disease develops similarly in non-induced plants and no difference in disease severity between diseased bacterized and diseased non-bacterized plants is evident.

It appears that bacterization reduces *Fusarium* wilt in radish by reducing and/or delaying fungal infection. The mechanism of this enhanced resistance is currently being investigated.

Analysis of the Role of Ethylene in Systemic Acquired Resistance

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Ethylene is a plant hormone that controls many aspects of plant growth and development, and also plays a role in stress response. Ethylene production increases during the hypersensitive response of Samsun NN tobacco to tobacco mosaic virus (TMV). The hypersensitive response is followed by the induction of the expression of genes encoding

pathogenesis related (PR) proteins and the development of a systemic acquired resistance (SAR) against viruses, fungi and bacteria.

The ethylene biosynthetic pathway is controlled by two key enzymes: 1-aminocyclopropane-1-carboxylic acid synthase (ACCS) and ethylene forming enzyme (EFE). ACCS converts S-adenosyl-methionine into ACC and EFE converts ACC to ethylene. Local and systemic induction of ACCS and EFE gene expression by TMV has been analysed. In a time course experiment total RNA was isolated from local and systemic TMV induced leaves. Northern blotting showed that the ACCS and EFE genes are locally induced by TMV. No systemic induction was observed. EFE genes were induced by treatment of the plants with ethephon, but ACCS genes were not. The cDNAs for ACCS and EFE have been transformed into Samsun NN tobacco plants in the sense or antisense orientation. Plants were transformed with the single gene or with a combination of the ACCS and EFE genes. Primary transformants have been obtained, and the expression levels of the transgenes were analysed. The primary transformants were selfed, and the progeny is currently used for further analysis. The effect of overexpression or silencing of ACCS and EFE on ethlene production and induction of PR genes and SAR is being investigated.

MEETING OF THE SECTION FOR PLANT CELL AND TISSUE CULTURE ON 17 MARCH 1995

Ins and Outs of Position Effects in Transgenic Plants

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Upon plant transformation, a large variation in transgene expression levels is observed. This variation shows our limited knowledge concerning the regulation of plant gene expression, hampers comparisons of constructs, and makes plant transformation much less efficient and predictable than desirable. The variation, commonly designated as 'position effect', is attributed to the supposedly random integration of the transgene in the plant genome and/or the transgene DNA configuration. The position effect is thought to reflect the influence of surrounding chromatin on gene expression. In animal systems, so-called matrix-associated regions (MAR elements) were shown to alleviate position effects. MAR elements interact with the nuclear matrix. When surrounding a transgene, the elements are supposed to create a loop of DNA that behaves as a unit of transcription that is less susceptible to the influences of the neighbouring chromatin.

The efficacy of one of the better characterized MAR elements, the chicken lysozyme A element, in reducing transgene variability was demonstrated in mature transgenic tobacco plants using the β-glucuronidase (GUS) gene as a reporter, driven by either a plant (Mlynárová, L. *et al.* 1994, *Plant Cell* **6**: 417–426) or a viral promoter. When cloned at the borders of the *Agrobacterium* T-DNA, encompassing both the GUS reporter and the NPTII selection gene conferring kanamycin resistance, the presence of this element reduced positional variation of transgene expression up to 30-fold. The presence of the A-element at the borders of the T-DNA does not cause meiotical instability. It is anticipated that this type of elements will improve the overall efficiency of the genetic modification of plants.

In vitro Tuberization and Carbohydrate Metabolism in Potato

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To enable the study of changes in carbohydrate metabolism during tuberization in potato, an *in vitro* tuberization system was developed, essentially as described by Hendriks, T. *et al.* 1991, *Plant Mol. Biol.* **17**: 385–394. Stem cuttings of potato plants grown under tuber-inducing conditions were put on a solid medium with cytokinin, 8% sucrose and a reduced nitrogen content. After 4–6 days in darkness, the axillary buds of almost all cuttings formed small tubers. In control treatments (same media but lower sucrose or added gibberellin), the buds developed into stolon-like shoots without any visible swelling.

At the point of visible tuberization, several enzymes display a remarkable change in activity. Invertase (both soluble and insoluble acid invertase) activity decreases whereas the activities of sucrose synthase, fructokinase and ADP-glucose pyrophosphorylase increase sharply. Simultaneously, the content of both glucose and fructose in the tubers drops. These changes do not occur in the control treatments.

The sharp changes in enzyme patterns and the contents of neutral sugars confirm that the *in vitro* tuberization system enables the study of changes in carbohydrate metabolism during tuberization. Currently, we are investigating the changes of other enzymes involved in carbohydrate metabolism and the changes in concentration of the related metabolites. Combined with the studies on gene expression during tuberization (in cooperation with the Department of Plant Breeding, WAU), we may be able to determine which events are related to tuberization, in which sequence they occur, and which genes are responsible for them.

Root Formation in Microcuttings of the Apple Rootstock 'Jork 9': Responsiveness to Different Auxins

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Auxins play a key role in adventitious root formation. We have determined the period of action of auxin during the rooting treatment, and the effectiveness of different auxins.

Adventitious root formation can be divided into three phases: dedifferentiation (during which the tissue becomes responsive to the rhizogenic stimulus), induction (during which cells becomes determined to form a root), and differentiation (outgrowth, during which the root is formed). We determined in microcuttings of *Malus* 'Jork 9' the timing of these phases by giving pulses with an auxin (isobutyric acid, IBA), a cytokinin (benzylaminopurine), an anti-auxin (*p*-chlorophenoxyisobutyric acid) or an inhibitor of auxin oxidation (caffeic acid). We assumed that inhibition by cytokinin and anti-auxin and promotion by auxin and auxin protector are maximal if they are applied during the induction phase. The times of action of the four compounds were similar. The timing of the three phases was found to be 0–24 h, 24–96 h, and from 96 h onwards, respectively.

For rooting, the auxins IBA, indoleacetic acid (IAA) and naphthylacetic acid (NAA) are mostly used. When applied during the induction period, these auxins resulted in a similar number of roots. However, when applied during the differentiation phase NAA resulted in highest inhibition. The auxin 2,4-dichlorophenoxyacetic acid (2,4-D) only induced a very few roots when applied during the induction period, but caused the strongest inhibition of rooting when applied during the differentiation phase. The results can be explained by the occurrence of two auxin-receptors: the first is related to root induction and has a high affinity to IAA, IBA and NAA, but a low affinity to 2,4-D. The second is related to the inhibition of root growth and has a low affinity to IAA, a medium affinity to IBA and NAA and a high affinity to 2,4-D.

Microspore-derived Embryos of Rapeseed as a Model System for Seed Development: Aspects of Lipid Metabolism

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Microspore-derived embryos (MDEs) are a suitable model system for studying seed development. MDEs are induced by giving isolated microspores a heat treatment for 3 days in a hormone-free but nutrient-rich medium (Gland, A. *et al.* 1988, *J. Plant Physiol.* **132:** 613–617). The embryos produced in this way have the typical advantages of a tissue culture system: ease of control and manipulation of conditions, and small size of experimental units. A number of studies showed that the morphological development of MDEs is similar to that of developing seeds up to the point of maturation. In other studies accumulation of triglycerides (oil) in MDEs has been demonstrated, but accumulation of storage proteins was reduced (Taylor, D.C. *et al.* 1990, *Planta* **181:** 18–26).

In our study we have compared the oil accumulation in seeds and MDEs in more detail with emphasis on the effects of temperature on oil accumulation and composition. Results show that seeds of the cultivar Reston grown at 25°C produce oil with reduced levels of erucic acid and a reduction in oil accumulation compared to seeds grown at 15°C. Erucic acid levels in oil from MDEs were lower than those from seeds, but the changes in oil composition as a result of changes in temperature were similar to those in seeds. Addition of abscisic acid to the medium of MDEs resulted in production of oil with a composition more like seed oil.

Investigations to Establish a System for Somatic Embryogenesis in *Arabidopsis thaliana*

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Through recent genetic studies, genes involved in the formation of zygotic embryos have been identified in *Arabidopsis*. However, for further study of the processes controlling embryogenesis, a system is needed

that reproducibly allows the development of somatic embryos *in vitro*. Apart from direct development of somatic embryos on explants, investigations were aimed at establishing embryogenic cell suspensions. In MS-medium, with a combination of 2,4-D and kinetin and using young seedlings, it was possible to directly initiate continuously growing suspension cultures that released small aggregates and putative proembryogenic masses to the medium. With NAA or dicamba as auxins the cultures contained very rough and less cytoplasm-rich aggregates, which led mainly to root formation. Patterns of secreted proteins are different in these different types of suspensions. However, LTP (lipid transfer protein, a marker for embryogenic *Daucus* suspensions and protoderm formation) was present in all cultures. From suspensions kept in 2,4-D, globular or triangle-like structures were obtained. Semi-thin sections of these structures showed similarities in the cellular organization of *Arabidopsis* zygotic embryos between the globular and triangle states. However, no further embryo development could be observed and these structures recallused and/or developed a short root or root hairs at the putative root pole. To overcome this arrest of development, cultures were transferred to conditioned media or were co-cultivated with *Daucus* somatic embryo cultures. The GUS expression under control of the AtLTP1 promoter was examined in transgenic cell cultures in order to screen conditions for the development of embryogenic cultures. Furthermore, suspension cultures of the embryo pattern mutants *gnom* and *keule* were established.

Freeze Fracture and Cryo SEM Observations During Androgenic Microspore Culture of *Brassica napus*

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For plant breeding, androgenic microspore cultures are an efficient way to obtain high amounts of haploids in a rather short time. Induction of the embryogenic pathway of pollen development instead of the normal gametophytic path is often obtained by exposing the microspores to a heat shock. Although some light microscopical and TEM studies have been performed (Hause, B. *et al.* 1993, *Cell Biol. Int.* **17:** 153–168; Zaki, M. & Dickinson, H. 1990, *Protoplasma* **156:** 149–162), no freeze fracture and cryo SEM work have been done. We have made observations on embryogenic microspore cultures of *Brassica napus*, using both methods. We focused on the structure and distribution of nuclear pore complexes, which have been reported to be indicative for cellular activity (Wagner, V. *et al.* 1990, *Planta* **181:** 304–309). The methods used are extremely suitable to study this in detail.

Various developmental stages of embryogenic microspores were sampled and plunged into liquid propane. Freeze fracturing was done at −130°C using conventional techniques. Replicas were cleaned in 50% chromic acid for 2–3 hours, and overnight in 4% sodium hypochlorite. They were examined with a JEOL 1200 EX TEM. For cryo SEM, a small droplet of concentrated microspores was put between two rivets and frozen in liquid propane. The sample was transferred into a cryo transfer unit (CT 1500 HF, Oxford Instruments, UK) which was connected with a JEOL 6300 F. After fracturing at −130°C and subsequent sputtering with 3 nm platinum the specimens were brought directly into the SEM chamber, which was maintained at −180°C. Some first results are presented that do not indicate remarkable changes in nuclear pore number or distribution. However, at high magnifications occasionally a tripartite substructure of the nuclear pore complex was found with both methods that cannot be explained by the current models (Akey, C. & Radermacher, M. 1993, *J. Cell Biol.* **122:** 1–19; Goldberg, M. & Allen, T. 1993, *J. Cell Sci.* **106:** 261–274).

Methyljasmonate inhibits dormancy development in lily bulblets regenerated *in vitro*

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Lily bulblets regenerating from scale explants *in vitro* develop dormancy: at harvest, 11 weeks after the start of tissue culture, they require a cold treatment to achieve rapid sprouting after planting in soil. This tissue culture system allowed us to identify the factors that control dormancy development. We found that abscisic acid (ABA) played a major role. Auxin, ethylene, gibberellin and cytokinin did not affect dormancy development.

We have observed previously that bulb formation and dormancy development are often concurrent processes, and other studies have shown that methyljasmonate (MeJa) promotes storage organ formation, so we examined the effect of MeJa on dormancy development in the Asian hybrid 'Connecticut King'. As expected, MeJa strongly promoted the relative bulb weight (bulb weight as a percentage of plant weight). However, bulblets regenerated in the presence of MeJa developed dormancy. At the optimal MeJa concentration (250 µl l^{-1}) 100% of the

bulblets sprouted without a cold treatment. Without addition of MeJa, this value was 20%. We also observed this effect of MeJa in *L. speciosum* 'Rubrum No. 10' and *L. longiflorum* 'Snow Queen'.

MEETING OF THE SECTION FOR PLANT SYSTEMATICS AND GEOGRAPHY ON 21 APRIL 1995

Seed Coat Structure and the Delimitation of the Geraniales

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In older classifications, e.g. that of A. Engler & K. Prantl (1931, *Nat. Pflanzenfam.* 19a), the order Geraniales was large and heterogenous, comprising families such as Rutaceae, Euphorbiaceae, Linaceae and Polygalaceae. This concept gradually changed in more recent classifications and the order was split up into a number of separate orders, such as Rutales, Euphorbiales, Linales and Polygalales. As a consequence, the remaining order Geraniales became much smaller. However, there is still no consensus concerning its composition. In most modern classifications, the Oxalidaceae are included in the order. Only Dahlgren (in Tan, K. 1989, *The Davis and Hedge Festschrift*: 249–260. Edinburgh Univ. Press) groups of Oxalidaceae in the Linales. This is in agreement with the seed anatomical data. Also, Zygophyllaceae are placed either in the Geraniales or in Linales. Most authors follow Engler & Prantl (1931) in placing the families Vivianiaceae, Ledocarpaceae, Rhynchothecaceae, Biebersteiniaceae and Dirachmaceae as related families close to the Geraniaceae, or even as genera under Geraniaceae. Ovule and seed coat structure of these taxa were studied in order to obtain additional characters useful in phylogenetic discussions.

The Geraniaceae *sensu stricto* are characterized by beaked fruits, campylotropous seeds and a well-differentiated seed coat with a crystal-containing endotesta and a palisade exotegmen. The Vivianiaceae, Ledocarpaceae, Rhynchothecaceae, and Biebersteiniaceae have rather undifferentiated seed coats and that of *Dirachma* is characterized by an exotesta and an antiraphal vascular bundle. Because all these seed coats differ from the geraniaceous one, these families do not seem closely related to the Geraniaceae *s.s.* and probably have to be excluded from the Geraniales. Vivianiaceae and Ledocarpaceae resemble each other by ovule and seed structure, and thick-walled endosperm. They are probably more closely related to the Linales.

Explosive Pods and Seed Dispersal of Caesalpinioid Trees in the African Rain Forest

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Explosive seed dispersal is a characteristic feature of Caesalpinioid tree species in Gabon, especially in the tribe Amherstieae. At least 74 Caesalpinioid tree species with explosive dispersal are indigenous to Gabon. One of these is *Tetraberlinia moreliana* Aubr. The dehiscence of pods of this tree sends the seeds away at an average angle of 17·3° upwards from the horizontal. There is a sharply defined maximum distance over which seeds can be projected. The furthest explosively dispersed seed of a 48 m tall tree was found 60 m away, measured horizontally from the edge of the crown. This is the longest distance for explosive seed dispersal ever recorded in literature. Seeds can pass over various neighbouring tree tops before they reach the ground. The explosive dispersal of large Caesalpinioid rain forest trees might be a balance between the dependence of seedlings on better growth conditions away from the parent tree, and their dependence on infection with ectomycorrhizae of the parent tree.

An Exploratory Study on Seed Morphology of *Miconia* Ruiz & Pavón (Melastomataceae), with Taxonomic and Ecological Implications

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The results of an exploratory study on seed morphology of *Miconia* Ruis & Pavón (Melastomataceae) is presented. Seeds of 75 species, mainly from north-west South America, including representatives of all 12 sections of *Miconia*, were examined, 57 of them by SEM. *Miconia* shows an appreciable diversity in seed structure. Twelve major seed types are defined and compared with the current sectional division of the

genus and with ecological data. The types do not coincide with the established sections. The defined seed types may well be of importance for phylogenetic classification, because they may indicate relationships. In general, variation in seed morphology in *Miconia* largely overlaps with variation found in the tribe Miconieae as a whole. Implications for seed dispersal and habitat characteristics are given. The importance of secondary dispersal is discussed: a few species are probably ant-dispersed after having been dropped in vertebrate faeces. Some of the seed types form a species group restricted to certain belts and certain precipitation zones.

Systematics of the Moss Family Hypopterygiaceae Mitt.

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The Hypopterygiaceae Mitt. are a small family of pleurocarpous mosses with mainly a Gondwanan distribution. They mostly occur in the humid forests of the warm-temperate to tropical areas of the world. They have at least partly complanate stems and branches with two rows of asymmetrical, lateral leaves and a single, ventral row of smaller, symmetrical amphigastria.

The Hypopterygiaceae were regarded as a separate, monophyletic family. However, during the last 20 years the classification and phylogeny of the Hypopterygiaceae have been discussed. Several authors regarded the Hypopterygiaceae as polyphyletic, others suggested the family to be monophyletic. There is no consensus on the relationships between the genera of the Hypopterygiaceae either within the family, or on the relationships between these genera and some other moss families.

After completing a taxonomic revision of the Hypopterygiaceae, the *c.* 160 validly published species and infraspecific taxa will be reduced to 26 species. The family shows its greatest diversity in the Indo-malesian Archipelago (10 species).

The phylogenetic relationships between the species of the Hypopterygiaceae and representatives of possibly related taxa were analysed with the computer program HENNIG86. The 57 characters of gametophyte and sporophyte that were used for these analyses were coded as multistate and treated as unordered. The analyses resulted, due to much homoplasy, in 234 most parsimonious trees (length 327 steps, CI 0·28, RI 0·54), which mainly differ in the topography of the paraphyletic genus *Hypopterygium* Brid. The results suggest that the Hypopterygiaceae are polyphyletic, because the genus *Cyathophorum* P. Beauv. seems to be closely related to the Hookeriaceae Schimp. *s.l.* However, the phylogeny of the Hypopterygiaceae *s.l.* is not completely satisfactorily resolved. For a definite transfer of *Cyathophorum* to the Hookeriaceae *s.l.* further research of the relationships of the Hypopterygiaceae *s.l.* is necessary, and should include other possibly related pleurocarpous moss families and the use of molecular techniques.

Tabernaemontana (Apocynaceae) and its Relatives

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The genus *Tabernaemontana* (Leeuwenberg, A.J.M. 1991, *A Revision of* Tabernaemontana, *The Old World Species*, Royal Botanic Gardens, Kew; 1994, *A Revision of* Tabernaemontana *2, The New World Species and* Stemmadenia, Royal Botanic Gardens, Kew) belongs to the tribe Tabernaemontaneae of the subfamily Plumerioideae of the Apocynaceae. It comprises 99 species and is represented throughout the tropical belt. It is by far the biggest of the nine genera in the tribe. All genera are woody plants growing in the model of Leeuwenberg. The entirely fertile, mostly triangular anthers, and the seeds with their deep hilar groove and ruminate endosperm are important characteristics of the tribe. All genera are so closely allied that it was difficult to make a key. This tribe shows a trend seen by several other tribes or even families in that there are many genera with few species in Africa and few genera and many species in America. Four genera are monotypic and African, *Tabernanthe* with two species is also African, as is *Callichilia* with seven species. *Voacanga* occurs with 12 species in the Old World and *Stemmadenia* with 10 species in the New World. The delimitation of *Tabernaemontana* has been disputable since Alfonse De Candolle (1844, *Prodromus* 8, Fortin, Masson & Sociorum, Paris), who made the first segregates. Monographs of all genera have been published.

Parkia biglobosa (Leguminosae: Mimosoideae) in West Africa

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Parkia biglobosa (Jacq.) R.Br. ex G.Don is one of the useful indigenous tree species in West Africa. A detailed study on its diversity was recently published (A.S. Ouédraogo, 1995: *Parkia biglobosa* (Leguminosae) en Afrique de l'Ouest: Biostystématique et Amélioration; WAU, IBN-DLO, CNSF,

Wageningen). Virtually all parts are used for food, medicine, or customary use. The fermented seeds provide a condiment, used in most sauces accompanying cereal dishes. The rich ethnobotany and folk taxonomy are proof of a long association with man, although the tree is not actually domesticated.

The tree is adapted to the Sahel, and various clines of morphological characters are detectable, running from east to west, such as in seed colour and thickness. On the other hand, intra-population diversity is usually larger than inter-population diversity and even larger than the differences between countries, pointing to cross-fertilization. The pollinators in the northern part of the area, such as in Burkina Faso, are mainly bees, towards the south bats are more important (Hopkins, H.C. 1983, *Bot. J. Linn. Soc.* **87**: 135–167). Flowers conform to the bat pollination syndrome.

Conservation of germplasm is required, as rejuvenation is affected by over-exploitation, and *Parkia* orchards are generally ageing. To this effect there is a European Union project on Germplasm Conservation and Improvement of *Paria biglobosa* for Multipurpose Use as a follow-up to the above-mentioned studies. Sibidou Sina (1995, in *Germplasm Conservation and Improvement of* Paria biglobosa *for Multipurpose Use*; E.U. Contract TS3*-CT92-0072; Second Annual Progress Report) characterized various populations by acrylamide gel and starch gel electrophoresis, and endeavours to establish the rates of gene-flow within populations. The genetic diversity (heterozygosity) is large, ranging between 0·36 and 0·52. The polymorphism is on average 92%, reaching 100% for six out of 11 populations from four countries, tested with PAGE. clustering on genetic distances (Prevosti) do not bear relation to geography.

Adaptive radiation in *Aeonium* (Crassulaceae)

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In the Macaronesian Archipelagoes (Azores, Madeira, Canary Islands, and Cape Verde Islands) the subfamily Sempervivoideae of the succulent plant family Crassulaceae comprises four genera, *Aeonium, Aichryson, Monanthes*, and *Greenovia* (Lösch, R. 1990, *Dissertationes Botanicae* **146**, J. Cramer, Berlin-Stuttgart). In particular, the species of *Aeonium* have markedly different growth forms, which have evolved through adaptive radiation (Carlquist, S. 1974, *Island Biology*. Columbia University Press, New York). However, in taxa that evolved through adaptive radiation excessive morphological divergence generally obscures phylogenetic relationships, e.g. a large polytomy comprising all but five of the 40 species of *Aeonium* is present in a cladogram based on morphological data.

Recently, the use of molecular techniques to generate independent datasets for phylogenetic reconstruction has greatly contributed to our understanding of adaptive radiation on oceanic islands. In many of the resulting phylogenies of these taxa, unresolved relationships are present (Baldwin *et al.*, 1990. *Ann. Missouri Bot. Gard.* 77: 96–109). Whether such polytomies result from rapid speciation or not is still a problem, because unresolved phylogenetic relationships can result from (i) a lack of variation (due to rapid speciation or too conservative data), (ii) homoplasy, or (iii) a too distant outgroup. For *Aeonium*, appropriate outgroups exist (Ham, R.C.H.J. van. 1994, *Phylogenetic implications of chloroplast DNA variation in the crassulaceae*. Ph.D. thesis, University of Utrecht) and therefore the unresolved relationships within the genus are due either to homoplasy or a lack of variation. We have tried to identify monophyletic clades within *Aeonium* at the infrageneric level as well as at (intra)specific levels. Identical monophyletic clades were found in phylogenetic analyses of all data sets, but relationships among the monophyletic clades were unresolved. Polytomies are always present and these probably result from rapid speciation. This is supported by the low genetic divergence of both the nuclear and chloroplast genome in *Aeonium*.

Systematics of *Aporosa* Blume (Euphorbiaceae)

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Aporosa Blume consists of small dioecious trees, common in the tropical rain forests of the Malay Archipelago. The inflorescences are simple spikes. The male flowers, characterized by the near absence of the pistillodia, are clustered in glomerules along the rachis; the female flowers are borne singly.

The *c.* 80 species are delimited by specific combination of characters. Five main groups are defined on the arrangement of the flowers along the rachis and the position of disc-like glands on the lower surface of the leaves. Stamen length, female pedicel length, locularity, stigma structure, leaf colour on drying, and stipule persistence can, a.o., be used for subdivision. Finally, species are separated on differences in size and indumentum.

Phylogenetic analysis yields thousands of different cladograms. Three of the five main groups are monophyletic, but their position switches. The paraphyletic remnant shows little consensus in its specific relations; they are divided geographically into the

Sundan species, which are intermediate in many characters and character combinations, and the New Guinea species, which show a complete rearrangement of all Sundan character combinations.

For a better determination of relationships, subanalyses were conducted using selections of taxa and characters. Majority consensus trees were then compared. They disagree in the position of some Sundan species, which are placed as sister-species to various taxa. This can be explained by assuming that these species are survivors of a once species-rich group that was decimated by a Pleistocene ice-age. The modern monophyletic groups radiated afterwards from now-extinct species.

The New Guinea species form a second source of contradiction. However, the same two or three species are indicated as ancestral in most analyses. It might be that two primitive lineages of *Aporosa* colonized New Guinea and hybridized as a reaction to the constantly changing habitats and the strong geological activity. This hybrid origin of the New Guinea species would appear as morphological intermediacy and as switching positions in the phylogenetic tree.

Taxonomy and Phylogeny of the Tribe Erismantheae (Euphorbiaceae)

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The Euphorbiaceae, the revision of which is now one of the main research items of the Rijksherbarium, is a very heterogeneous family. This can easily be demonstrated with the tribe Erismantheae. The Erismantheae are characterized by opposite leaves and interpetiolar stipules, characters typical for the Rubiaceae. The plants are monoecious. The tribe comprises three small genera. The genus *Erismanthus* (two species) has flowers of both sexes on different inflorescences, a single pistillate flower per inflorescence and the staminate flowers contracted into a catkin, but with very long pedicels. The petals (absent in pistillate flowers) are smaller than the sepals. *Moultonianthus* (one species) also has the sexes on different inflorescences, but with several pistillate flowers per inflorescence. The petals (also present in pistillate flowers) are longer than the sepals. *Syndyophyllum* (two species) has the sexes united into one inflorescence, one pistillate flower and several staminate flowers per node. As in *Erismanthus*, the petals are shorter than the sepals and absent in pistillate flowers.

The nodal meristems of the Erismantheae show displacements. As a result, not only interpetiolar stipules are present, but the axillary buds can also be found in different places. *S. excelsum* has, per leaf pair, one axillary bud halfway the leaf petiole; the other bud is in the axil and develops into side branches and flowers. The axillary buds in *M. leembruggianus* are present between stipules and petioles, while in *Erismanthus* one bud of every leaf pair is present in the axil of a stipule instead of a leaf.

Phylogenetic analysis of the Erismantheae, together with the tribes Cheilosae (outgroup) and Chaetocarpeae, shows that all tribes are monophyletic. The monoecy, leaf and stipule position are typical for the Erismantheae. Within the Erismantheae the three genera are monophyletic with *Erismanthus* and *Syndyophyllum* as sister genera.

