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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR VEGETATION RESEARCH OF 4 APRIL 1984

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Some ecological theories and their consequences for the study of vegetation dynamics.

Recent developments in the study of relations between ecological phenomena have consequences for studies of succession and species richness.

GRIME (1979) gives an ordination of plant species with respect to the strategies with which they are adapted to classified environments. The environments are characterized by 'stress' (S), 'disturbance' (D) and 'competition' (C). In this 'environmental triangle' phytocoenoses can be classified as well. When relating this ordination with vegetation dynamics and species richness, some problems are met. According to Grime there is a development from 'D', mostly over 'C', to 'S'. Since species diversity is low in an 'S'-environment, there should be maximum species richness in an intermediate stage of succession. Indeed this is in accordance with theories and observations of Connell and others, but in contradiction with e.g. WHITTAKER's (1975) observation of a decrease with time of nutrient- and microclimate-stress and an increase of diversity.

The theories with respect to species diversity and vegetation dynamics as they are proposed by Huston, van Leeuwen, Newman and Whittaker, can be compiled in a model. If a positive relationship between 'disturbance' and 'environmental heterogeneity' is supposed, this model also includes the observations on which Grime's and Connell's theories are based. This model can help to understand temporal and spatial variation in species richness. Own observations on *Calluna*- and *Erica tetralix*-heath vegetation give an example of the possibilities of the model. To obtain values which can be regarded as 'α'-diversity, it is necessary to correct the number of species in a quadrat with species-heterogeneity.

Some researchers use mathematical models and microcosms (in-vitro ecosystems) to obtain observations on properties of a system under controlled conditions. Mostly it is assumed that the final state is a 'steady state' or an 'equilibrium' which is static or in balance. Another assumption on which this research is based is the irreversibility of the disappearance of a species from the system. GRUBB (1982) states that the equilibrium-assumption with respect to plant communities is not realistic. A species can re-enter a system after having been competed out (regeneration). When we look at the results of observations in permanent plots, we can frequently observe a species 'skip' one or two years. If Grubb's theory is correct, these skips should not be regarded as errors of the observer, but as an essential property of the system studied.

A problem to be solved is still whether a system – after a catastrophy – tends to go to a fixed steady state (which is determined by macro-abiotic conditions only) or to a variable steady state (being determined by initial biotic conditions as well). Grubb's theory might have far-reaching consequences for our understanding of vegetation dynamics

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Towards a heathland succession model

A model is developed to simulate heathland regeneration after fire. The model is based on field observations. Charts of a plot were digitalized and used to test Markovity by LIPPE et al. (1984), who observed a systematic trend in transition probabilities.

The basis of our model is a grid in which individuals of species establish, grow and die: Any grid square can be occupied by one individual, one individual can occupy more than one square. Establishment is varying from year to year and individuals of three species (*Empetrum nigrum, Calluna vulgaris* and *Erica tetralix*) are placed at random in the grid. Growth is related to the area occupied by an individual and restricted at larger sizes. Any species has its own growth pattern, related to a growth constant and size parameters. Area growth is translated to probabilities of occupying adjacent squares in the grid. Competition is modelled as competition for space. Death is more likely when individuals are young, when they are old and when they are restricted in growth by competition. In all three cases growth is low, so death probability of an individual is related to its actual growth. Each individual is traced in the model from establishment till death.

The first runs with parameters estimated from field data resulted in growth curves for the three species resembling well the real ones. The model is partly validated because parameters were estimated on the basis of charts made during the first years after a burn. Cover percentages estimated from digitalized charts are all, except *Empetrum* and bare ground in 1976, enclosed in the 90% simulation envelope. Response to growth and establishment parameters is almost linear in a restricted range, but *Empetrum* seems to be almost at its maximum cover (70%) and *Erica* is not pushed down to much less than 2% cover. *Calluna* response is linear over the whole tested range.

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Developments in botanical research for nature conservation and management

Some three decades ago the botanical research aimed at conservation of natural values was by and large limited to inventory, description and mapping. The persons involved accumulated an invaluable empirical knowledge of (rare) plant-environment relations. However, this type of knowledge is not easily transferred and therefore strongly 'personbound'. Between 1970 and 1980 the number of biologists employed to provide information for nature conservation and management increased rapidly. The permanent staff of the Botany Department of the National Research Institute for Nature Management (RIN) more than doubled, from eight in 1972 to 19 staff members in 1984. At the same time virtually all provincial administrations appointed their own biologists. This situation caused a shift in emphasis regarding research being carried out for nature conservation and management.

The important questions to be answered are and will remain:

- Where are the areas situated with actual high natural values; how are these values threatened and what should be done to maintain or improve the values? (the conservation aspect).
- Where are the areas situated with potential high natural values, what has to be done to bring about these values and what management practices should be applied to maintain them? (the habitat-creation aspect).

High natural values can in many cases be explained as 'providing the living conditions for rare and/or threatened organisms'. Apart from the increased number of scientists and assistants now working in the field concerned, the following developments should be considered:

- An ever increasing stream of information (from international and national published literature, 'grey' reports, personal communications, etc.).
- The environment being increasingly threatened (air and water pollution, road construction, lack of money for the proper management practices, etc.).

- Disappearance of areas that could serve as a reference (where conditions are as they should be).
- Increasing facilities as provided by modern technology.

Against this background the botanical research for nature conservation and management at the national level is shifting its emphasis from inventorying-descriptive (with predictions based on empirical knowledge, strongly 'person-related') to causal-analytical using a models approach (with predictions based on relations that are or can be proved and wherever possible an imput from databanks). The inventory role is largely taken over by the provincial workers and other local groups. This inventory-monitoring work is therefore still continued, but research groups working at a national level are increasingly putting emphasis on:

1. Development of methods for (field-)data collection and processing, using new technological facilities.
2. The establishment of databanks for storage and retrieval of (impersonal) knowledge useful for nature conservation and management.
3. Process-studies using a models' approach and allowing for generalisation of the findings in space and time.

A point urgently requiring attention is the exchange of information and integration of knowledge as at present gained by the many individuals and research groups active in this field, local, provincial and national, in the private sector, at governmental research institutes and universities. Keeping track of the existing knowledge is becoming a challenge in itself, for the researchers, but even more so for the persons in charge of the actual management practices.

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Some remarks on the use of integrated vegetation surveys in land evaluation projects

The distribution of plant communities in the north of the Netherlands was studied in relation to the hydrology of the Drenthian Plateau. The vegetation surveys were carried out in 1982 and 1983 in the catchment area of the 'Drentsche A' and in the heath-reserve 'Dwingelderveld' which forms an upstream infiltration area on top of the Plateau. The stream 'Drentse A' flows through several seepage areas, where large quantities of ground water discharge. Here hay meadows are quite common.

In the vegetation surveys over 160 vegetation types were distinguished (EVERTS et al. 1980, 1984; EVERTS & DE VRIES 1984). The vegetation types were delimited by using the Braun-Blanquet method. Their distribution and the distribution of some 50 selected phreatophytes (marsh plants) was assessed on a 1:5000 scale. During the survey geomorphological and hydrological information was gathered to add to the already existing geomorphological and hydrological investigations of the Drenthian Plateau (W.R.G.O.D. 1978; STREEFKERK & VAN HOORN 1984). The vegetation maps and the distribution of phreatophytes were related to rough estimations of ground water discharge, frequency of floodings and human interferences with the hydrology.

We found that in the catchment area of the 'Drentse A' the distribution of single species and that of vegetation types were linked to different hydrological phenomena. *Carex acutiformis*, for instance, was found in all (potential) seepage areas with $0 < \blacktriangle\varnothing < 3$ m. ($\blacktriangle\varnothing$ = difference in water potential of deep and undeep ground water). The *Angelico-Cirsietum oleracei*, a *Calthion palustris*-community characterized by a dominant occurrence of *Carex acutiformis* in the research area, was restricted to seepage areas with a very large discharge of ground water ($1 \leqslant \blacktriangle\varnothing < 3$ m.) and with convex shaped peat layers.

We, therefore, concluded that the abundant occurrence of *Carex acutiformis* is restricted to those seepage areas, where no barriers, such as clay layers were present under the valleys. Here large quantities of ground water from the main aquifer discharge. In seepage areas with barriers in the subsoil only small quantities discharge, possibly from layers above the main aquifer. Here *Carex acutiformis* is found in small numbers and within quite a number of different plant communities. We also found that interferences with the hydrology were indicated more specifically by the distribution of vegetation types than by the distribution of species. However in a landscape-ecological analy-

sis both must be included, for they give different, but additional information on the hydrology.

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Interpretation of the results of hay-making without fertilization in grassland communities

Authorities in charge of nature management practise hay-making without fertilization aiming at impoverishing the soil, a subsequent decrease of standing crop and higher species diversity. Their underlying starting-point is that these goals are reached better the longer the management practice continues. Hardly any long lasting permanent plot data, however, are available to test this idea. We therefore compared lots under different periods of hay-making with permanent plot data in the nature reserve Drentse A.

Most lots consisted of dry sandy parts with infiltration and wet peaty parts with seepage. Clustering of permanent plot data concorded with these abiotic differences. Dissimilarities between the first year and subsequent years revealed that wet and dry subsets of permanent plots changed equally after a short period by hay-making. The dry subsets changed more than the wet subsets after a longer period of hay-making. Dissimilarities between subsequent years decreased, indicating that the rate of change decreased in all subsets of permanent plots.

The trend of changing plant communities was related to indicator values, since no soil chemical analyses were available. All occurring plant species were assigned to three classes: indicating nutrient poor soil conditions, medium- and nutrient rich soil conditions. The ratio of species indicating nutrient poor and nutrient rich soil conditions (poor/rich ratio) was taken as a measure for the nutrient status of the soil. The poor/rich ratio was lowest in the dry subsets and did not increase. In the wet subsets, however, the poor/rich ratio increased after a short period of hay-making, but sometimes decreased after a longer period of hay-making.

Species diversity was higher in wet than in dry subsets of permanent plots. In the dry subsets also species indicating nutrient rich soil conditions ingressed. An other anomaly was the high species diversity in some wet subsets combined with a larger standing crop of thin stands of *Juncus acutiflorus* than in subsets with a lower species diversity. Perhaps the amount of light reaching the soil was more important than the standing crop on its own.

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Vegetation research as applied to the management of nature reserves

MEETING OF THE SECTION FOR PLANT TAXONOMY AND PHYTOGEOGRAPHY ON 13 APRIL 1984

This meeting was completely devoted to current research on Begoniaceae

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Taxonomy of the (African) Begoniaceae, an introduction

The family Begoniaceae is commonly accommodated in the order Violales (Parietales, Cistales). Within this order it shows advanced characters and, more in particular, affinities with Datiscaceae and Loasaceae. The Begoniaceae number about 900 species. At present three genera are recognized, viz. *Hillebrandia* Oliv., *Begonia* L. and *Symbegonia* Warb. *Hillebrandia* represents a monotypic genus confined to the Hawaii-archipelago. *Symbegonia*, with about 10 species, is only found on New Guinea. The large genus *Begonia* is pan-tropical and shows its greatest development in the Neotropics. On the African continent it is rather poorly represented by some 130 species. A recent account for Madagascar and the Comores (KERAUDREN-AYMOUNIN 1983) recognizes 52 species. Of these only a single species, *B. cladocarpa* Baker (= *Menzierea c.*) is probably conspecific with a continental one, viz. the widely distributed *B. oxyloba* Welw. ex Hook. f. All other species are endemic.
The family does not occur in Australia.

IRMSCHER (1925), the last monographer of the family as a whole, recognized c. 60 sections mainly based on fruit and placentation characters. Section *Begonia* (= *Begoniastrum* A. DC.) excepted, all these sections are restricted to a single continent and often only to a small region. Most workers on Begoniaceae accept such a division into sections, some disagree, however. KERAUDREN-AYMONIN (1983) concluded that for the species of the Madagascar region a treatment based on the existing sections was not warranted.

The present work on Begoniaceae unequivocally leads to the conclusion that on the African continent sections are natural taxa and useful working-units. The concept of a division into 9 sections, already proposed by WARBURG (1894), is in essentials tenable although a number of rather drastic modifications seems unavoidable. Sections *Sexalaria, Augustia* and *Rostrobegonia* are probably to be merged into a single section; for species like *B. baccata* Hook. f. and *B. thomeana* C. DC. new sections are to be erected. The delimitation of sections *Scutobegonia* and *Loasibegonia* needs further research. Series *Longicaules*, accommodated by ENGLER (1921) in *Scutobegonia*, needs to be transferred to section *Filicibegonia*.

Analysis of African *Begonia* species, in comparison with those of *Hillebrandia* and *Symbegonia* leads to the recognition of 16 characters which show trends in specialization. It appears that *Hillebrandia* represents a distinct and very original genus. *Symbegonia*, on the other hand, scores high in advanced characters but can probably only claim sectional status within *Begonia*. Convergence of characters is found to be a common feature in different phylogenetic lines.

Among African Begoniaceae two distinct groups are recognized. A first group shows exalate fleshy fruits and a placentation which is either parietal or septal. In this group epiphytism in a common trend of specialization. A second group is characterized by alate, predominantly dry fruits in which the placentation is always axillary. All species in this group are terrestrial. Both groups show a development towards flowers with two perianth-segments. While the first group developed fruit- and seed characters which are functional for zoochory, the second group specialized in anemochory. An exception forms the successful section *Scutobegonia* (34 species), rainflorest dwellers of which the very small seeds are probably passively disseminated via adherent mud on the legs of big mammals (epizoochory), and perhaps also hydrochoric.

The typical anemochorous group, containing sections *Augustia, Sexalaria* and *Rostrobegonia*, finds its main development in the often drier and more open regions in Africa.

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Pollen morphology of Begonia in relation to taxonomy

Pollen morphology can be a useful tool for elucidating relationships within a taxonomically difficult

group. Genera, sections and even species can be characterized by an unique pollen type and hypotheses of phylogeny may be proposed, based on pollenmorphological data.

In *Begonia* the pollen grains are nearly always 3-colporate, (per)prolate and striate. Their size ranges from 15 to 30 μm. An important character is the absence or presence of a margo, a zone along the colpi where the striate ornamentation is replaced by an irregular pattern. Further characteristics include the size and shape of the endoaperture and the outline of the grain in equatorial view, where the sides may be convex, straight and, occasionally, concave.

The occurrence of different character states in these features makes it possible to postulate several trends, from a primitive basic type towards more advanced types. A small, nearly spherical grain without a margo and with a small elliptical endoaperture without costae represents the primitive condition in *Begonia* pollen. Several lines of development can be recognized leading to an increase in size, enlargement of, especially, the polar axis, acquisition of a margo and of a relatively large, more or less oblong endoaperture with costae. The more primitive type(s) occur in the sections *Mezierea, Baccabegonia* and *Tetraphila* p.p. More advanced types are found in the sections *Squamibegonia*, the remaining part of *Tetraphila*, and in *Augustia* and *Scutobegonia*. The sections *Sexalaria* and *Rostrobegonia* can hardly be distinguished from *Augustia*, pollen morphology thus reinforcing the combination of these three sections on macromorphological grounds.

A deviating development took place in *Scutobegonia* where the concave sides of the grains and the extremely elongate endoaperture (endocolpus) can be considered as advanced traits, although a margo is lacking. The overall pattern shows many correspondences with the conclusions of macromorphological taxonomy. A major difference is the position of the sections *Cristasemen* and *Filicibegonia* which seem, pollenmorphologically, rather primitive but represent derived sections in the macromorphological scheme. A comparitive study of the pollenmorphology of the other two genera of the Begoniaceae, *Hillebrandia* and *Symbegonia*, showed the former to resemble certain primitive types of *Begonia* pollen (conform the exceptionally primitive status of *Hillebrandia*), while the latter possesses a strikingly different pollen type: a small, spherical grain with irregular echinate ornamentation, and as could be studied with transmission electron microscopy, an unique wall structure consisting of only two layers, the infratectal columellar layer being absent. Contrary to the taxonomist's opinion that *Symbegonia* can be considered as a mere section of *Begonia*, pollenmorphologically the generic status of *Symbegonia* seems to be well warranted.

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Placentation in African Begonias

Form and structure of the ovary, especially the type of placentation, have always played an important role in the taxonomy of *Begonia*, e.g. Irmscher made use of the form of the placentae to subdivide his continental groups of sections.

In order to analyse the placentation found in African begonias the extensive collection of living begonias present in the greenhouse of the Department of Horticulture of the Agriculture University at Wageningen could be used. Fifty three species, representing all African sections (except the section *Filicibegonia* s. str.) have been investigated. Analysis of the ovary cross-sections yielded the following results.

1. In the sections *Mezierea, Tetraphila, Squamibegonia* and *Baccabegonia*, the placentation changes from the bottom to the top of the ovary. For example, in an ovary of a representative of the section *Tetraphila* we encounter axillary, septal (with the placentae localized halfway the septa), parietal and, again, septal placentation, respectively. This transition of placentation-type may be deceptive and lead to wrong interpretations. Therefore it is always necessary to make cross-sections at different levels in an ovary.
2. On the ground of placentation characters, the African begonias can be divided into two groups. Group A, characterized by parietal placentation and unwinged ovaries (sections *Mezierea, Tetraphila, Squamibegonia* and *Baccabegonia*) and group B, showing axillary placentation and winged ovaries (*Augustia, Rostrobegonia, Sexalaria, Scutobegonia, Filicibegonia, Cristasemen, Loasibegonia*). This implies that c. 40% of all African begonias show parietal placentation, much more than was

assumed before. Of all species with axillary placentation, 11% have bifid and 80% entire placentae. Preliminary investigation of c. 20 Asiatic and c. 60 American *Begonia* species revealed much less variation in placentation-type: nearly all showed axillary placentation.

3. It appears that the type of placentation provides an indication of the phylogenetic level of a species, species-group or section. Using this character, a better understanding of the evolutionary relationships in the genus can be obtained. Following Gauthier parietal placentation is the primitive condition within the Begoniaceae; as a consequence the sections of group A are relatively primitive, while group B is more advanced. Within group A we discern a development in placentation from parietal (*Mezierea*), via septal (*Baccabegonia, Tetraphila*) to pseudo-axillary (*Squamibegonia*) (pseudo-axillary because the centre of the ovary consists of placental tissue instead of carpellar tissue as in true axillary placentation). In the same progression we observe an increasing inward extension of the fused adjacent carpels. Consequently, the placentae shift towards the centre of the ovary.

We may state that in group A *Mezierea* is the most primitive section, whereas *Squamibegonia* shows the most advanced types of placentation (pseudo-axillary). Between these, the sections *Baccabegonia* and *Tetraphila* form a rather wide and variegated link. Real axillary placentation as occurs in group B is a result of the fusion of the margins of the same carpel in the centre of the ovary. Phylogenetically, in the course of the process of fusion, the placentae are first bilamellate, and then, when the fusion is more complete, simple. Accordingly, the sections *Scutobegonia, Loasibegonia, Cristasemen* and *Filicibegonia* may be regarded as more advanced than the section *Sexalaria* and the bilamellate species of the sections *Augustia* and *Rostrobegonia.*

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The importance of seed morphology for the classification of African Begonia sections

The seeds of Begoniaceae are characterized by the presence of specialized testa cells (collar cells), which have a function during germination: they split along their middle lamellae, which results in the release of the micropylar-hilar part as a seed lid.

There is a great diversity in shape and micromorphology of the seeds within the family, which characters appear to be important for the delimitation of species and sections of the African begonias. On the basis of the seed structure of the about ninety species examined, three major groups can be distinguished.

1. The *Augustia-Sexalaria-Rostrobegonia* group. The dry, dehiscent fruits have wings. The seeds are of medium size (0,4–0,6 mm) and exhibit a distinct cuticular structure which functions in wind dispersal. The seeds of species in these sections show much resemblance with those of Asiatic and American representatives. A distinction between the three originally described sections did not prove to be possible.

2. The *Mezierea-Squamibegonia-Tetraphila* group. These sections are characterized by the reduction or absence of a cuticular structure, which corresponds with the presence of fleshy fruits and a zoochorous form of dispersal. Within this group there is a trend towards larger seeds (up to 2 mm and more). The three sections are well-discernable from each other. Section *Mezierea* seems to be the most original. The seeds in section *Tetraphila* are provided with a funicular aril. Although the species *B. baccata* and *B. crateris* clearly belong to this complex, they do not fit into one of the three secions.

3. The *Filicibegonia-Scutobegonia-Loasibegonia* group. The general characters of these sections are the small seeds (to less than 0,3 mm), the comparatively large collar cells, especially in *Filicibegonia* and, particularly in *Scutobegonia,* the pronounced cuticular structure. The section *Loasibegonia* is indiscernable from *Scutobegonia* as regards the seed structure. Hydro(ombro)chory and epizoochory are probably the most important ways of dispersal. The seeds of *B. socotrana* and *B. thomeana* differ clearly from those of all species belonging to the sections just mentioned and both species are possibly best placed in separate sections. Our cognisance of the seeds of the American and Asiatic sections is still too scanty to permit a theory on intercontinental relationships.

The data on seed morphology not only clearly support the systematic classification but also lead to a better understanding of the several ways of dispersal.

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Karyology of African Begonias

The somatic chromosomes of the African species of *Begonia* are small: their length varies from about 0.5 to 2 μm. Primary constrictions are generally not distinct. In those cases, where they have been observed it can be concluded that the chromosomes are (sub)metacentric. Consideration of the 2n numbers found for the various species leads to the following basic numbers for the different sections:

	Sexalaria	x = 11	*Tetraphila*	x = 18
	Augustia	x = 13/14	*Filicibegonia*	x = 18
	Rostrobegonia	x = 13/19	*Cristasemen*	x = 19
	Mezierea	x = 12/13	*Scutobegonia*	x = 17/19
	Baccabegonia	x = 18	*Loasibegonia*	x = 16
	Squamibegonia	x = 18		

It is possible to construct karyograms for the various species on the basis of the relative length of the chromosomes. When this is done, four groups according to karyotype similarity can be distinguished. They are:

1. Sections *Sexalaria, Augustia, Rostrobegonia.* The section *Sexalaria* with x = 11 is the most advanced.
2. Section *Mezierea.* This section is distinct by its symmetrical ('primitive') karyotype.
3. Sections *Squamibegonia, Baccabegonia, Tetraphila.* In this group the karyotypes are very similar and the most asymmetric of the African begonias. *Squamibegonia* has a slightly more asymmetric karyotype than the other two.
4. Sections *Filicibegonia, Cristasemen, Scutobegonia, Loasibegonia.* Regarding their basic number these sections appear to be similar to the sections of group 3, however, their chromosomes are smaller and the karyograms less asymmetric.

Polyploid taxa have been observed for *B. oxyloba/B. pycnocaulis* (sect. *Mezierea*); *B. squamulosa* aggr. (sect. *Tetraphila*), a group of morphologically similar taxa (2x and 4x) with strong genetic barriers; *B. sessilifolia* and *B. macrocarpa* (sect. *Filicibegonia*); various taxa of sect. *Scutobegonia.* In this section the polyploid taxa have triploid and tetraploid numbers according to the basic numbers x = 17 and 19. In all other sections the polyploid taxa are exclusively tetraploids.

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Domestication of Begonia

A brief summary will be given here of the history of the improvement of *Begonia,* as an ornamental, in which hybridization took an important part. Four main groups of hybrids can be discerned: tuberous begonias, semperflorens-begonias, winter-flowering begonias and begonias with ornamental foliage. In temperate regions tuberous and semperflorens-begonias are mainly grown in the summer border etc. outside, while winter-flowering and begonias with ornamental leaves are cultivated as indoor plants. The tuberous begonias originated shortly after 1865 through repeated hybridization of *B. boliviensis, B. veitchii, B. rosiflora* and *B. pearcei,* all from South America. These products of hybridization soon became tetraploids. Further hybridization (c.1880) with *B. baumannii, B. davisii* and *B. froebelii* failed, because only sterile triploids were raised. The reproduction in this group is by means of seeds.

The semperflorens-begonias were at first selected from the polymorphous, Brazilian species *B. semperflorens* (rightly named *B. cucullata*). Afterwards crosses were carried out with *B. schmidtiana,* which came also from Brazil. From these crosses arose, amongst others, a tetraploid group, the 'Gracilis'-type. At this moment all semperflorens-begonias are triploids, while seeds are continually produced by crossing diploid and tetraploid strains. There are two groups of the winter-flowering begonias, *B.* × *hiemalis* and *B.* × *cheimantha,* both originated through crossing with *B. socotrana,* an endemic species from the island Socotra, introduced in 1881. The other parents were the tuberous begonia and the South-African *B. dregei,* respectively. From those primary hybrids several races were made by back-crossing with *B. socotrana* and also by selection of mutants. Well known old races in the *B.* × *cheimantha* group are 'Gloire de Lorraine' (1892) and 'Konkurrent' (1911). Recent-

ly several new forms were made, partly with methods developed at Wageningen. Several races in the winter-flowering group are triploid. The begonias with ornamental foliage also mainly comprise two hybrid groups. The 'Rex'-begonias originated through crossing *B. rex*, imported in 1857 from India, with other, mostly also Asian, species. More recently a number of chiefly Mexican species were used for hybridization, which produced a second group of begonias with ornamental leaves. Members of the 'Rex'-group are usually polyploids, while as far as known, all hybrids of the Mexican group are diploid. In both cases, propagation is by means of leaf-cuttings.

MEETING OF THE SECTION FOR PLANT TAXONOMY AND PHYTOGEOGRAPHY ON 16 NOVEMBER 1984

W. VAN DELDEN *(Vakgroep Genetica, Biologisch Centrum, Kerklaan 30, 9751 NN Haren)*

The use of allozyme variation in taxonomy

Both population genetists and taxonomists are interested in intra- and inter-specific genetic differences. Both disciplines have to cope with the problem that often either the genetic differences are concealed by phenotypic uniformity or that, on the contrary, in the case of genetic uniformity, phenotypic differences are induced by environmental influences. This holds especially for morphological characters, which are often used for taxonomic purposes. Such characters have in general polygenic inheritance and are subject to environmental modification. Which may interfere with proper taxonomic classification.

The finding in population genetics that populations of most animal and plant species are highly polymorphic at enzyme loci has created possibilities for the comparison of populations and species for genetic differences, without the interference of environmental influences. These allozyme variants at a locus originate from nucleotide substitutions in the DNA, giving rise to amino acid substitutions, accompanied by changes in charge. These charge differences are detected on the protein level by means of electrophoresis and the subsequent application of specific staining methods. Determination of the occurrence of allozyme variation (which gives an underestimation of the genetic variation present at the DNA level) provides a suitable way to quantify the amount of variation existing within a population. The method further allows a quantitative comparison of populations and species for their genetic resemblance. A measure often used is Nei's genetic identity (I) or its derivative: the genetic distance (D), based on the comparison of allele frequencies in the taxa studied. Application of relevant statistical clustering methods allows the construction of dendrograms, indicating the genetic relationships between several taxa. As D is the cumulative number of codon differences per locus, detectable by electrophoresis, it is – with certain restrictions – possible to introduce a time axis in such a dendrogram.

Species can often easily be distinguished in a qualitative way as particular alleles at some – diagnostic – loci appear to be species-specific. This enables an unambiguous distinction between members of different species e.g. in the case of sibling species which are otherwise hard to separate. Data on genetic distances between populations, subspecies, species and genera are now available for many plant and animal species. Above the genus level the genetic differences are generally too great to allow meaningful application of the allozyme technique. It further appears that the average D-values between species e.g., are not uniform but vary considerably depending on the systematic group involved. Nevertheless the use of allozyme variation as a tool in taxonomy can be considered as highly valuable. Its usefulness is especially apparent in cases as the following: the analysis of various stages in the process of speciation, in the description of biogeographical distributions, in hybridization studies and in the elucidation of specific genetic events involved in speciation.

S. B. J. MENKEN *(Vakgroep Bijzondere Plantkunde, Kruislaan 318, 1098 SM Amsterdam)*

Possibilities and impossibilities of biochemical systematics

Biochemical systematic studies usually start with an analysis of the allozyme variation pattern at various loci in samples taken from natural populations. From the resulting frequency table genetic

identity/genetic distance values (e.g. NEI 1972) are computed for all pairwise comparisons. From the matrix of such values dendrograms can be constructed by means of several computer programs (e.g. SWOFFORD & SELANDER 1981; this program allows also the construction of distance Wagner trees). It should be clear that the higher the number of loci studied the more reliable the dendrogram is. Two questions now arise 1) How stable is the dendrogram? 2) How representative are the samples?

ad 1. As in a genetic identity/distance matrix all allozymic data are lumped (with a concomitant loss of information) the resulting tree may represent only an overall picture. For example, taking at random 10 loci out of a total of 50 and generating dendrograms based on such subsets will result in different trees if the matrix contains contradictory information. In such cases the congruence among 'subset dendrograms' will be low and the classification is unstable.

ad. 2. Highly substructured populations should be sampled adequately in order to prevent biased sampling. Moreover, one needs insight in the pattern of allozyme distribution over the geographic range of the species under study. In cases of uniform patterns one population can be regarded as a good representative for the species. Usually, genetic identities for conspecific populations are high: populations are fixed for the same allele at monomorphic loci and show comparable variation build-up at polymorphic loci. Occasionally, populations can be fixed for different alleles at a locus (caused by lowered gene flow – dispersal of pollen and seeds is low in most cases –, drift in small populations and/or differential selection) resulting in lower identity values.

In cases of (almost) invariable species or species remotely related to all other species under consideration one single individual is sufficient to obtain the correct topology of a dendrogram. Closely related species, on the other hand, can be separated only by one or a few characters (diagnostic loci). Hybrids between them can easily be detected as allozymes which exhibit codominant expression. Allozyme analysis proves to be a superior technique in discriminating sibling species (MENKEN 1982).

In a multidisciplinary approach to the systematics and phylogeny of a group of organisms different classifications based on morphology, bio-chemistry, karyology, phenology etc. are compared; none of these should be considered as a panacea. Especially the congruence between morphological and allozymic classification can be low.

It is stressed that in plants crosses are indispensable to understand the genetic basis and to interpret reliably the banding patterns observed on the gels.

The objective technique of allozyme analysis allows us, without a priori weighting of characters that are homologous if they have the same specific catalytic function, to calculate relative similarities based on an exactly known amount of genetic information. Unfortunately only living (or deep frozen) material can be used. Further disadvantages of the technique are 1) technical difficulties, 2) restricted resolution power a) chance identity in band mobility (a *Taraxacum* species will have a genetic identity of 5% or so when compared with a cow purely due to chance alone b) not detected protein differences (one band on a gel can be heterogeneous because of electrophoretically undetectable mutations), 3) high saturation rate (mutation accumulations after a fixed difference are not 'seen') and 4) biased sample of loci (mainly water soluble dehydrogenases, dehydrogenase-linked enzymes and hydrolases, as for these proteins simple assays are available).

Despite these drawbacks allozyme analysis has proved to be a valuable tool in systematics, evolutionary biology and many other areas of research (TANKSLEY & ORTON 1983).

MENKEN, S. B. J. (1982): Biochemical genetics and systematics of small ermine moths (Lepidoptera, Yponomeutidae). *Z. zool. Syst. Evolut.-forsch.* **20**: 131–143.

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SWOFFORD, D. L. & R. B. SELANDER (1981): Biosys-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Heredity* **72**: 281–283.

TANKSLEY, S. D. & T. J. ORTON (1983): *Isozymes in Plant Genetics and Breeding, Part A and B.* Elsevier, Amsterdam.

K. BACHMAN *(Vakgroep Bijzondere Plantkunde, Kruislaan 318, 1098 SM Amsterdam)*

Reconstruction of the evolution of Microseris pygmaea from a founder specimen

Microseris pygmaea of Central Chile is the only species of the genus in South America. The centre of diversity of *Microseris* is the corresponding region of mediterranean climate in Western North America, and the two close North American relatives of *M. pygmaea* (*M. bigelovii* and *M. elegans*) represent the genus directly on the Pacific coast. The distribution of this genus parallels that of more than 130 other plant genera which have centers of diversity in one of the two ecologically similar regions and one or a few dispersed species in the other one. The most likely explanation for these patterns is very rare long-distance dispersal between the two regions, most likely by migrating shore birds. *M. pygmaea* differs from the other annuals of the genus by having 10 rather than 5 (or fewer) pappus parts. The genetics of this difference has been analyzed in detail. It involves a single-gene mutation to 10-determination followed by gene duplication. Obviously then, all *M. pygmaea* are derived from a single founder specimen carrying the 10-determining allele that became established in Chile. The evolution of the species from this founder specimen can be reconstructed, and mutations in enzyme genes demonstrable by gel electrophoresis play a key role in this reconstruction. We can order the nine available populations of *M. pygmaea* in geographical sequence from northern coastal ones to southern inland ones over the 400 km distribution range, and find a progressive increase in new enzyme alleles that are shared by all populations South of the point of the presumed mutations. The consistency of the data from six enzyme genes lends strong support to our hypothesis of the origin and evolution of *M. pygmaea*. The direction of the evolution of the species is confirmed by a gene for glutamate oxaloacetate transaminase, which is represented by the same allele in all North Amercian annuals of *Microseris* and therefore should be the ancestral one. This same allele occurs in three coastal populations of *M. pygmaea* but gets replaced by a new 'Chilean' allele in all inland populations. Since the other genes that we have studied are polymorphic in North America, the precise ancestral allele for them can not so easily be inferred. We plan to extend this analysis to the examination of variation at the DNA level. Such an analysis can easily yield any number of mutational markers and thereby provide statistical certainty for our phylogenetic hypothesis if it is true.

W. T. STAM *(Vakgroep Mariene Biologie, Biologisch Centrum, Kerklaan 30, 9751 Haren)*

The use of DNA-DNA hybridization for taxonomy

DNA-DNA hybridization is one of several techniques which can be used to establish genotypic relationships between organisms. These relationships enable taxonomists to build up a classification based on a biological (or genetic) species concept. Such classifications should reflect possible common ancestry of the organisms involved, in contrast to classifications which are merely based on morphological (phenotypical) relationships.

The degree of genotypic relationship is assessed by two methods. First, by determination of the number of hybridized products and, secondly, by examination of the stability of these products by determination of their thermal stability.

Taxonomic implications of hybridization studies with species belonging to the genera *Anabaena* (blue-green algae), *Chlorella* (green algae), *Osmunda* (ferns) and *Atriplex* (angiosperms) are discussed. Within the genus *Anabaena*, morphological similarities and differences are correlated with genetic similarities and differences, respectively (STULP & STAM 1984). The genus *Chlorella* is genotypically heterogenous, so that for some species their assignation to this genus is questionable (KERFIN & KESSLER 1978). For both *Osmunda* and *Atriplex*, the recognition of subgenera, based on morphological grounds, is not confirmed by DNA-DNA hybridization results. For *Atriplex* the suggested polyphyletic origin of the C4-metabolic pathway is at least questionable (STEIN et al. 1979; BELFORD & THOMPSON 1981).

Current DNA-DNA hybridization research at the Department of Marine Biology at Groningen involves the prokaryotic algal genera *Synechococcus* and *Prochloron* and the eukaryotic *Laminaria, Scytosiphon, Lomentaria* and *Cladophora*. The eukaryotic genera are being studied within the framework of the research project 'Experimental biogeography of seaweeds'.

BELFORD, H. S. & W. F. THOMPSON (1981): Single copy DNA homologies in Atriplex. II. Hybrid thermal stabilities and molecular phylogeny. *Heridity* **46**: 109–122.

KERFIN, W. & E. KESSLER (1978): Physiological and biochemical contributions to the taxonomy of the genus Chlorella. XI. DNA hybridization. *Arch. of Microbiol.* **116**: 97–103.

STEIN, D. B., W. F. THOMPSON & H. S. BELFORD (1979): Studies on DNA sequence in the Osmundaceae. *J. Mol. Evol.* **13**: 215–232.

STULP, B. K. & W. T. STAM (1984): Genotypic relationships between strains of Anabaena (Cyanophyceae) and their correlation with morphological affinities. *Br. phycol. J.* **19**: 287–301.

J. VAN BREDERODE *(Vakgroep Populatie- en Evolutiebiologie, Transitorium III, Heidelberglaan 8, 3584 CH Utrecht)*

Molecular variation in Silene pratensis

In *Silene* the variation at macroscopic, microscopic and molecular levels of various characters of as much as possible individuals of many populations has been studied. Much emphasis has been placed upon the variation in secondary compounds. In higher plants, next to the polygenetic determined morphological adaptation, the potency to synthesize secondary compounds is of importance for the interaction of the plant with its environment. The genes governing the biosynthesis of these compounds have also been identified and their regulation studied. Without proper genetic and ontogenetic studies the pitfalls caused by convergence cannot easily be circumvented.

At the macroscopic level variation in capsule (30 populations, 15 plants/population), flower (90 populations, 15 plants/population), pollen morphology (30 populations, 15 plants/population) and seed morphology (110 populations, 15 plants/population) was established. At the microscopic level, the variation in banding pattern of chromosomes has been studied. At the molecular level the variation in 7 isozymes of 100 populations has been studied, of each population 100 plants have been investigated. The flavone variation in 120 plants of each of the 358 populations studied has been determined. Convergence and differential regulation appear to play an important role both with the flavone and isozyme variation. The variation in flavone substitution, seed capsule and pollen morphology showed a geographical component, the variation in isozyme patterns is strongly influenced by environmental factors, e.g. microclimate in green house.

A genomic library has been synthesized and the sequences of the genes coding for plastocyanin, ferredoxin and the small subunit of rubisco (sequences often used in evolutionary studies) have been determined. Comparison of these sequences by means of cluster analysis with the known sequences of other species have been performed.

The study of restriction fragment length polymorphism has started. Antibodies have been raised against the enzymes involved in flavone biosynthesis. From the cross reactions, conclusions have been drawn upon the evolutionary relations between these enzymes.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY OF 26 OCTOBER 1984

ANNE MIE C. EMONS and J. A. TRAAS *(Botanisch Laboratorium, Universiteit Nijmegen, 6525 ED Nijmegen)*

Coated pits and coated vesicles in plant cells

The role of coated pits, nor the path coated vesicles follow is known for plant cells. In animal cells they function in receptor-mediated endocytosis and specific intracellular transport of membrane proteins.

This study was undertaken as a preliminary to research on coated structures in plant cells. Plant cells from different origins were used: growing root hairs of *Equisetum hyemale. Raphanus sativus, Lepidium sativum:* full-grown root hairs of *Equisetum hyemale*; meristematic root cortical cells of *Raphanus sativus*; elongated root cortical cells of *Raphanus sativus, Urtica dioica, Limnobium stoloniferum* and *Ceratopteris thalictroides.*

The structures were visualized by the dry-cleaving technique (TRAAS 1984), which proved to be an appropriate method to study the occurrence of coated pits, their density, distribution and dimensions.

Occurrence: In all cells three classes of coated structures were observed: planar coated pits, bud-

ding – or fusing – coated pits and coated vesicles often attached to microfilaments.

Density: The density of the coated pits varies greatly between cells: from 23 to 600 pits/100 μm^2. Plasma membranes of growing cells contain more pits than plasma membranes of full-grown cells, even in areas (hair tube) where no cell expansion takes place. A high density of pits might suggest high activity as well as suppression of pit invagination or evagination.

Distribution: Coated pits are distributed rather evenly over the membrane, though groups of two or four pits occur as well as pits associated with a vesicle.

Dimensions: The planar pits are built up of hexagons and are 30 to 170 nm in diameter. The budding – or fusing – pits range from 92 to 124 nm and consist of hexagons and occasionally a pentagon. The coated vesicles measure 72 to 96 nm. The center-to-center distance of hexagons in the coated pits is approx. 23 nm; the sides of the hexagon measure 7 to 8 nm in width.

J. DERKSEN, J. A. TRAAS and E. PIERSON *(Botanisch Laboratorium, Universiteit Nijmegen, Toernooiveld, 6525 ED Nijmegen)*

The cytoskeleton in plant cells

The microtubular skeletons in various plant cells were studied by means of immunohistochemical techniques. In meristematic and elongating cortex cells the microtubules are perpendicular to the cell axis. In elongated cortex cells the microtubules show large variations in orientation. In root hairs, which are growing at their tip only, the microtubules are often parallel to the cell axis and mostly do not protrude into the uttermost tip. In pollen tubes of lily and tobacco, microtubules are oriented parallel to the cell axis or they are slightly S-helical. They do not protrude into the tip region. In the generative cell of the pollen tubes the microtubules are mainly parallel to the cell axis, forming a basket enclosing the generative nucleus and probably a large part of the plasma of the generative cell. During development 'in vitro' the microtubular skeletons do not undergo important changes. In tobacco, however, when the generative cell passes the vegetative nucleus, microtubular orientations become distorted. When passage is completed the microtubules are no longer axial, but show angles of about 45° with the cell axis. The dense microtubular skeleton in the generative cells of lily and tobacco may have a real skeleton function protecting the generative cell against the shearing forces of the streaming plasma of the vegetative cell. In general microtubules may be involved in various cell functions, like plasma distribution and transport and maintainance of cell polarity.

P. BAAS *(Rijksherbarium, Postbus 9514, 2300 RA Leiden)*

Ecological wood anatomy of trees and shrubs from Israel and adjacent regions

A summary was given of an ecological analysis on the wood anatomy of about 170 dicotyledonous species from various desert habitats, the mediterranean maquis, and hydrophilic localities in Israel and adjacent regions. For full details see BAAS et al. (1983) and FAHN et al. (1985).

BAAS, P., E. WERKER & A. FAHN (1983): Some ecological trends in vessel characters. *IAWA Bull.* n.s. 4: 141–159.

FAHN, A., E. WERKER & P. BAAS (1985): *Wood anatomy and identification of trees and shrubs from Isreal and adjacent regions.* Jerusalem, Israel Academy of Sciences (c. 350 pp., in press).

P. BRAAT, J. A. TRAAS and J. DERKSEN *(Botanisch Laboratorium, Universiteit Nijmegen, Toernooiveld, 6525 ED Nijmegen)*

The cytoskeleton in meristematic and differentiated cortical cells from root tips

Cortical microtubules in meristematic and differentiated cortex cells from root tips of various plant species were studied using immunofluorescence for light microscopy and dry cleaving for electron microscopy. Orientations, lengths and densities of the microtubules were measured in micrographs from dry cleave preparations, using a Kontron videoplan computer. Microtubules in meristematic cells of *Raphanus sativus* are oriented perpendicular to the cell axis. The average length of these microtubules ranges between 0.9 and 1.3 μm. Their maximum length is about 5 μm and their density

varies between 1.7 and 3.2 μm/μm^2. In the differentiated cortex cells of *Raphanus*, orientations are highly variable, from transverse to longitudinal. Lengths vary between 2.6 and 6.7 μm and the densities between 0.9 and 1,9 μm/μm^2. Similar observations were made in *Limnobium stoloniferum* and *Ceratopteris thalictroides*.

The results indicate that important changes occur during differentiation of meristematic cells into cortex cells.

ANJA VAN DER NEUT and R. J. BINO *(Vakgroep Plantencytologie en -morfologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen)*

Histochemical analysis of enzyme activity in anthers of cytoplasmic male sterile petunia

In petunia it was demonstrated that changes in mitochondrial DNA are associated with cytoplasmic male sterility (KOOL et al. 1985). Investigations of translational products from isolated mitochondria have revealed several unique polypeptides. This distinct polypeptide composition possibly influences sterile microsporogenesis and may ultimately result in abortion of the tapetal and sporogenous cells (BINO 1985). Cytochrome oxidase may be localized at the ultrastructural level, using the diamino benzidine method (BINO et al. 1985). In the present study cytochrome oxidase activity is demonstrated in the cristae and at the membranes of the mitochondria. We could not detect any difference in staining specificity, correlated with sterility.

BINO, R. J. (1985): Histological aspects of microsporogenesis in fertile, cytoplasmic male sterile and restored fertile Petunia hybrida. *Theor. Appl. Genet.* (in press).

BINO, R. J., S. J. DE HOOP & A. VAN DER NEUT. (1985): Cytochemical localization of cytochrome oxidase in anthers of cytoplasmic male sterile Petunia hybrida (Hook.) Vilm. In: M. T. M. WILLEMSE & J. L. VAN WENT (eds.): *Sexual reproduction in seed plants, ferns and mosses.* Pudoc, Wageningen (in press).

KOOL, A. J., J. M. DE HAAS, J. N. M. MOL & G. A. M. VAN MARREWIJK (1985): Isolation and physicochemical characterization of mitochondrial DNA from cultured cells of Petunia hybrida. *Theor. Appl. Gen.* **69**: 223–233.

C. H. THEUNIS and J. L. VAN WENT *(Vakgroep Plantencytologie en morfologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen)*

Embryo sac ultrastructure before and after fertilization in Cucumis sativus

The composition and development of ovule and embryo sac of *Cucumis sativus*, from anthesis up to fertilization has been studied. The mature ovule of *Cucumis* is anatropous, bitegmic and crassinucellate, and the embryo sac is of the Polygonum type. Development, composition and ultrastructure of the embryo sac generally resemble the pattern as already described for many other angiosperm species. The pollen tube grows through the micropylar nucellar tissue towards the embryo sac and strongly proliferates. After discharge of part of its contents into one of the synergids, the pollen tube is disconnected from the degenerated synergid by the deposition of a plug (VAN WENT et al. 1985).

J. L. VAN WENT, C. H. THEUNIS & A. P. M. DEN NIJS (1985): Embryo sac ultrastructure before and after fertilization in Cucumis sativus. In: M. T. M. WILLEMSE & J. L. VAN WENT (eds.): *Sexual reproduction in seed plants, ferns and mosses.* Pudoc, Wageningen (in press).

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON 16 NOVEMBER 1984

PH. BOXUS *(Station des Cultures fruitières et maraichères, Centre de Recherches agronomiques, B-5800 Gembloux, Belgium)*

Micropropagation of woody plants

Since 1967, investigations in the Gembloux tissue laboratory are devoted to the micropropagation of woody species, mainly fruit trees (cherry, apple, cultivars and rootstocks), but also some some

forest trees (wild cherry, larch, poplar, oak, elm, eucalyptus), and a few shrubs (rhododendrons).

Meristem-tip culture was well developed to assure:

1. bacterial and virus eradication. Prune dwarfs virus, european rusty mottle virus, and chlorotic necrotic ringspot virus were successfully eliminated from *Prunus* sp. On the other hand, the removal of nepo viruses and nectrotic ringspot virus partly failed. Eradication of bacteria was generally good.
2. respect of genetic stability and quick return to juvenility.

In meristem culture and the phases: axillary branching, elongation and rooting of the most woody species, the same unspecific basic medium is used: Lepoivre's salt medium, enriched by hormonal balance, vitamin mixture and sugar. Proliferation rate is about 4 or 5 per month, but can be perturbed by the 'vitrification' phenomenon. It was difficult to improve the rooting rate, but today it reaches 80 to 90 per cent. Use of riboflavin and proline improve the root quality.

Shoots can be stored *in vitro* in the dark at +2 °C during more than four years.

The first own-rooted apple trees don't show genetic instability. Nevertheless, they look stronger than the grafted-ones. Field behaviour of different cultivars, common and compact types, is being studied.

Industrial micropropagation of wild cherry, new dwarf cherry rootstocks, and rhododendrons is now effective.

B. P. A. M. KUNNEMAN *(Proefstation voor de Boomteelt en het Stedelijk Groen, Postbus 118, 2770 AC Boskoop)*

In vitro propagation of nursery stock

Since 1978 tissue culture techniques are used for vegetative propagation of woody species and some perennials at the PBSG (Research Station for Nursery Stock and Urban Horticulture Boskoop). At this moment the research program includes vegetative propagation of *Acer, Alnus, Malus, Paeonia, Rhododendron, Skimmia, Tilia* and *Wisteria* as well as in vitro selection for fireblight resistance in *Pyracantha.*

Two problems which occur in culturing many of the species are: Isolation of non-contaminated explants, and the after-effects of certain treatments (pre-treatments) in later stages of culture.

Isolation of bacteria-free explants of *Paeonia* was achieved by raising the mother material in perlite at a temperature of c. 25 °C in combination with applications of fungicides and antibiotics. In *Malus*, a two-step sterilization procedure is used to eliminate contamination in meristems excised from dormant buds. The first step is the sterilization of intact buds, the second step a light sterilization of these buds after removing two-third of the bud scales and leaf primordia.

Effects of pre-treatments on rooting have been observed in several species. In *Rhododendron* a reduction of the cytokinin level at the end of the propagation cycle improves root formation in the rooting medium. In *Acer lobelii* pretreatment with active charcoal has similar effects. In *Ulmus* 'Dodoens' the auxin-cytokinin balance in the pre-treatments is an important factor in the subsequent root formation. Occurrence of these pre-treatment effects can interact with effects of the rooting medium itself. Therefore it is difficult to differentiate between effects due to the pre-rooting medium and effects caused by the rooting medium itself.

P. W. EVERS *(Rijksinstituut voor Onderzoek van Bos- en Landschapsbouw 'De Dorschkamp', Postbus 23, 6700 AA Wageningen)*

Vegetative propagation of forest trees in vitro

To prevent the outbreak of new diseases due to the use of a limited number of clones in forestry, tissue culture methods are adapted to multigenotypical applications. The main problems in culture are dormancy, lack of rejuvenation and lack of rooting. The applications and species can be grouped in three categories:

1. Micropropagation of broadleaved trees of which it is possible to make grafts or cuttings (*Populus, Salix, Ulmus, Platanus*). Tissue culture of *Populus* and *Salix* is initiated with buds from hydroculture cuttings, *Ulmus* with tips of cambial shoots and *Platanus* with buds of growth regulator pre-treated trees.
2. Micropropagation of broadleaved trees using families of genotypes (*Quercus, Alnus*). Bud culture

and embryo culture are tested. Pulse and drop techniques are used to activate axillary buds. Dormancy is less a problem when BAP drops are applied to the cultures.
3. Micropropagation of conifers (*Pseudotsuga*). In this case, tissue culture is merely used as an extension of the seed orchard programs. The seed production of well adapted genotypes of exotic species in The Netherlands is not enough to cover the inland need.

L. LEFFRING[1], C. R. VONK[2] and S. A. RIBÔT[2] *([1]Proefstation voor de Bloemisterij, Linnaeuslaan 2a, 1431 JV Aalsmeer, [2]Centrum voor Agrobiologisch Onderzoek, Postbus 14, 6700 AA Wageningen)*

Hormonal effects on shoot formation of Cordyline cultivars

During the multiplication stage the tissue seems to produce endogenous hormones influencing the growth. Polar shoots will form callus at the base after some weeks. Removal of the apical dominance by cutting off the end meristem gives more and faster new axillary shoots. However, after some weeks, when these shoots are growing, callus will be formed at the base.

Apolar shoots with or without apex produce no or very few callus whereas the former also produce less shoots than the latter. This phenomenon can perhaps be ascribed to the production of hormones during the growth of the shoots. To clarify this, shoots without apex have been checked for IAA and ABA contents during the start and after two and four months. In all cases the explants came from an auxin-free medium.

Plant material (± 250 mg fr. wt.) was homogenized under liquid nitrogen and fortified with ^{14}C-4-phenylbutyric acid as an internal standard for determination of recoveries. The homogenized material (in: methanol/ethyl acetate containing butylated hydroxy toluene) was purified by means of Elut-X and Sep-pak columns and methylated. Antibodies against IAA, raised from rabbits, were used for determination of the methyl-IAA by indirect enzyme immuno-assay. Detailed description about purification procedure and immuno-assay are in preparation. Results showed an increasing amount of IAA and ABA in the shoots during the growing period.

Transport of IAA downwards causes callus formation at the base and prevents further axillary shoot formation as it antagonises the kinetin in the medium. However, also ABA can be antagonistic to the cytokinin. On the other hand few or no callus will be formed at the base of apolar shoots, which leads to the conclusion that the callus formation can be due to the endogenous IAA. More experiments will follow.

G. STARITSKY and E. A. ZANDVOORT *(Vakgroep Tropische Plantenteelt, Landbouwhogeschool, Postbus 341, 6700 AH Wageningen)*

In vitro propagation and genetic conservation of tropical woody crops

Research at the department is concentrated on two aspects of the in vitro culture of tropical woody crops, i.e. the vegetative propagation and the genetic conservation. The approach is somewhat different from that of research centres working with temperature trees and moreover, more applied than one would expect from a university institution. This situation is caused by:
- the large number of crops of which hardly any research data on in vitro culture are present
- the diversity in use and culture
- the difficulties in obtaining basic material
- the distance between research worker and culture area
- the absence of tropically oriented research institutes and stations in The Netherlands.

The choice of the crops is guided by interest of colleagues and requests from society. International contacts, for instance with the International Board for Plant Genetic Resources (IBPGR) prevent overlap in research and promote a certain co-operation at international level.

At the moment research is concentrated on cashew, Jack-fruit, cinchona and sago palm. Special attention is paid to the aseptical isolation of starting material, mass propagation and adaptation of in vitro propagated plantlets to nursery conditions.

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Relationships between research, practice and management of in vitro culture of agricultural and horticultural crops