Acta Bot. Neerl. 27(2), April 1978, p. 139–156.

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY ON AUGUST 26, 1977*

T. BARETTA-KUIPERS (*Instituut voor Systematische Plantkunde, Utrecht*)

Aspects of wood anatomy in Leguminosae, with special reference to the Pithecellobium complex (Mimosaceae)

Many species of the *Pithecellobium* complex have been transferred to other genera by a number of authors time and again, depending on the characters used for the segregation. The wood of *Pithecellobium* species shows some diversity as seen in transverse section, but many species are strikingly alike in their ray structure as seen in tangential section. In other large genera, for instance *Inga* of the Mimosaceae, the ray structure has proved to be of great value in classifying the species; the same seems to be true for *Pithecellobium*. There are many species with very low, widely spaced, and predominantly uniseriate rays; an unusual combination in Mimosaceae. Most species have thin-walled fibres.

Several segregated "genera" show the same type of wood and, therefore, their segregation does not seem justified. *Pithecellobium saman* (Jacq.) Benth., recently included in *Pithecellobium* again, shows an essentially different wood structure and should better be treated as the genus *Samanea*. *Zygia*, included in Bentham's section *Caulanthion*, and now incorporated in *Pithecellobium* again, also differs in its wood structure from this genus. This indicates that *Zygia* should be reinstated on the generic level. or at least as a distinct section of *Pithecellobium*.

Archidendron, by some authors regarded as a primitive section of *Pithecellobium* on account of its apocarpy, shows a rather different wood structure. It seems better, therefore, to keep it a separate genus.

The wood anatomy of *Pithecellobium* has convinced the present author that the wholesale lumping of all species into one very large genus is not advisable. It seems best to retain the bulk of the species in *Pithecellobium*, but to exclude a few groups and give them generic status. However, a great deal of research remains to be done.

G. J. C. M. VAN VLIET (*Rijksherbarium, Leiden*)

Wood anatomy of the Combretaceae

The Combretaceae have been the subject of thorough macromorphological and leaf anatomical studies (EXELL & STACE, 1966) providing a solid working base for wood anatomical research. The present study was aimed at a comprehensive survey of the wood anatomical diversity of the family, in order to aid in solving some taxonomic problems. In addition an ultrastructural study using scanning electron microscopy was undertaken in order to test the taxonomic significance of several types of vestured pits occurring in the family.

In Combretaceae three types of vesturing were found in the pit chamber of the vessel wall pits: 1. Vestures of more or less equal thickness, attached to the roof of the pit chambers and branching profusely to fill the pit chambers with a compact mass of filaments; 2. Vestures composed of thick filaments attached to the roof of the pit chamber nearby the pit canal, branching into filaments of decreasing thickness. In this type of vestured pit some thin, branched filaments may be present on lower parts of the roof; 3. As in type 2, but with

* All papers read at the meeting were dedicated to Dr. Alberta M. W. Mennega, on the occasion of her retirement as head of the Department of Systematic Wood Anatomy at the University of Utrecht.

only very little branching of the thick filaments. Intermediates between the three types have sometimes been found. In addition to the vesturing described above, vestures may occur on the apertures, irrespective of the type present in the pit chambers. Though constant for the species and the genus, the type of vesturing was not related to any taxonomic subdivision of the family. The taxonomic significance of type of vestures is therefore highly restricted in the Combretaceae.

A classification of the family, taking into account all wood anatomical characters, agrees well with the classification proposed by EXELL & STACE (1966). Wood anatomically four groups can be recognised:

1. *Strephonema*; outstanding because of its ray type (heterogeneous II) and fibre type (fibre-tracheids). The other genera have heterogeneous III to homogeneous rays and libriform fibres.

   Combretum, *Calopyxis*, *Calocopteris*, *Guiera*, and *Thiloa*; characterized by the occurrence of two distinct sizes of the vessels and by the presence of radial vessel in the rays. The latter character has never been recorded before for any dicotyledonous family (cf. VAN VLIET 1976).
3. All remaining genera outside 1 and 2 are rather heterogeneous for certain characters such as parenchyma distribution, and crystal type, but overlap strongly in most qualitative characters. The genera *Laguncularia*, *Lumnitzera*, and *Macropteranthus* stand out from this assemblage by a statistically significant difference in fibre length/vessle member length ratio with the other genera.
4. *Anogeissus*, *Bucida*, *Buchenavia*, *Conocarpus*, *Finetia*, *Pteleopsis*, *Ramatuella*, *Terminalis*, and *Terminaliopsis*, the remaining genera, can be kept together because they strongly overlap in anatomical features and lack any of the characters of the three groups mentioned above.

Group 1 coincides with subfamily Strephonematoideae. Group 2 coincides with subtribe Combretinae of the tribe Combretae (subfamily Combretoideae). Group 3 coincides with the tribe Laguncullariae (subfamily Combretoideae). Group 4 is composed of genera of the subtribes Pteleopsidinae (only genus *Pteleopsis*) and Terminaliinae of the tribe Combretae. This indicates that the subtribe Pteleopsidinae, which is macromorphologically more or less intermediate between the Terminaliinae and the Combretinae is wood anatomically closer to the former subtribe.

This study was financially supported by the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.).

EXELL, W. A. & C. A. STACE (1966): Revision of the Combretaceae. *Bol. Soc. Brot.* **40**: 4–25.
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J. KOEK-NOORMAN and B. J. H. TER WELLE (*Instituut voor Systematische Plantkunde, Utrecht*)

Systematic wood anatomy of the Blakeeae (Melastomataceae)

The Melastomataceae are divided into three subfamilies. The largest one, the Melastomatoideae, are characterized by the presence of libriform fibres, often septate, sometimes with parenchyma-like patches or bands of thinner-walled cells with conspicuous intercellular spaces; by the predominantly uniseriate rays, mostly composed of square and upright cells; by the presence of parenchyma in narrow vasicentric rings or in single paratracheal strands; and by the absence of included phloem.

Within the Melastomatoideae the tribe Blakeeeae is characterized by the presence of long axial rows of thin-walled, enlarged cells containing crystals; the idioblasts are situated in tangential parenchyma patches; and by the 2-3-seriate rays which are often over 1,500 μm high.

The ratio between the length of the parenchyma strands and the vessel member length lies between 1.19 and 1.30. This is not in accordance with the concept, that both parenchyma strand length and vessel member length represent the length of the cambium initials.

Up till now a suggestion about the affinities between the Blakeeae and other Melastomatoideae can not be given. The wood samples studied so far seem to suggest a rather isolated position of the tribe in the subfamily.

D. F. CUTLER (*Jodrell Laboratory, Royal Botanic Gardens Kew, U.K.*)

Teaching Plant Anatomy

An understanding of plant anatomy is basic to many other botanical disciplines, notably physiology, plant pathology, taxonomy and morphology. It is also of importance in pharmacognosy, the identification of archaeological remains of plant origin and of plant fragments from various sources. A knowledge of wood anatomy enables us to understand the properties of wood, and helps us to make the proper application of different types of wood for particular purposes. The study of plant anatomy is also of intrinsic value in its own right.

Unfortunately the modern university syllabus is so crowded that less and less time is given to plant anatomy. This is probably due in part to the fact that we do not give enough stress to the applied and economically important aspects of the subject. Our short courses give little scope to elaborate on variability, and the vast range of interest that lies in plant structure. Students struggle to become familiar with the terminology and unless strongly motivated, lose interest.

Briefly, three levels of training are commented on.

a) Undergraduate.

Students who are allowed to cut up the whole plant (preferably an herbaceous weed) and prepare their own microscope slides as a first exercise rapidly grasp the idea that the plant is a real, three dimensional object. Selection of a weed unrepresented in textbooks forces the student to draw what he sees! Difficulty experienced in making good sections helps the student to value and treat with due care prepared slides given for study later in the course.

Rather than work from the cell to the tissues, I prefer to discuss whole systems first, e.g. mechanical, water and food distributional, and follow this by a closer examination of the cell types involved. Development, cell contents, secondary tissues etc. fit into place later in the course. By starting from the "known" and moving in easy stages to the "unknown" I have found that many students develop a lasting interest in the subject, their minds become open to enquiry and they often want to look at a wider range of plants than would normally be seen during a course.

It is most important to show how anatomy can be applied to solving practical economic problems.

b) Postgraduate lecture courses.

Anatomy forms an integral part of pure and applied plant taxonomy courses. Here the range of variability of characters in the vegetative parts can be explored in some detail. This in itself can be enough to stimulate many students to select anatomy as their course project, but again emphasis on the practical and applied aspects, with examples, is most important. Emphasis could, for example be given to wood anatomy, or the anatomy of medicinal plants or those used as food or animal feed.

Students at this level benefit from being taught how to record anatomical data and how to write in the various styles required for scientific papers, monographs, diagnoses and so on.

c) Doctoral theses.

There are still numerous plant families where an anatomical study on its own is sufficient for a thesis. However, I feel that such studies are best left to the postdoctoral level, and these days a student should be encouraged to conduct an integrated study for the doctoral thesis. A well circumscribed and conveniently small group studied from anatomical, palynological, taxonomic, cytological and phytochemical standpoints will help the student to develop a balanced and critical view. Since it is increasingly difficult to obtain a post as a pure anatomist, it is sensible to train on as broad a base as possible.

The subject of the thesis should be as relevant as possible to the proposed future career. Students from overseas may benefit particularly from a study involving plants from their

own country, and if convenient, of some economic significance.

In conclusion, plant anatomy has a great deal of interest to offer students at all levels. It can lead to an intelllectually stimulating and worthwhile career. Those of us fortunate enough to be working in and teaching anatomy should ensure that the message is put over well.

R. M. DEN HARTOG-VAN TER THOLEN (*Rijksherbarium, Leiden*)

Epidermal characters of the Celastraceae (Hippocrateaceae included)

Leaf epidermal characters of 100 species belonging to 35 genera of Celastraceae (including Hippocrateaceae) have been studied. Hairs are mostly absent. In numerous species crystalliferous epidermal cells occur, containing either druses or rhomboidal crystals. Five stomatal types were found: anomocytic, anisocytic, cyclocytic, paracytic, and laterocytic. The laterocytic type has been newly introduced for stomata with more than two subsidiary cells on at least one of each of the (lateral) sides of the guard cell pair. In general all characters of Celastraceae sensu stricto also occur in the former Hippocrateaceae. There is a trend for crystalliferous epidermal cells to be more common in the "Hippocrateaceae". In Celastraceae sensu stricto the stomatal type shows a greater diversity than in "Hippocrateaceae". The latter group only shows laterocytic and cyclocytic stomata as dominant type. On the whole the leaf epidermal characters support the inclusion of Hippocrateaceae in Celastraceae.

The broad genus concept of *Cassine*, including 5 genera as recognized by some authors, may need revision because three groups with different stomatal types can be distinguished on the basis of leaf anatomy. The affinities of *Euonymus globularis* from Australia with *Brassiantha* and *Hedraianthera* as suggested by macromorphological similarities are strongly supported by their shared stomatal type – different from that found in other *Euonymus* species. The genera *Goupia* and *Siphonodon* both show laterocytic stomata and are therefore not aberrant in Celastraceae on the basis of their leaf epidermal characters.

A detailed account of all results will be published elsewhere (VAN DER THOLEN & BAAS, *Blumea*, in preparation).

R. W. DEN OUTER (*Botanisch Laboratorium, Wageningen*)

Comparative bark anatomy of Angiosperms

The anatomical features of the secondary phloem of 444 species of woody dicotyledons, belonging to 24 families, all from the Ivory Coast, West-Africa, were used to find correlations among the different types of secondary phloem characteristics as indicated by Zahur. Both companion-cell type A (short companion cell, one per sieve-tube member) and sieve-tube type I (long sieve-tube member with compound sieve plates with ten or more sieve areas) are correlated with phloem-parenchyma type $^{+}$ (abundant, forming the ground tissue), mechanical-tissue type I (sclereids or no mechanical tissue at all) and to a certain extent with sieve-area type a (many, well developed sieve areas on the side walls of the sieve-tube member, equally spaced). Also both companion-cell type C (long companion cell, septated to form a strand of cells) and sieve-tube type III (short sieve-tube member with simple sieve-plates) are correlated with phloem-parenchyma type^{-} (not abundant, usually arranged in tangential bands), mechanical-tissue type 3 (true fibers, arranged in tangential bands) and to a certain extent with sieve-area type c (sieve areas absent or obscure). Thus it appears that more advanced sieve tube- and companion cell types, are correlated with less advanced other characteristics of the secondary phloem or the other way round.

In secondary phloem of a species sieve-tube type I is often associated with companion-cell type C, or sieve-tube type III is associated with companion-cell type A. This combination of the two features makes it difficult to predict what other anatomical characters to expect in the bark on the basis of sieve-tube type present.

L. GOOSEN-DE ROO (*Botanisch Laboratorium, Leiden*)

Electron microscopy of the cambial zone of Fraxinus excelsior L.

Relatively few ultrastructural studies have been carried out on cambial zones. Three different reasons can be mentioned for this: technical difficulties in sampling relatively undeformed cambial zones from actively growing trees; difficulties in fixation for electron microscopy and in embedding and sectioning certain groups of cells in a defined plane; and the lack of conformity in defining cambial initial and cambial zone.

Sampling of cambial zones as described by BURGGRAAF (1973) appeared to yield material suitable for investigation.

A provisionally acceptable fixation procedure of the samples is: 24 hours at room temperature in 6.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, followed by washing in the same buffer. Thereafter, post-fixation in 1% osmium tetroxide in the same buffer for 24 hours at room temperature (VAN SPRONSEN, pers. comm.).

BURGGRAAF (1974, 1976) developed a model that describes cell-division sequences and cell production in the cambial zone. This model, which is based on light-microscopical investigations of cambial-zone sections, permits a rather precise definition of the cambial initial with respect to its location.

With this model as a basis, ultrastructural investigations of the cambial zone are now in progress. These studies aim at answering the following questions:

1. Can the site of the future cell-division plane in the cambial initial or in mother cells be predicted from, for instance, the distribution of cell organelles (preprophase band, phragmosome)?
2. Are the cambial initials characterized by special ultrastructural features?
3. What are the growth possibilities of cell walls in the cambial zone? To answer this question the presence of cellulose microfibrils in the walls and thus the distribution of peripheral microtubules in the cambial zone are being investigated.

Some preliminary results of this work have been illustrated.

BURGGRAAF, P. D. (1973): On the shape of developing vessel elements in Fraxinus excelsior L. *Acta Bot. Neerl.* **22**: 271–278.

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P. BAAS (*Rijksherbarium, Leiden*)

The peculiar wood structure of Leptospermum crassipes Lehm. (Myrtaceae)

Leptospermum crassipes is a small woody plant from swamp flats in West Australia with distinctly swollen stem bases. Its juvenile secondary xylem is normal and consists of vessels, thick-walled fibre-tracheids, "normal" heterogeneous rays, and scanty axial parenchyma. The later-formed wood, however, is characterized by a homogeneous ground tissue of wide, thin-walled "vessel members" and "vascular tracheids" of similar shape and size, traversed by extremely low rays of strongly procumbent cells. Axial parenchyma is absent. The length-on-age graphs for vessel member and tracheid length deviate from normal curves in showing a steep decline in the first-formed secondary xylem. A comparison with other *Leptospermum* species of normal habit, as well as with other plants with a comparable habit or habitat lead to the conclusion that the situation in *L. crassipes* is unique, and defies all general trends established for wood anatomical correlations with taxonomic classification, habit and habitat. Yet the juvenile secondary xylem of *L. crassipes*, together with its macromorphology clearly indicate its affinity to other *Leptospermum* species. A more detailed account, including a discussion of the relevance of these findings with respect to current ideas on xylem evolution has been published elsewhere (*IAWA Bulltin* **1977/2**: 25–30).

MEETING OF THE SECTION FOR PLANT TAXONOMY AND -GEOGRAPHY ON APRIL 9, 1976

J. H. IETSWAART

(Biologisch Laboratorium, Vrije Universiteit, Amsterdam)

Some results of a taxonomic revision of the genus Origanum L. (Labiatae)

Within the genus *Origanum* three subgenera (*Amaracus*, *Majorana*, *Origanum*) are distinguished, groups which have been considered before either as sections in the genus, or as separate genera (BRIQUET 1895; BOISSIER 1879). The differences between the subgenera mainly concern size and colour of spikes and bracts, and shape of calyces. The genus is divided into ten sections in all, of which several are new. The subgenus *Amaracus* is divided into the sections *Amaricus*, *Anatolicon*, *Brevifilamentum* and *Longitubus*, the subgenus *Majorana* into the sections *Chilocalyx* and *Majorna*, and the subgenus *Origanum* into the sections *Campanulaticalyx*, *Elongatispica*, *Origanum* and *Prolaticorolla*. This classification especially rests on differences in shape of calyces and corollas, and in length of staminal filaments (IETSWAART 1977, in preparation).

The genus comprises ca. 45 species, many of which have an East Mediterranean distribution. Several others occur in the adjacent Pontic and Irano-Turanian parts of Turkey. Some species are found in the West Mediterranean subregion and a few others (section *Origanum*) in the Euro-Siberian region, outside the Pontic province (ZOHARY 1973). Many species are endemic, especially in the East Mediterranean subregion, and restricted to only one mountain (range) or island. Several of them can be indicated as Oro-Mediterranean elements, and some as chasmophytes. Distribution patterns of (endemic) *Origanum* species in Turkey agree with the general picture (DAVIS 1965 & 1971).

At the moment fourteen, for the greater part intersubgeneric, hybrids are known. A very few excepted, these hybrids have been found only very locally. Besides, hybrid swarms occur most probably between some pairs of closely allied species. These must be considered as varieties of one of the parents, as long as their hybrid character has not been clearly proved.

The chromosomes of only a few species from each of the three subgenera have been studied. In all cases (except a few populations of *O. vulgare*) a number $2n = 30$ has been established (MIEGÉ & GREUTER 1973). In some related genera, like *Micromeria*, *Satureja* (s.s.) and *Thymus* also a number $2n = 30$ has been found (JALAS & KALEVA 1967; TUTIN 1972).

The species of *Origanum* show a great diversity in types of corollas and stamens, doubtless correlated with comparable variation in pollinating insects. One of the most obvious examples is the upside-down position of the flowers found in the subgenus *Amaracus*.

Gynodioecism is established for many species in the subgenera *Majorana* and *Origanum*. It is very uncommon in the subgenus *Amaracus*, however.

Much confusion in nomenclature, which appears from the many synonyms, is found in the sections *Majorana* and *Origanum*. For this confusion several reasons can be given: unfamiliarity of former authors, especially those of the earliest post-Linnean period, with each other's work; overestimation of slight morphological differences as distinguishing criteria (e.g. concerning length, hair-covering and colour of flower-spikes); the occurrence of hybrids, and lastly the morphological modifications caused by growing Mediterranean species in temperate regions (e.g. in indumentums and degree of branching).

From the related genus *Thymus*, *Origanum* can be distinguished by the more erect, longer stem with more, roundish or ovate leaves, more branches in the upper parts and consequently more spikes, and by the less acute to absent teeth in the lower lips of the calyces. From *Satureja* (s.l.) *Origanum* differs in the clear compact spikes with well developed bracts. The limits between *Origanum* and the allied genera are not sharp throughout. For instance in the section *Campanulaticalyx*, *Origanum* approaches *Satureja* very closely.

Geographical differentiation and hybridization most probably played (and actually play)

a prominent part in speciation in *Origanum*. Not only mutual hybridization of *Origanum* species but also hybridization with species from related genera in the tribe *Saturejeae* can be considered in this respect.

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MEETING OF THE SECTION FOR PLANT TAXONOMY AND -GEOGRAPHY ON MARCH 26, 1977

J. BOKDAM (*Laboratorium voor plantensystematiek en -geografie, Landbouwhogeschool, Wageningen*)

Seedling morphology of some African Sapotaceae and its taxonomical significance.

The seedling morphology of 46 African species representing 25 genera has been studied. Two major seedling types can be distinguished, a primitive type with strongly enlarged, foliaceous cotyledons and an advanced type with cotyledons not enlarged and fleshy. In the taxa studied, the seedling structure appears to be correlated with the following characters of the generative plant:

- the level of insertion of the stamen in the corolla tube;
- the number of locules in the ovary;
- the number of developed seeds in the ripe fruit;
- the position, shape and dimensions of the scar on the testa;
- the quantity of endosperm in the ripe seed.

The seedling type has certainly taxonomical significance in Sapotaceae. In this family the progressive loss of endosperm and the evolution of fleshy cotyledons from foliaceous ones seems to have taken place in several subfamilies. Using the seedling type or a character closely correlated with it for the delimitation of subfamilies in Sapotaceae, leads towards unnatural, heterogeneous units, which only share the same stage of evolutionary advancement of the seedling type.

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M. S. HOOGMOED (*Rijksmuseum van Natuurlijke Historie, Leiden*)

Species diversity in northern South America as a consequence of climatic fluctuation

Vegetations in northern South America, especially in the Guianas can be divided into two great groups: forests and savannas. The latter category also includes swamps, which share many physical features with savannas. The Amazonian forest is not one continuous rainforest, but encloses many savannas. Most of them are situated in a belt with lower rainfall connecting the Venezuelan Llanos with the caatinga and cerrado regions of C. and NE. Brazil (PRANCE 1973). Until recently it was thought that these savannas were man-made, but from their fauna and (to a lesser extent) flora it appears that they are natural entities, which existed before man entered the region (conservatively estimated at 7000 years ago). Man's influence was only secondaryly. It has been established that the climatic fluctuations of Holocene and Pleistocene not only influenced the climate in temperate regions, but also in tropical lowlands (SIMPSON & VUILLEUMIER 1971). Their effect was a lowering of the temperature with 3–4 °C (VAN DER HAMMEN 1974), more important was their effect on precipitation. As a result dry and wet periods alternated. Probably the wet periods coincided with warm interglacials, the dry periods with cold glacials. Both palynological and geomorphological data prove that areas which are today covered with forest, had experienced dry climates in the past (stone lines) (VANZOLINI 1970). Within such regions there are areas in which stone lines are absent, which means that during the dry periods these areas were covered with forest.

Distributions of animals can be divided into three groups:

1. Those distributions that indicate a former connection between the Amazonian rainforest and that of coastal SE. Brazil (*Anolis punctatus, Bothrops bilineatus, Lachesis muta*).
2. Those that indicate a former connection of the now isolated savannas in the Amazonian region with each other and with the open formations north and south of the hylaea (*Crotalus durissus, Tropidurus torquatus*).
3. Those distributions that indicate that complex processes occurred in the hylaea. Examples can be found in several birds which formed superspecies-complexes in the area (*Psophia, Rhamphastos, Selenidera, Pipra*) and mammals (*Saguinus, Cebus, Alouatta*) (HAFFER 1974). Several species of butterflies (*Heliconius erato* and *H. melpomene*) form numerous subspecies in Amazonia. These are all deep forest animals for which open spaces constitute barriers (BROWN et al. 1974; HAFFER 1974). However, the boundaries between the several species or subspecies are within the forest, sometimes coinciding with rivers, sometimes formed by wider or narrower contact zones. Forest species of the plant genera *Stephanopodium, Cariocar, Cariniana* and the species *Hymenaea oblongifolia* and *Couratari macrosperma* are distributed in the hylaea and in the SE. Brazilian forests, this also suggesting a former connection between the two areas (PRANCE 1973). Similar data come from linguistic research. For instance the aforementioned belt of lower precipitation is inhabited by Carib speaking people, surrounding areas by Arowak and Tupi-Guarani speaking people (MEGGERS 1975).

To explain these facts the hypothesis of the forest refugia was advocated (HAFFER 1969, 1974, 1977). This hypothesis takes the line that the climatic fluctuations directly affected the vegetation. During wet periods the forest expanded at the cost of the savannas, which were forced back into refuges (areas with lower rainfall and certain edaphic characteristics). During dry periods the reverse happened, the savannas expanded and the forest was broken up into small parts which could maintain themselves in areas with higher rainfall. It is also assumed that the rainfall pattern in the Quaternary did not essentially differ from that of today, only in dry periods the total amounts were lower. This hypothesis provides the basis for understanding how species in the seemingly uniform Amazon Basin could develop. The forest-dwelling animals during dry periods retreated into the forest refuges and thus individuals formerly belonging to one population became geographically separated and could develop into different taxa. When during wet periods the forests expand again and come into contact the populations from different origins may behave like good species, semi-species, subspecies or they may have remained unaltered. The position of the supposed forest refugia

was decided by taking into account distribution patterns of animals (mammals, birds, reptiles, butterflies, flies), position of secondary contact zones between species, position of rainfall maxima and geomorphological features (HAFFER 1974). Decisive evidence from crucial areas still has to be provided by palynology and geomorphology. However, so far the postulated refuges serve well to explain present day distribution patterns in a variety of animal groups, in a number of plants and even of linguistic groups in man. The events responsible for the fluctuations in vegetation cover in Amazonia occurred only recently. The last arid period lasted from 3500 to 2000 years ago. Other dry periods occurred 11 000 and 21 000–13 000 years ago. When assuming an extension rate for the forest of 100 m/year, the present day situation could have been reached in about 2000 years, starting from the postulated refuges.

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M. M. J. VAN BALGOOY (*Rijksherbarium, Leiden*)

Flora and vegetation of the Langkawi Islands

The Langkawi Islands are situated at 6°20′ N. and 99°50′ E. at c. 20 km off the northwestern part of Malaysia (Malaya). The area is just over 400 sq. km and the highest elevation is c. 1000 m. The island group is subject to a seasonal climate. Annual precipitation amounts to c. 2,500 mm, with rainfall peaks in May and October and a pronounced dry season in January/February.

Geologically the islands are very interesting: highly metamorphic rocks of Cambrian age in the W, sedimentary sandstone of Carboniferous to Permian age in the centre and in the E the so-called Setul Limestone (Ordovician and Silurian) and the Chuping formation (Permian limestone). These limestone rocks are responsible for the fantastic karst topography of a great part of the islands. There are also several scattered granite intrusions.

The forest is relatively well preserved especially on the limestone. The total number of native vascular plant species on record so far is 1036 belonging tot 563 genera, numbers expected to increase drastically with more exploration. The vegetation over limestone is stunted and xeric, unlike that over other rock types. The floristic composition is also quite different.

A sinkhole basin on Pulau Langgun contains a lake of 5 ha (pH = 8). It supports a dense submerged vegetation of *Nitella*, *Utricularia*, and *Najas*. Scattered tree trunks were found rooting in the bottom of the lake with their broken end near the water surface. Apparently the basin was once covered with forest which was killed when the drainage was blocked and

the basin filled with water. A heavy marshy forest surrounds the lake, the vegetation rapidly assuming a xeric character away from the lake on the surrounding limestone rocks. The dominant tree here is *Pentaspadon curtisii* (Anac.), a species rare elsewhere. In the undergrowth the most conspicuous species is *Impatiens mirabilis* (Bals.) with a "trunk" 40 cm across at the base and up to 2.5 m tall.

MEETING OF THE SECTION FOR PLANT TAXONOMY AND -GEOGRAPHY ON NOVEMBER 5, 1977

R. J. SCHEFFER and J. C. M. DEN NIJS (*Hugo de Vries Laboratorium, Universiteit van Amsterdam*)

Preliminary note on the polyploid Rumex acetosella-complex in the Balkans

The linnean species *Rumex acetosella* L. has proved to be a polyploid complex with the haploid number x = 7.

On the basis of karyogenetic studies LÖVE (1944) suggested that the degree of ploidy and the morphological characters are correlated. He accordingly recognized the following species within the complex: *R. angiocarpus* (diploid, 2n = 14) supposed to be angiocarpous in contrast to all other species (angiocarpy being the coalescence of the inner three perigone lobes with the exocarp at seed maturity), *R. tenuifolius* (tetraploid, 2n = 28), *R. acetosella* sensu stricto (hexaploid, 2n = 42), and *R. graminifolius* (octoploid, 2n = 56). As differentiating characters of the last three species the various leaf-shapes, the habit form, and the dimensions of floral parts and the fruits were used. The octoploid, only known from arctic regions will not be discussed here because of its absence in the area explored.

Previous studies of populations in West and South Europe had already shown that angio- and gymnocarpy may occur at all ploidy levels, and, also that the variability of the leaf characteristics, among others, is appreciable and not consistently correlated with the ploidy level, so that such data cannot be maintained in the older classification (compare, e.g., HARRIS 1973; DEN NIJS 1976).

As a sequal to the previous investigations, a start was made with a survey of Balkan populations. Owing to her singular geomorphological structure, geological history, and geographical position, the Balkans have been an important area for the development and distribution of many taxa, and e.g. HARRIS (1973) expressed the expectation that the Balkan region may have played a key role in the recent evolution and geography of the *R. acetosella*-complex. As an initial approach an extensive series of population samples was collected in Bulgaria, which became augmented by some material from northern Greece contributed by fellow botanists.

The preliminary results are roughly as follows:

1) diploids, tetraploids, and hexaploids were encountered; multifidy, i.e., the bifid to multifid basal lobing of the leaves, is common at all ploidy levels;
2) hexaploids are of rare occurrence in Bulgaria and Northern Greece. All available material from the Aegaeans proved to be hexaploid, however;
3) tetraploid (consistently gymnocarpous) are very wide-spread in Bulgaria and the Greek mainland;
4) gymnocarpous diploids were only found to be present in the South-bulgarian mountains (Rila and Pirin);
5) angiocarpous diploids were found in the mountains of Northern Greece.

These findings strengthen the surmise that diploid populations are the remnants of a number (two?) of old population groups, at present – as far as the gymnocarpous ones are concerned – restricted in their occurrence to glacial refugia, and that, among other ones, various tetraploid lineages have spread from these refugia, most probably on account of a somewhat different ecological preference. No arguments have been found pleading in favour of the hypothesis that the complex has spread especially from the Balkans all over Europe.

In order to clear the role of the Balkans more thoroughly investigations are planned in Rumania, Turkey and remnant parts of Greece.

HARRIS, W. (1973): Leaf form and panicle height variability in Rumex acetosella. *New Zeal. Journ. Bot.* **11**: 115–144.
LÖVE, A. (1944): Cytogenetic studies on Rumex Subgenus Acetosella. *Hereditas* **30**: 1–136.
NIJS, J. C. M. DEN (1976): Biosystematic studies of the Rumex acetosella complex II. The Alpine region. *Acta Bot. Neerl.* **25**: 417–447.

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON NOVEMBER 15, 1977

J. VAN DEN HEUVEL (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Formation of phytoalexins in French bean leaves infected by *Botrytis cinerea*.

Primary leaves of French bean (*Phaseolus vulgaris* L. "Dubbele Witte z. dr.") were inoculated with three pathogenic and two nonpathogenic isolates of the fungus *Botrytis cinerea* Pers. ex Pers. The infected tissues, which contained either spreading lesions (caused by the pathogenic isolates BC-1, BC-3 and BC-4) or lesions limited in size (caused by the nonpathogenic isolates BC-5 and BC-6), were investigated for the presence of phytoalexins. To this end, lesions, together with an adjacent green, 3 mm wide zone, were excised from the leaves; the collected material was lyophilized, extracted with 60% methanol, and purified by column chromatography (Sephadex LH-20) and thin-layer chromatography (TLC). Phytoalexins and other antifungal substances were detected by bioassays on developed TLC plates and by UV-spectrometry. Quantitative analysis was carried out by in situ densitometry.

In most cases the phytoalexins phaseollin, phaseollidin and phaseollinisoflavan, the phaseollin metabolite, 6a-hydroxyphaseollin, and a few unidentified antifungal substances were detected; phaseollin was predominant. The concentration of phaseollin accumulating in leaves infected by the nonpathogenic isolate BC-5 was about twice as high as that in infections produced by the pathogenic isolates. In contrast, leaves infected by the nonpathogenic isolate BC-6 only contained low concentrations of phaseollin.

P. C. SCHEEPENS (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Mitochondrial electron transport in Peronosporales

Mitochondrial electron transport of *Phytophthora infestans* was compared with that of *P. erythroseptica* and *Pythium debaryanum*. The three fungi represent increasing levels of physiological specialization within the Peronosporales (class Oomycetes, lower fungi).

As compared with the two other fungi, the electron flux through the main electron transport chain of *P. infestans* was only limited; a narrow-pass in this chain was presumably located in the cytochrome b region. However, externally added NADH was oxidized much faster by mitochondria of *P. infestans* than endomitochondrially generated NADH and succinate. In this case, the electrons of NADH were introduced directly into the cytochrome c region of the main electron transport chain, thereby circumventing the postulated narrow-pass. This abbreviated electron transport was insensitive to rotenone and antimycin A, but sensitive to cyanide. The pathway for abbreviated electron transport was also present in *P. erythroseptica* and *P. debaryanum*, but here it did not contribute much to the oxidation of NADH.

A low energy yield and a shortage of precursors for important biosynthetic pathways, which are possible consequences of the anomalous electron transport in *P. infestans*, are proposed as the cause of its inability to grow on basal nutrient media.

H. R. VISSCHER (*Proefstation voor de Champignoncultuur, Horst*)

Factors affecting fructification in the cultivated mushroom, Agaricus bisporus (Lge.) Imb.

Fructification in the cultivated mushroom only occurs in presence of a casing soil. From 1960 onwards laboratory experiments have revealed the existence of a relevant microflora in this soil in absence of which no fructification in the mushroom occurs. This flora can thrive on volatile metabolites of the mushroom mycelium as the sole carbon source.

Own experiments on the structure of casing soil, on supplementation at casing with soybean meal and on steaming of soil are explained in connection with this flora: interventions considered as to stimulate this flora caused enhanced fructification and yield and vice versa.

Application of excess mycelial metabolites at different times after casing caused depression or stimulation of number and yield of mushrooms, interpreted as the effect of either CO_2 or of other metabolites, considered in the past as a substance X.

Studies on the nature of substance X revealed enhanced fructification in deep petridishes after application of ethylene, notably at a concentration of 1 ppm in interaction with the CO_2 level of the ambient air. An ethylene producing fungus e.g. *Fusarium oxysporum* f. sp. tulipae, strain B1 caused the same phenomena. The results throw a new light on recent and older experimental data and enable a better understanding of the function of the casing as a medium, a carrier in which mushroom mycelium and microflora give rise to mushrooms as a result of symbiosis.

F. J. GOMMERS (*Laboratorium voor Nematologie, Landbouwhogeschool, Wageningen*)

Metabolism of giant cells and syncytia as determined by ultra-micro analytical techniques

Metabolite concentrations were determined in giant cells and syncytia induced by *Meloidogyne* and *Heterodera* with Lowry's micro analytical techniques (LOWRY & PASSONNEAU 1972). Samples of 5–50 ng were dissected from lyophilized frozen sections and weighed on a quartz fibre fishpole balance. Metabolites were quantitatively converted to pyridine nucleotides. These were subjected to "enzymatic cycling" and measured fluorometrically. Giant cells and syncytia in soybean had concentrations of ATP, glucose-6-phosphate, and protein comparable to those of meristematic root tip tissue (control) but about 4× more glucose. Free amino acids were more concentrated in giant cells (about 6× that of controls) than in syncytia (about 1.7× that of controls). Giant cells of garden balsam had higher concentrations of ATP, glucose-6-phosphate than controls, 4× more glucose, and free amino acids only slightly more concentrated than controls. It is concluded that both pathogen and host influence the altered metabolite concentrations in giant cells and syncytia. It is clear that the methods used are applicable to the analysis of many plant pathological problems. This summary is essential an abstract of an article published elsewhere (GOMMERS & DROPKIN 1977).

GOMMERS, F. J. & V. H. DROPKIN (1977): Quantitative histochemistry of nematode-induced transfer cells. *Phytopathol.* **67**: 869–873.

LOWRY, O. H. & J. V. PASSONNEAU (1972): *A flexible system of enzymatic analysis.* Academic Press, New York and London.

B. J. M. VERDUIN, W. M. A. M. VAN DONGEN and K. M. KRÜSE (*Laboratorium voor Virologie, Landbouwhogeschool, Wageningen*)

The effect of age of infection on the properties of cowpea chlorotic mottle virus and comparison with quinone modified virus.

Cowpea chlorotic mottle virus (CCMV) is isolated in the presence of EDTA from 1, 2 and 3 weeks infected *Vigna unguiculata* (L) Walp. var. Blackeye Early Ramshorn with yields of 0.5, 0.4 and 0.3 mg per gram of fresh tissue, respectively. The dissociation of this purified virus is increasingly inhibited with age of infection and resembles the dissociation behaviour of CCMV modified by quinones (CCMV-Q). CCMV-Q, obtained after incubation of CCMV from one week infected plants with chlorogenic acid and tyrosinase contains, on average, 1 molecule of bound chlorogenic acid per two coat protein subunits and is no longer infectious. CCMV from 2 and 3 weeks old infections retains approximately 70 and 60% of the infectivity found with one week old infection.

Dissociation of virus in sodium dodecyl sulphate (SDS) reveals increasing heterogeneity of the RNA with longer infection periods or after modification by quinones, both in gradients and polyacrylamide gels. This heterogeneity is lost after incubation of virus in the presence of SDS, a reducing agent and pronase (WYATT & KUHN 1977). The presence of EDTA during the incubation with pronase is essential to prevent degradation of RNA by reducing agents.

No significant amounts of dimers or polymers of the coat protein subunit are found with either CCMV-Q of CCMV from plants after long infection periods. Although both long infection periods and modifications by quinones cause inactivation of CCMV by cross-linking protein and RNA, no breaks in the RNA occur. The results suggest that the *in vitro* modification of CCMV during the oxidation of polyphenols also applies to the *in vivo* modification of virus during long infection periods.

WYATT, S. D. & C. W. KUHN (1977): Highly infectious RNA isolated from cowpea chlorotic mottle virus with low specific infectivity. *J. Gen. Virol.* **35**: 175–180.

P. VAN DER ZWEEP, A. C. VAN ELSEN and H. J. VAN MAANEN (*Laboratorium voor Virologie, Landbouwhogeschool, Wageningen*)

The use of granulosis virus for the control of the codling moth, Laspeyresia pomonella (L.)

Orchard tests in Germany, Switzerland, Australia and Canada have indicated that a granulosis virus, discovered in 1964, may have potential as an applied agent for the control of the codling moth, *Laspeyresia pomonella* (L.). In 1977 studies were performed to assay its efficacy in controlling the codling moth in The Netherlands.

The experiments were carried out in the experimental orchard of TNO at Lienden. The trial orchard (360 trees, var. Golden Delicious) was divided into six parallels and each parallel into six plots; these plots received different treatments with virus or insecticide, applied at two weekly intervals.

Treatment	No. of plots	% of apples injured	% reduction in injury
Control	2	19.1	–
Virus 2x	1	9.3	51.3
Virus 3x	1	10.0	47.6
UV-R virus 2x*	1	11.1	41.9
Azinfos-methyl	1	0.9	95.3

* A virus strain reported to be less susceptible to UV inactivation.

From the results (see table) it is evident that the virus treatments were not as effective as the chemical insecticide in reducing fruit injury. This is because it took 2–6 days for a larva to die after ingestion of the virus, during which time the fruit was injured. However, there was a great reduction in deep entries in the virus treated apples, most of the injuries being

shallow entries or stings. Such injuries would reduce the value of the apples as fresh fruit but should cause less loss of crop value for other purposes. A more pronounced effect is observed in the number of dead larvae found in the sprayed apples. About 60% were dead.

These data indicate that further study with the granulosis virus is necessary, especially as a component in integrated control programs for the codling moth.

TH. L. J. DUINEVELD and J. C. M. BEIJERSBERGEN (*Laboratorium voor Bloembollenonderzoek, Lisse*)

The influence of soil-stabilizers on the depot function of fungicides

In bulb culture a tendency is present to use mixtures of several fungicides, for example benzimidazoles and dithiocarbamates, to disinfect bulbs before planting. To prevent the formation of considerable quantities of fungicide dust, soil-stabilizers e.g. polyvinylacetate, are used to fix the dry fungicides onto the bulb surface.

The influence of these soil-stabilizers on the depot function of systemic fungicides (benzimidazoles) was studied by descending paperchromatography. Using 1% polyvinylacetate the depot of Benlate quickly diminished while that of Topsin M and Bavistin was not influenced under the experimental conditions.

The influence of the soil-stabilizers on the activity of captafol (Difolatan 4F) and dithiocarbamate (Aatulsan) was studied on microscope slides. These slides were partly covered with a thin layer of dry fungicide in order to imitate the conditions in which the fungicide is present on the bulb surface. Based on the results with spore germination tests it was concluded that on a glass surface the soil-stabilizer induces a depot function of the dithiocarbamate. This means that the dithiocarbamate is set free less easily when the soil-stabilizer is present. This phenomenon has not been found with captafol.

G. J. BOLLEN and M. M. VERF (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Steamed potting soil as a source of Aspergillus fumigatus spores in the air of greenhouses

The Office of Health for Civil Servants requested an analysis on *Aspergillus fumigatus* spores in the air of a greenhouse in Wageningen. The air-spora was investigated with an Andersen sampler and a malt agar medium, supplemented with an oxytetracyclin to prevent bacterial growth. The optimum temperature for growth of the fungus was 37 °C, but incubation of the plates at this temperature was not practical, because fast-growing colonies of other fungi masked growth of *A. fumigatus*. However, the competing species like *Monilia crassa*, *M. sitophila* and a *Trichoderma* species were less heat-tolerant than *A. fumigatus*. When the plates were incubated at 45 °C, the colonies of the latter species could be counted.

In samples of air taken at different sites in de greenhouse, the number of *A. fumigatus* spores varied from 33 to 2250 per cubic metre. Extremely large numbers were found in the air of the potting room. The concentration of spores amounted 6000 to 4.5 million per cubic metre and depended on how often potting soil was used. This substrate was found to be the source of the spores. It was stored indoors after being steamed. In the inner layers of the soil, the temperature remained between 36 and 40 °C. Under those conditions, the fungus grew profusely. At 14 days after steaming viable spores were counted in four samples of soil by means of the soil dilution plate method. The number of spores varied from 6.15×10^6 to 8.61×10^8 per gramme of dry soil. This high density resulted in a high concentration of spores in the air when the soil was used by staff.

A survey of the air-spora in other greenhouses revealed that the incidence of *A. fumigatus* was dependent mainly on temperature in the steamed potting soil during storage.

P. REINECKE (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Influence of Aureobasidium bolleyi and Phoma eupyrena on pathogenic fungi of the haulm base of cereals in the field

Among isolates from the haulm base of cereals (especially winter wheat) not only the well-known foot rotting fungi *Pseudocercosporella herpotrichoides* (Fron.) Deighton, *Fusarium* spp., and *Rhizoctonia solani* Kühn could be found, but sometimes also several other micro-organisms occurred in high frequency and percentage. Two of these were *Aureobasidium bolleyi* (Sprague) v.Arx (Syn. *Microdochium bolleyi* (Sprague) de Hoog & Hermanides-Nijhof) and *Phoma eupyrena* Sacc.

In artificial infection experiments with *A. bolleyi* and *P. eupyrena* on spring wheat (cv. Kolibri) the first one showed no symptoms on the haulm base, the second showed only weak symptoms, but both reduced natural infection by seed-borne or soil-borne *Fusarium* spp.

In two field trials with winter wheat (cv. Diplomat and cv. Topfit) and one with winter rye (cv. Kustro) inoculation with *P. eupyrena* had no influence on natural infection by pathogenic fungi producing symptoms at stage 11.2 (Feekes' scale). *A. bolleyi* had a differential effect on the pathogens. *P. herpotrichoides* was reduced slightly but not significantly. On the other hand *Fusarium*-symptoms were reduced in one wheat field (cv. Topfit). This possible antagonistic behaviour of *A. bolleyi* agains *Fusarium* spp. was confirmed by the significant reduction of the percentage of *Fusarium*-isolations from the haulm base in all fields tested. *F. solani* was present only in very low quantities.

N. J. FOKKEMA (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Preliminary research on biological control of Septoria nodorum and Cochliobolus sativus in wheat.

Different methods were compared to enhance the growth of the saprophytic mycoflora of field-grown wheat up to a population density high enough to be antagonistic to *Septoria nodorum* and *Cochliobolus sativus*. *Sporobolomyces roseus* and *Cryptococcus laurentii* var. *flavescens* are dominant phyllosphere yeasts and have proved to be antagonistic to many necrotrophic pathogens in controlled environments. A mixture of cells of these yeasts (3×10^7/ml) with and without nutrients, nutrients alone, or water was sprayed on just unfolded flag leaves of wheat. Only spraying with yeasts in combination with 2% sucrose and 0.1% yeast extract increased the yeast population within a week from 10^2 to 10^4 cells/cm^2 leaf surface. The population densities obtained with the other treatments did not differ much from those of water-sprayed leaves and remained below 10^3 cells/cm^2 in this period. The yeast population on the water-sprayed leaves reached the density of 10^4 cells/cm^2 *c.* 2 weeks later by natural colonization. By this time the yeast population on the leaves treated by yeasts and nutrients amounted to $1–2 \times 10^5$ cells/cm^2.

During this period inoculations of field-grown flag leaves with *S. nodorum* and *C. sativus* resulted in significantly less infection of the leaves treated with yeasts and nutrients compared to the infection of the control leaves.

A side-effect of stimulating the leaf-surface-mycoflora might consist of accelerated senescence of the leaves. During this senescence, however, no difference in the reduction of the chlorophyll content was found between leaves with an enhanced mycoflora and those with a reduced mycoflora (by captafol).

IDA BLOK (*Instituut voor Plantenziektenkundig Onderzoek, Wageningen*)

The occurrence of two uncommon Pythium species in lettuce

Isolations, made from lettuce plants with "black rot" symptoms, revealed, in addition to *Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia* spp., sometimes considerable amounts of *Pythium* spp. One of the isolated species has rather large, spiny oogonia and appeared to be a new species. It will be described as *P. uncinulatum*. Another interesting species, found in only a few samples, is *P. tracheiphilum*, described by MATTA (1965) as pathogen of lettuce.

Tests with seedlings of several plant species have shown that *P. uncinulatum* can be very pathogenic to lettuce and somewhat less to cucumber and tomato. *P. tracheiphilum* is very pathogenic to cucumber and cauliflower, somewhat less to lettuce, tomato and pea, and slightly to endive, radish and flax. The pathogens did not affect carrot, leek and wheat.

Whether the forementioned *Pythium* spp. do play a role in the "black rot" symptom of lettuce is not yet certain.

MATTA, A. (1965): Una malattia della Lattuga prodotta da una nuova specie di Pythium. *Phytopath. Mediterr.* **4**: 48–53.

G. M. TICHELAAR (*Instituut voor Plantenziektenkundig Onderzoek, Wageningen*)

Two new root diseases of wheat in The Netherlands

Winter wheat grown on very light sandy soils in The Netherlands often show a premature falling-off leading to reduced yields, even when major diseases such as take-all and *Fusarium* root-rots are absent. In a preliminary experiment set up to find the causal factors for these phenomena, roots of stunted plants were examined microscopically in order to detect fungi that cannot be grown on agar media as well. Patches of stunted plants were prevalent in the non-irrigated part of the field. Most of the roots of these plants were infected by *Microdochium bolleyi* (= *Aureobasidium bolleyi*) which could be recognised by its dark brown microsclerotia in the root cortical cells. In normal plants mycelium had penetrated the lateral roots but sclerotia had not yet been produced.

DOMSCH & GAMS (1968) reported that cultivation of wheat increased the population of *M. bolleyi* in the soil and that the fungus can cause reductions in root dry weight of about 25% in subsequent wheat cultures. FITT & HORNBY (1975) also found that infections by *M. bolleyi* decreased plant dry weight. In the irrigated part of the field *M. bolleyi* was less abundant but here the lateral roots showed a low level of infection by *Polymyxa graminis*. This fungus could not be found in the non-irrigated part.

The importance of these so-called minor diseases in The Netherlands is not yet known but it may be more damaging than is at present thought, especially in sandy soils.

DOMSCH, K. H. & W. GAMS (1968): Die Bedeutung vorfruchtabhängiger Verschiebungen in der Bodenmikroflora. I. Der Einfluss von Bodenpilzen auf die Wurzelentwicklung von Weizen, Erbsen und Raps. *Phytopath. Zeitschr.* **63** (1): 64–74.

FITT, B. D. L. & D. HORNBY (1975): Effects of root-infecting fungi on transport of materials by young wheat plants. *Rothamsted Experimental Station. Report for 1975*, Part 1: 248–249.

H. D. FRINKING (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Research on rose powdery mildew (Sphaerotheca pannosa), some aspects of colony development.

Rose powdery mildew in the Netherlands is called a "cosmetic" disease on an economically important glasshouse crop. Research on the disease is done by means of field-, glasshouse- and climateroom-experiments.

One of the basic questions is: "How does a mildew colony develop on a rose-leaf and is there a way to influence the development without using fungicides".

Growth of mildew colonies is observed by placing inoculated leaves into a micro-wind-tunnel. The development of the colony is expressed by means of spore-production. No spores are expected to stay on the conidiophores or in the colony area after ripening. Mature spores are blown off by wind-force.

The development appears to have a squaring declination, changing itself after about 10–12 days into a linear one.

Interesting points of the growth-curves are: rate of linear growth, the time of greatest spore-production etc.

More data like production-capacity, conidiophore-density etc. are needed for a good interpretation and simulation of colony-growth.

Research is focused on the environmental factors, which can be used to influence the normal growth. From field-experiments we learned, that radiation, more or less in combination with RH, is one of the main factors. In case of spore dispersal after formation and ripening a third factor closes the triangle: wind-force.

This factor especially attracts the attention viewed in the light of glasshouse-experiments, where only very low wind-velocities occur.

J. A. G. VAN LEUR (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Homeostasis between plant and pathogen in pathosystems untouched by man: Limonium vulgare, rust and mildew on the Boschplaat, Terschelling, The Netherlands.

This research is carried out on the Boschplaat, the eastern part of the island of Terschelling. The Boschplaat is the largest nature reservation area of The Netherlands. A major part of it is formed by sedimentation of clay from the floodwaters. The sedimentation started in 1936 after the formation of a sand dike at the northern side of the area. The turbulent North Sea waters could no longer reach it and vegetation became possible. The succession of this vegetation has been an object of study for several research workers.

On the Boschplaat, large areas of vegetation are dominated by *Limonium vulgare* Mill., which is followed in the succession by a vegetation dominated by *Festuca rubra*. *Limonium vulgare* is a salt-tolerant, rosette forming plant which expands by rhizomes. *Limonium vulgare* is parasitized by various organisms of which *Uromyces limonii* and *Erysiphe communis* are the most conspicuous. A different susceptibility of the plants to these pathogens can be noted in the field.

The objective of our research is to answer the following questions:

- Is the difference in susceptibility inheritable?
- Do the rust and mildew pathosystems have physiologic specialization?
- Does the disease influence the seed production of the plant?
- Does the disease influence the competitive ability of the plant?

By solving these questions we hope to find evidence of a balanced pathosystem, and we hope to analyze the balancing mechanism which is possibly a form of genetic homeostasis.

Project financed by ZWO-BION.

B. SCHIPPERS and F. PRUIS (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Fungistatic volatiles released from alkaline soil.

Botrytis cinerea spores were incubated on membrane filters in sealed Conway vessels ± 4 mm above the surface of 15 g non-sterile alkaline soil. The soil was located in the outer ring of the vessel surrounding the central well filled with a weak buffered salt solution (BSS), pH 5.7. The membranes with spores were saturated with BSS. After 24 hr, germination of spores above soil was significantly inhibited to upto 50% of the controls without soil. Inhibition did not occur if the central well surrounded by soil contained 0.04 N H_2SO_4 instead of BSS. The volatile fungistatic factor released from soil apparently is trapped in the H_2SO_4 solution and could be ammonia (NH_3).

Effect of NH_3 was analyzed by incubating spores above a dilution series of $(NH_4)_2SO_4$ in water in Conway vessels. During the 24 hr incubation period the NH_3 was released from $(NH_4)_2SO_4$ by connecting this solution in the central well through a piece of filter paper (inserted just before closing) with the outer ring containing 0.004 N NaOH. Germination was inhibited to upto 50% above the 10 ppm $(NH_4)_2SO_4$, while no germination occurred from 100 ppm onwards. If all NH_3 is released from the 10 ppm solution, the maximum concentration of NH_3 in the Conway vessel atmosphere is ± 320 μg/litre.

NH_3 content of soil atmosphere was estimated by passing NH_3-free air through a 9 litre soil column and then through 40 ml 0.04 N H_2SO_4. The trapped NH_3 was estimated colorimetrically and amounted to 2–4 μg NH_3/litre calculated soil atmosphere.

Germination percentage of *B. cinerea* spores in BSS decreased from ± pH 6.5 onwards being 50% or less at pH 7.0. The pH of membrane filters with spores was found to be 6.8–7.0 after 24 hr incubation above 10 ppm $(NH_4)_2SO_4$. Inhibition of spore germination above this solution therefore could be explained by pH increase. However, the pH of filters with spores incubated above soil, did not increase appreciably.

Although the experimental data suggest that ammonia can act as a fungistatic volatile in soil, they do not fully explain the inhibition of germination of *B. cinerea* spores above the alkaline soil.