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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT PATHOLOGY ON 27 OCTOBER 1983

H. J. M. LÖFFLER (*Willie Commelin Scholten, Phytopathologisch Laboratorium, Javalaan 20, 3742 CP Baarn*)

The effect of ammonia on chlamydospore formation in Fusarium oxysporum

Ammonia has been shown to inhibit germination of conidia of several fungi in concentrations apparent in some soils. Formation of resting structures as well as germination can be considered as differentiation processes; therefore the possible role of ammonia in chlamydospore formation was investigated.

In 25 ml erlenmeyer flasks containing 10 ml Tris-HCl (50 mM) + 0.05% $MgCl_2$ (pH 7.5), pregerminated conidia of several isolates of *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium oxysporum* f. sp. *raphani* produced chlamydospores abundantly within 8 days. Addition of 0.05, 0.5 or 5 mM NH_4Cl did not influence the amount of chlamydospores, but somewhat retarded their formation. High concentrations of NH_4Cl (50 or 500 mM) reduced the amount of chlamydospores, but this effect could be ascribed to the chloride ions or the high molarity. To exclude a possible nutrional effect of NH_4Cl masking an inhibition of chlamydospore formation, the non-metabolisable analogues methylamine and trimethylamine were tested. The results were the same as with NH_4Cl. Chlamydospores formed in the presence of different concentrations of NH_4Cl were equal in size and morphology to those of the control. After 8 weeks there was no variation in persistence and viability of these chlamydospores buried in soil.

Addition of 0.01, 0.1 or 1% NH_4Cl to soil reduced chlamydospore formation by 50, 70 and 100% respectively in ungerminated conidia of *F. oxysporum* f. sp. *dianthi* on membrane filters, buried for 4 weeks.

In conclusion, no effect of ammonia on the amount and quality of chlamydospores has been shown *in vitro*, whereas in soil, ammonia possibly reduces chlamydospore formation.

G. JAGER and H. VELVIS (*Instituut voor Bodemvruchtbaarheid, Postbus 30003, 9750 RA Haren (Gn)*)

Biological and integrated control of Rhizoctonia solani in potato fields

Biological control of *R. solani* in potato fields by inoculation of seed tubers with the hyperparasite *Verticillium biguttatum* proved successful in soils with a rather low inoculum density of *R. solani*. In soils with a relatively high inoculum density of *R. solani* inoculation of seed tubers with *V. biguttatum* produced yields on which, on average, the sclerotium indices were insufficiently reduced.

Some chemicals are available now which reduce the activity of *R. solani* in the soil. The effect of three of them on *V. biguttatum* was tested in laboratory experiments, viz. Monceren (Pencycuron, Bayer), Rhizolex (Tolclofos-methyl, Sumitomo) and Campogran D (Furmecyclox, BASF). Monceren showed the least inhibitory effect on V. biguttatum in vitro and no inhibition, but even a stimulating effect in soil at the recommended dosage rates.

In field experiments Monceren was used at 20% of the dosage rate recommended for practical use. It was applied to the soil one week before the tubers were planted. The treatment as such did not reduce the sclerotium index of the new tubers. Combined with inoculation of the seed tubers with *V. biguttatum* (M73), however, it gave comparatively the best results. A reduction of the disease index and of the percentage stolon pieces with living *R. solani* and an increase in the percentage

stolon pieces with *V. biguttatum* and a denser population of the hyperparasite thereon, and ultimately a reduction of the sclerotium index was achieved. On one field this reduction amounted to 70%; on another it was 50%. The tubers of these experiments were harvested three weeks after chemical desiccation of leaves and stems, conditions leading to maximum development of sclerotia.

Tubers of plants dying off naturally had low sclerotium indices. The combination of *V. biguttatum* and Monceren reduced them further.

L. C. DAVIDSE and M. BOEKELOO (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 6709 PD Wageningen*)

Elicitation and suppression of necrosis in potato leaves by culture filtrate components of Phytophthora infestans (Mont.) de Bary

Phytophtora infestans (Mont.) de Bary, when grown in liquid synthetic medium (HENNIGER, 1959), produces substances, which upon injection into the intercellular spaces of potato leaflets cause necrosis of the tissue, and hence may be called toxins. The toxins elute in void volume fractions of Sephadex G-25 columns, are heat-stable and sensitive to treatment with periodate, pronase and NaOH. Quantities as low as 5 μg of the high-molecular weight material per injection site are able to induce necrosis. Toxins from race 1, 4 and 3.4.7 have similar effects on potato cultivars with R_1 and R_3 as major genes for resistance. Toxin sensitivity of cultivars with non-specific resistance to *P. infestans* increases with the level of resistance. Gel filtration of dialysed and freeze-dried culture filtrates on Sephadex G-100 columns with water as eluent yields a number of active fractions including the void volume fraction. Fractions eluting at the column volume are inactive. When active fractions are diluted with the latter fractions in a 1:20 to 1:200 ratio, the combined fractions are less active or inactive in inducing necrosis whereas dilutions of the active fractions with water in the same ratios are highly active. This indicates that the action of the toxins can be suppressed by other components of the culture filtrate. The suppressive effect was observed with preparations of race 1 and 4 on cultivars carrying the major genes R_1 and R_3, respectively, indicating the absence of race or cultivar specificity. Toxins are also present in infiltration fluids from diseased leaves but not in fluids from healthy leaves or leaves inoculated with an avirulent race. The ability to induce necrosis is independent of the compatible combination of cultivar and race which had been used to raise the infiltration fluid or the cultivar being used in the bioassay. Our data are compatible with the idea that necrosis within the late blight lesions is caused by the action of toxins and that by suppression of their action which is assumed to occur along the edge of the lesion, the fungus is able to delay cell necrosis which otherwise would lead to limitation of biotrophic growth. In our model varying levels of non-specific resistance in potato cultivars to *P. infestans* are based on differential levels of sensitivity to the toxin. No evidence was found for a role of the toxins or suppressors in race-specific resistance of potato to *P. infestans*. Definite conclusions can only be drawn when toxins and suppressors will have been purified to homogeneity and their biological activities in potato cultivars differing in race-specific and non-specific resistance to *P. infestans* are known.

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P. J. G. M. DE WIT*, J. E. HOFMAN*, G. C. M. VELTHUIS* and J. A. KUĆ** (* *Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 6709 PD Wageningen, Nederland;* ** *Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, 40506, U.S.A.*)

Isolation, partial characterization and specificity of a necrosis-inducing protein produced by Cladosporium fulvum in vivo[1]

Specific elicitors of chlorosis and necrosis were obtained from intercellular fluids of compatible race-cultivar interactions of *Cladosporium fulvum* and tomato. The elicitors specifically induced chlorosis and necrosis in resistant but not in susceptible plants. The specificity of the elicitors was not determined by the gene(s) for resistance present in the susceptible cultivar from which the intercellular fluids were obtained but by the race of the invading fungus, i.e. by the virulence gene present in the race. The fungus is most probably the producer of the specific elicitors.

Using the tomato cultivar Sonatine (resistant to all races of *C. fulvum* except race 2.4.5.9) for assaying the necrosis induction we isolated and partially characterized an elicitor occurring in the intercellular fluids. The elicitor of necrosis was heat-stable (10 min. 100 °C), did bind to CM-Sephadex, but not to DEAE-Sephadex. The elicitor of necrosis was sensitive to Pronase and Protease but not to other proteases like α-chymotrypsin and trypsin. When the partially purified elicitor was electrophoresed under low pH-conditions on 15% acrylamide gels, necrosis-inducing activity was associated with a fast moving protein band. The necrosis-inducing activity could be eluted from the gel after gel-slicing. The specificity of this basic necrosis-inducing protein has been discussed.

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J. P. M. KOUWENHOVEN, I. M. J. TOMA, A. FUCHS and P. J. G. M. DE WIT (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 6709 PD Wageningen)*

Induced systemic resistance in tobacco, cv. Samsun NN

In a previous investigation (MOL et al. 1983) it was shown that induced systemic resistance in tobacco, cv. Samsun NN, was only achieved with TMV against TMV. With all other combinations of inducer and challenge agents tested, including *Pseudomonas syringae, P. tabaci*, TMV, and $HgCl_2$, no systemic resistance was observed. We have extended these previous experiments to include some other *Pseudomonas* species (*P. lachrymans*) and strains (three strains of *P. tabaci*), two fungi (*Cercospora nicotianae* and *Thielaviopsis basicola*) and two elicitors isolated from *Phytophthora infestans* (arachidonic acid, eicosapentaenoic acid; BOSTOCK et al. 1981). In all these instances, TMV was used as the challenge agent. Only in two cases (one strain of *P. tabaci* and eicosapentaenoic acid) slight protection against TMV was obtained.

Induction of systemic resistance is assumed to be mediated by (a) signal (s) (cf. SEQUEIRA 1983), the chemical nature of which, however, remains elusive. Time-course experiments, in which the time interval between induction of lower leaves and challenge of upper leaves, both with TMV, was varied from 1 to 10 days, showed the systemic resistance in upper leaves to be expressed 1-2 days after the inducing inoculation. The best results were obtained with an experimental set-up in which not the induced leaves but the challenged leaves were of the same age, thus eliminating the intrinsic effect of leaf age on TMV lesion size. Reduction of lesion size in upper leaves due to prior inoculation of lower leaves increased from 43% using a 2-day interval to 75% with a 10-day interval. Whether the signal is persistent in the upper leaves for a longer period or has to be produced continuously in the lower leaves awaits further investigation.

As a result of inoculation or treatment with elicitors, in tobacco leaves fungitoxic sesquiterpenoid stress compounds are formed. Among the sesquiterpenes isolated so far from TMV-inoculated leaves of cv. Samsun NN were capsidiol, solavetivone, 3-hydroxysolavetivone, 3-hydroxylubimin, rishitin, epirishitin, glutinosone and 5-hydroxyglutinosone plus two isomers with elemental composition of $C_{15}H_{26}O_2$ (M.W. 238) (FUCHS et al. 1983). Since then, small amounts of four other sesquiterpenes, namely a third isomer with M.W. 238, lubimin, phytuberol and 2-keto-α-cyperone, have been isolated; of these, phytuberol was present in amounts too low to be recovered in the TLC-bioassay (HOMANS & FUCHS 1970) with *Cladosporium cucumerinum* being used for the detection of fungitoxic sesquiterpenes (cf. FUCHS et al., 1983). In general, sesquiterpene formation reflected symptom expression: the more and larger the lesions, the greater the amounts of sesquiterpenes being formed.

Time-course experiments, in which the production of sesquiterpenes was followed for up to 13 days after TMV-inoculation of the upper leaves of TMV-induced and control plants, showed that sesquiterpenes appeared at the same time (2-3 days after inoculation), irrespective of whether the lower leaves were inoculated or not. In both instances, sesquiterpenes increased to a distinct maximum at 4 days after inoculation, and decreased again afterwards. This suggests that the lag-time of sesquiterpene synthesis in upper leaves is independent of the presence of a possible signal substance. Parallel with decreased symptom expression (less and smaller lesions), overall-levels of sesquiterpenes were much lower (less than 10%) in the challenged upper leaves of TMV-induced plants.

Thus, systemic resistance seems to be associated neither with a decreased lag-time nor an increased rate of sesquiterpene synthesis.

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E. N. FLACH, R. M. J. KRUTZEN and A. FUCHS (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 7609 PD Wageningen*)

Induced systemic resistance in tobacco, cv. White Burley

Previous investigations on induced systemic resistance in tobacco and the possible role of sesquiterpenes in it have been confined to one cultivar, Samsun NN (FUCHS et al. 1983; MOL et al. 1983; KOUWENHOVEN et al. 1984). Since then, we have extended our investigations to include White Burley, a cultivar which is of considerable economic importance. Concomitantly, we studied the occurrence and possible role of sesquiterpenoid stress compounds.

Preliminary experiments showed inoculation with TMW and treatment with arachidonic acid to cause neither necrosis nor sesquiterpene formation, whereas symptoms elicited by *Cercospora nicotianae* and ethrel were only mild, with little or no sesquiterpenes being formed. TNV, *Pseudomonas lachrymans* and *Thielaviopsis basicola*, on the other hand, all caused severe necrosis together with formation of small (*P. lachrymans*) to large (TNV, *Th. basicola*) amounts of sesquiterpenes.

Induced systemic resistance was therefore examined using TNV, *P. lachrymans* and *Th. basicola*. Inducing and challenge inoculations of lower and upper leaves were carried out as described by KOUWENHOVEN et al. (1984), with all nine combinations of virus, bacterium and fungus. Both TNV and *Th. basicola* offered good protection against challenge inoculation with *Th. basicola*, whereas slight protection was found with TNV against TNV and *P. lachrymans*, with *P. lachrymans* against *P. lachrymans* and with *Th. basicola* against TNV. With the other combinations no systemic resistance was obtained. When protection was insignificant and thus lesions strongly developed, there was extensive production of sesquiterpenes of the 5-hydroxyglutinosone, capsidiol and phytuberol pathways (cf. MASAMUNE et al. 1978; MOL et al. 1983) in the case of TNV and *Th. basicola*; with *P. lachrymans* as the challenge organism, however, only sesquiterpenes of the first two pathways were formed and these only to a small extent. Protection, on the other hand, was revealed by suppression of necrotic lesions, and was accompanied with the formation of (still) smaller amounts of sesquiterpenes.

In conclusion, as in Samsun NN (KOUWENHOVEN et al. 1984) systemic resistance could be induced in the cultivar White Burley in several, but not all, combinations of inducing and challenging agents. Sesquiterpenoid stress compounds did not seem to be causally related with the systemic resistance observed.

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R. VANTOMME, M. GOOR, I. MERCHIE, M. VANDENBERGHE, A. OVERSTIJNS*, J. SWINGS, K. KERSTERS and J. DE LEY (*Onderzoekscentrum voor Fytobacteriosen, Sektie II, I.W.O.N.L., Laboratorium voor Microbiologie en microbiële Genetica, RUG, Gent, Belgium; * Voorlichtingscentrum voor Land- en Tuinbouw, Beitem-Rumbeke, Belgium*)

Pseudomonas species isolated from cultivated mushrooms

Pseudomonas tolaasii is generally known as the causal agent of brown blotch on cultivated mushrooms. In this study we have compared twenty-eight bacterial strains isolated from cultivated *Agaricus bisporus* and *Pleurotus ostreatus*, showing typical brown blotch symptoms, together with the following 35 culture collection strains isolated from mushrooms: *Pseudomonas tolaasii* (13); *Ps. agarici* (9); *Ps. gingeri* (4); *Ps. reactens* (3); *Ps.* species causing mummy disease (3), and *Ps. aeruginosa* (3). Other *Pseudomonas* strains, not isolated from mushrooms, i.e. *Ps. fluorescens* (4), *Ps. aeruginosa* (1) were also included.

The pathogenic capacity of each strain was tested. Eight *Ps. tolaasii* strains from the NCPPB, the strain *Ps. aeruginosa* NCPPB 2197 and ten of our own isolates caused chocolate-brown blotches on mushroom caps. Slide agglutinations were performed with an antiserum prepared against the type strain of *Ps. tolaasii* NCPPB 2192. All strains causing brown blotch, except NCPPB 2197, were positive. The white line test proposed by Wong and Preece for the identification of *Ps. tolaasii* was applied: the 3 *Ps. reactens* strains, 12 of our isolates and *Ps. tolaasii* NCPPB 1311 produced white lines with pathogenic *Ps. tolaasii* strains.

The numerical analysis of 275 biochemical, serological and phytopathological data on 68 strains showed that seven phenons could be clearly distinguished. Phenons I, II and III correspond respectively to *Ps. aeruginosa, Ps. fluorescens* and *Ps. gingeri*. Phenon IV contained 11 *Ps. tolaasii* strains and 16 own isolates and phenon V comprised all the strains reacting in the white line test with *Ps. tolaasii* NCPPB 2192. Phenons VI and VII comprised respectively 2 strains causing mummy disease, and 8 *Ps. agarici* strains. This analysis showed that phenons III, IV and V are phenotypically closely related, wherease phenons I, II, IV and VII are not.

In order to determine the resistance against bacterial brown blotch, two highly virulent *Ps. tolaasii* strains were inoculated on 13 different mushroom races from *Agaricus bisporus* (12) and *Psalliota edulis* (1). All commercially available white mushroom races were very susceptible.

A. F. L. M. DERKS and J. L. VINK-VAN DEN ABEELE (*Laboratorium voor Bloembollenonderzoek, Vennestraat 22, 2160 AB Lisse*)

Identification of viruses with elongated particles in bulbous iris

In The Netherlands four viruses with elongated particles have been described in bulbous iris, viz. iris mild mosaic viris (IMMV), iris severe mosaic virus (ISMV), iris mild yellow mosaic virus (IMYMV) (all three: ASJES 1979), and bean yellow mosaic virus (BYMV; DERKS et al. 1980).

All cultivars grown commercially in The Netherlands were invariably found to be infected with IMMV, which made it difficult to separate and identify the viruses. This was further complicated by the variable reactions of the test plants after inoculation with the various isolates of the same virus.

Virus isolates and antisera were exchanged with A. Brunt (Littlehampton, U.K.; BRUNT & PHILLIPS 1980) to explain the differences between his and our results. The following conclusions could be made:

- IMMV is similar to the iris severe mosaic virus of Brunt
- ISMV is serologically related to his 'potyvirus II' isolated from *Iris* cv. Professor Blaauw.
- IMYMV is probably the same as narcissus latent virus
- there is no confusion about BYMV
- the iris mild mosaic virus of Brunt has not yet been detected in The Netherlands.

After mechanical inoculation of virus-free *Iris* cv. Professor Blaauw with IMMV, the same symptoms were observed as those described by Asjes (1979). ISMV and IMYMV alone caused the same type of symptoms in *Iris reticulata* as the combination of the respective viruses with IMMV in *Iris* cv. Professor Blaauw.

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A. R. VAN SCHADEWIJK and J. EGGINK (*Bloembollenkeuringsdienst, Postbus 300, 2160 AH Lisse)*

Detection of tulip breaking virus (TBV) in tulips by means of ELISA

Inspection of plants on viruses is one of the most time-consuming tasks of the Dutch Bulb Inspection Service. Due to a new quality program, this task will extend considerably in the near future. For both economical and practical reasons, efforts are made to replace large parts of the visual field inspections by serological tests for the economically most important viruses.

For a good detection of TBV in tulip bulbs by means of ELISA (CLARK & ADAMS 1977), several adaptations are necessary. Although the TBV-antisera are of high quality (DERKS et al. 1982), high background absorbances due to tulip bulb substances occur. These can be reduced by increasing the Tween-20 concentration in bulb extracts to 1.0%. Addition of 1% normal horse serum and 0.3% Tween-20 to the conjugate solution prevents non-specific attachment of the conjugate.

The ELISA-absorbance values will be reduced by incubating plant extracts at a pH lower than 7.0. Therefore, an extraction buffer of 0.1 M PBS, pH 7.4 is necessary.

In most cases, ELISA-absorbance values obtained with extracts of TBV-infected tulips are suboptimal. This can be explained by the presence of clusters of TBV particles bound to plant material, as observed with the electron microscope. Enzymes are capable of releasing virus particles from plant material, as has been demonstrated for lily symptomless virus in lilies (BEIJERSBERGEN & VAN DER HULST 1980). The absorbance values increase strongly when cellulase, hemicellulase or pectinase is added to tulip bulb extracts. Also, an additional increase can be obtained with 0.1% Tween-20 in the bulb extract. In tulip leaves, TBV can also be released from clusters by heat treatment. A 30 minutes incubation of leaf sections in water at 50°C (VAN SLOGTEREN & DE VOS, 1966) can result in a 20-fold increase of the absorbance values. This effect varies with the physiological condition of the plants and between cultivars.

In general, primary infections with TBV are not detectable in leaves of tulips, but are in newly formed bulbs of the same plants. TBV remains detectable in tulip bulbs during at least 9 months of dry storage at 17–20°C.

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D. PETERS and J. ELDERSON (*Laboratorium voor Virologie, Landbouwhogeschool, Binnenhaven 11, Postbus 8045, 6700 EM Wageningen)*

Some observations on the mechanism of the persistent virus transmission – a study with potato leafroll virus

Some plant viruses are efficiently transmitted in long inoculation feeding periods after a long acquisition period. Between acquisition and inoculation an incubation period occurs in which the virus

circulates through its vector. The mechanism of this type of transmission, designated persistent or circulative, is only partly understood. The long acquisition and inoculation feeding periods used in experiments can obscure some aspects which may help to elucidate the mechanisms involved in this transmission.

This report summarizes some results of studies on the moment at which the plant is inoculated during the inoculation feeding period, the median effective acquisition access period, and the availability of virus for acquisition in plants showing different degrees of symptoms.

Aphids, *Myzus persicae*, which had acquired virus in a period of five or six days were used to test the length of the median inoculation period. In three experiments this period had a length of 45 to 95 min. These results are interpreted such that the aphid inoculates the plant at the moment at which the stylets penetrate the phloem. Retraction of the stylets and re-penetration will give the aphid a new opportunity to infect a plant.

Groups of aphids which could acquire virus in periods of different lengths were tested for their infectivity. It appeared that the aphids were able to acquire so much virus in 12 h that 50% infected a plant in the subsequent feeding period of five days.

It is also obvious from our experiments that the virulence of the aphids varied from aphid to aphid and from group to group. This variation is perhaps partly determined by the amount of virus that can be acquired from the source. In one type of experiment aphids could acquire virus from plants which were infected with different grades of intensity. After 24 h of acquisition the LP50 was determined. Aphids which had acquired virus from plants which were infected by one aphid in two h had a shorter latent period than those aphids which had fed on plants which were infected by five aphids in 24 h and showed, therefore, more serious symptoms. It was concluded from these results that more virus is available for acquisition in plants which have been infected with a low intensity and were less effected by the infection.

We conclude from our experiments that the transmission of persistent virus has to be explained more in terms of stylet penetration into the phloem and availability of virus than in use of long acquisition and feeding periods.

W. T. M. SMITS (*Laboratorium voor Fytopathologie, Binnenhaven 9, 6709 PD Wageningen*)

The horizontally perforated soil system used for mycorrhizal studies of dipterocarps

For experiments with *Anisoptera marginata* (Dipterocarpaceae) it appeared that dipterocarps are obligately ectomycorrhizal and that this relation may be specific. Besides uptake of nutrients the mycorrhizal fungus seems to produce thiamin for the dipterocarp tree. This obligate ectomycorrhizal relationship can give an explanation for many features of dipterocarps in primary as well as logged-over forest.

The horizontally perforated soil system is a non-destructive root observation method that can be useful for mycorrhiza research. By means of an intrascoop VAM as well as ectomycorrhizas can be seen up to 150 × enlarged on the roots in the soil. Samples of roots without attached soil particles can be taken from single roots without disturbing the soil or the other roots in the same hole. Root and mycelium growth can be followed in space and time. There is the possibility of inoculating roots on chosen positions for experimental purposes with very little material of fungal cultures.

The method can also be used with soil samples taken from the field in their original condition.

P. H. J. F. VAN DEN BOOGERT (*Instituut voor bodemvruchtbaarheid, Postbus 30003, 9750 RA Haren (Groningen)*)

Colonisation of subterranian parts of crops by Verticillium biguttatum

MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND -ANATOMY ON 4 NOVEMBER 1983

A. C. VAN AELST and H. J. WILMS (*Vakgroep Plantencytologie en -morfologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)

Pollen tube growth and structure of Impatiens in vitro

In this study the effect of monosaccharides and oligosaccharides on growth rate, ultimate length and ultrastructure of the tube tip of *Impatiens walleriana* Hook f. pollen tubes have been investigated. Both growth rate and ultimate length become submaximal with sugar concentrations higher than 0,5 M.

The results indicate that the pollen tubes in these media become isotonic after a certain time of growth, after which growth ceases. At the same time plasma streaming in the pollen tube stops, while the cytoplasmic zonation and polarity are maintained.

After cessation of the pollen tube growth, there is thickening of the wall at the tube tip, indicating that pollen wall formation continues.

Media with high monosaccharide concentration result in thickening of the wall at the tube tip after cessation of pollen tube growth. Similar oligosaccharide concentrations result in thickening of the wall at the tube tip, as well as at the adjacent part of the lateral wall.

R. J. BINO (*Vakgroep Plantencytologie en -morfologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)

Ultrastructual analysis of cytoplasmic male sterility in Petunia hybridia var. Blue Bedder

Cytoplasmic male sterility (CMS) is a common form of male sterility and is inherited via the cytoplasm of the mother plant. The trait has important application in plant breeding, particularly in the production of commercial hybrids.

In the present study microsporogenesis is compared in a sterile and a fertile strain of *Petunia hybrida*, Blue Bedder Sterile (BBS) and Blue Bedder Fertile (BBF), respectively.

The differences in the microsporogenesis are found to occur in the leptotene stage of profase I. During this stage the tapetum of the BBF in general becomes binucleate. In contrast to this normal condition, the tapetum of BBS starts to degenerate, a process expressed by an increase of the number of ribosomes, a deformation of the nucleus and the lysis of mitochondria and plastids.

In many cases further development of the microspores is arrested and degeneration has started. Sometimes microsporogenesis continues until the beginning of meiosis II. Later in anther development the aborted mass consists of crushed microspore cells encased in callose and degenerated tapetum cells.

R. BAKHUIZEN, L. GOOSEN-DE ROO and P. C. VAN SPRONSEN (*Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden*)

Cytokinesis in fusiform cambial cells of Fraxinus excelsior L: comparison to tip growth

In the elongated highly vacuolated fusiform cambial cells of *Fraxinus excelsior*, the ash, the phragmoplast appears to be surrounded by a relatively thick layer of cytoplasm which mainly contains dictyosomes and mitochondria. Initially the phragmoplast with surrounding cytoplasm is ring-shaped, later on it divides into two halves each of which migrates to a cell tip within a thin layer of transvacuolar cytoplasm coated by the tonoplast. This layer was found in the future plane of cell division and is called phragmosome. Phragmosomes traverse the whole cell during preprophase

and karyokinesis and persist during cytokinesis until they are integrated in cell plate with adjacent cytoplasm (GOOSEN-DE ROO et al., submitted).

The layer of cytoplasm surrounding the phragmoplast differs from normal parietal cytoplasm by a conspicuous zonation pattern of cell organelles. Its apical region hardly contains any larger cell organelles; underneath is a zone with many mitochondria interspersed with some dictyosomes. The lowest zone at first mainly contains dictyosomes; later on the distribution of cell organelles is as in parietal cytoplasm.

This zonation of mitochondria and dictyosomes in the phragmoplast surrouding cytoplasm resembles the zonation pattern found in cells with tip growth, namely in root hairs and pollen tubes.

This similarity in zonation suggests that: a. cell plate formation in fusiform cells is comparable to tip growth in other elongated cells; b. cell plate formation involves also the whole zone of specialized cytoplasm which surrounds the phragmoplast.

GOOSEN-DE ROO, L., R. BAKHUIZEN, P. C. VAN SPRONSEN & K. R. LIBBENGA (1983): The presence of extended phragmosomes containing cytoskeletal elements in fusiform cambial cells of Fraxinus excelsior L. (submitted).

H. T. H. M. MEEKES (*Botanisch Laboratorium, Toernooiveld, 6525 ED Nijmegen*)

Differentiation of root hair cells of Ceratopteris thalictroides (L.) Brongn.

Young isodiametric undifferentiated rhizodermal cells stretch longitudinally up to ten times their width, to become either atrichoblasts or trichoblasts.The trichoblasts (root hair cells) can be distinguished from the atrichoblasts by their vacuole content after fixation with glutaraldehyde or potassium permanganate for electron microscopy (an electron dense material is visible). This feature offers the possibility to examine root hair cells at a stage preceeding swelling, which is the first stage of root hair emergence. No structures or processes were observed that preceeded or indicated the site of swelling. The site of swelling lies at about 0.28 of the total cell length from the apical end of the trichoblast. During this stage the nucleus lies almost exactly in the middle of the cell. Therefore the observation by other workers (POTAPOV & FILIPPENKO 1976) that the swelling occurs in the vicinity of the nucleus is not confirmed for this species. After the swelling has occurred the nucleus migrates to the root hair base, invades the root hair tube and follows the growing tip of the root hair base, invades the root hair tube and follows the growing tip of the root hair with a somewhat increasing distance. The inner surface of the swelling is covered by an extracellular membraneous system which might function in enzymatic weakening of the cell wall to enable root hair outgrowth.

In the nuclei of all root cells (including root hair cells) crystalloids were observed. They might be protein, but their function is unknown. The crystalloids seem to originate from globoid structures in the nucleus.

POTAPOV, N. G. & V. N. FILIPPENKO (1976): Formation of root hairs by the wheat Triticum vulgare Host. trichoblasts. *Acta biol. Acad. Sci. Hung.* 27(2–3): 81–91.

J. A. TRAAS (*Botanisch laboratorium, Toernooiveld, 6525 ED Nijmegen*)

The membrane bound cytoskeleton of plant cells.

The membrane bound cytoskeleton of plant cells can be studied by means of a new technique called dry cleaving (MOSLAND 1981; TRAAS 1983).

Young, growing roots of different plant species were fixed in a mixture of 1% GA, 0,2% tannin and 5 mM EGTA in NaCacodylate (0,1 M, pH 7,1). In order to loosen the connections between the cells, the fixed material was subsequently treated with Driselase (1–5%) and EGTA (10 nM). Following this treatment, rows or bundles of epidermal and cortical cells could be torn from the roots. These were attached to poly-L-lysine coated grids, post fixed in OsO_4 (1%) and positively stained with uranyl acetate (1%). After dehydration in an ethanol series, the material was critical point dried. Finally the cells were cleaved by inverting the grids on adhesive tape and removing them with a forceps.

After cleavage only the cell wall, the plasma membrane and the cytoplasmic structures near the membrane remained on the grids. The preparations showed a three dimensional cytoskeletal network of 5–10 nm filaments and microtubules, resembling the membrane bound cytoskeleton of animal cells. In addition many coated pits and vesicles could be observed on the membrane. The first results obtained with this method indicate, that length, direction and density of the membrane associated microtubules change significantly during the development of cortical root cells.

MOSLAND, D. A. M., H. SPIELE & E. ROOS (1981): Membrane associated cytoskeleton and coated vesicles in cultured hepatocytes visualized by dry cleaving. *Exp. Cell Res.* **132**: 169–184.

TRAAS, J. A. (1983): Visualization of the membrane bound cytoskeleton and coated pits of plant cells by dry cleaving. *Protoplasma*, in press.

ANNE MIE C. EMONS (*Botanisch Laboratorium, Toernooiveld, 6525 ED Nijmegen*)

Particle rosettes in the plasma membrane of root hairs of Equisetum hyemale

Apart from randomly distributed particles, particle rosettes, consisting of six intramembranous particles arranged in a rosette, are visible in the protoplasmic fracture face (PF) of the plasma membrane of *Equisetum hyemale* root hairs by means of the double replica freeze-fracture technique. Rosettes measure 26 ± 5 nm in diameter from the outer edges of particles on opposite sides. These complexes are hypothesized to play a role in the synthesis of the cellulose microfibrils, the structural wall component of these hairs. In *Equisetum hyemale* root hairs the particle rosettes do not form hexagonal patterns nor rows or strings or any other known pattern.

Plasmolysis often occurs during handling of the hairs, as is apparent from a layer of amorphous ice between the cell wall and the plasma membrane and a corresponding absence of microfibril imprints on the extraplasmic fracture face (EF) of the plasma membrane. On the PF face of the plasma membrane of plasmolysed cells no particle rosettes are present.

Even a slight fixation with glutaraldehyde causes the microfibril imprints to disappear together with almost all of the particle rosettes, an observation which might explain the absence of particle rosettes in many studies on plant cell plasma membranes.

C. J. VENVERLOO, L. GOOSEN-DE ROO and P. C. VAN SPRONSEN (*Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden*)

The influence of 2,6-dichlorobenzonitrile and colchicine on cell division in epidermis cells of Nautilocalyx explants

Mitosis in the highly vacuolated epidermis cells of leaf explants of *Nautilocalyx* is preceded by the appearance of a phragmosome (PS) and a band of microtubules (BMT). Both the PS and the BMT have been found to give an early indication of the plane of cell division and the exact place were the new cell wall will meet the parental cell walls.

We investigated light microscopically (Nomarski) to what extent the formation of PS and BMT was influenced by 2,6-dichlorobenzonitrile, a substance known to interfere with cell plate formation, and by colchicine which interferes with microtubules.

Results showed that 2,6-dichlorobenzonitrile interfered with the termination of cell plate formation, this producing binucleate cells with a partial cell plate or without a cell plate. But this substance did not change the formation of the PS or BMT or the plane of cell division.

Colchicine given in a high concentration (125 μM) for a short period of time or in a low concentration (5–25 μM) for a longer period inhibited the migration of the nucleus to the centre of the cell as well as the formation of a premitotic PS. These inhibitions as well as the inhibition of mitosis could be reversed by a short illumination (366 nm). Normal mitoses were found directly after illumination. The plane of cell division was slightly changed as a result of the premitotic colchicine treatment.

These results suggest that microtubules are involved in the nuclear migration as well as in PS formation, and that the absence of microtubules may lead to a change in the plane of cell division.

W. L. A. HETTERSCHEID (*Vakgroep Plantensystematiek en -geografie, Postbus 80.102, 3508 TC Utrecht*)

Venation-pattern analysis, soral characters, and their bearing on the systematics of microsorioid Polypodiaceae.

The systematics of the fern family Polypodiaceae is hampered by a multitude of views regarding the delimitation of especially its genera. Traditionally these genera are based on one or a few striking characters, which at the time have not been considered as possibly the result of parallel development. It is argued that a cladistic analysis of available characters provides a powerful tool in tracing natural groups as well as parallel developments.

A well-known example is the occurrence of acrostichoidy in leptosporangiate ferns. Based on this character a great number of species has been lumped in the genus *Leptochilus*. Subesequent research proved the genus to be a highly unnatural assemblage. At present the greater part of its species is accomodated in a number of different genera outside the Polypodiaceae. The remaining species of *Leptochilus* are Polypodiaceae, and have been attributed to the genera *Christiopteris, Dendroglossa, Leptochilus s. str.*, and *Paraleptochilus*.

A preliminary cladistic analysis of the types of venation patterns occurring in these genera, suggests the latter three genera to be intimately related to each other, and as a group, related to the microsorioid genus *Colysis*. If true, the acrostichoid condition and strong sterile-fertile dimorphism of this genera-group, arose independently from the same conditions in *Christiopteris*, providing a clear case of parallel development.

Further research was concentrated on the question how the acrostichoid and dimorphic conditions could have developed, starting from sorus- and frond-shape as present in *Colysis*. It appeared possible to construct a complete transformation series from c. round sori on fronds isomorphic with sterile ones, to c. completely acrostichoid fronds, very differently shaped from the sterile ones. A number of *Colysis* species represent different stages of this transformation. Also, atavistic fronds in species of the normally fully acrostichoid and dimorphic genera *Dendroglossa, Leptochilus*, and *Paraleptochilus*, show the transition to the conditions in *Colysis*. It is noteworthy that the fertile fronds of *Colysis hemionitidea* (Wall. ex Mett.) Presl, proved to be very plastic relative to the conditions mentioned, covering the complete transformation as obtained from the study of all *Colysis* species and the three acrostichoid genera.

A cladogram is selected, depicting relative phylogenetic relationships amongst species of the three acrostichoid genera and *Colysis*, suggesting a monophyletic origin of the acrostichoid genera. Starting from the monophyly of these three genera, the next higher-level monophyletic group includes all three genera and part of *Colysis*. In order to maintain strictly monophyletic (natural!) genera, we will have to merge the species of *Dendroglossa, Leptochilus s. str.* (but see below!), and *Paraleptochilus* in *Colysis*. Keeping them separate as genera or as one genus, would render *Colysis* a paraphyletic group. It is admitted that the origin of acrostichoidy and sterile-fertile dimorphism in *Leptochilus axillaris* (Cav.)Kaulf., the type-species of *Leptochilus*, is still not completely solved, and thus may represent a third parallel transformation to these conditions within the microsorioid Polypodiaceae.

M. T. M. BOSMAN (*Rijksherbarium, Postbus 9514, 2300 RA Leiden*)

Some effects of decay and weathering on the anatomical structure of the stem of Phragmites australis Trin. ex Steud.

An anatomical study on factors influencing the durability of thatching reed was made in co-operation with the Department of Building Technology of the Technological University in Delft. Samples were taken from stems already used for thatching and from recently harvested stems and studied using light and scanning electron microscopy.

Biological degradation probably starts with bacterial attack, causing a discolouration and detachment of the inner cell wall layers in some sclerenchyma and lignified parenchyma cells. Towards the exposed basal part of the stems fungi cause a considerable loss of cell wall constituents. Morphometric determinations show a more or less gradual decrease resulting in more than fifty per cent

loss at the base of the stem. In sclerenchyma and parenchyma tissues this effect is visible as diamond shaped cavities, spirally arranged in the S2-layers of the cell walls (following the micro-fibrillar structure). These cavities strongly resemble those brought about by soft-rot fungi in wet timber (WILCOX 1970). The subepidermal layers of sclerenchyma, as well as tissues surrounding the air-cavities hardly suffer from this form of decay (partly in contrast to suggestions by STANT, 1953). At the exposed parts of the stem superficial weathering by ultra-violet radiation causes oxidation of lignin (cf. CHANG et al. 1982). Thus the middle lamella region desintegrates and the outer cell-layers peel off, forming a suitable substrate for green algae.

Futher research on the causes of resistance against soft-rot fungi in the subepidermal sclerenchyma and on swelling properties (in relation to soft-rot demands) of various kinds of reed, is necessary to establish a reliable criterion for testing the quality of thatching reeds.

CHANG, S. T. et al. (1982): Photodegradation and photoprotection of wood surfaces. *Wood and Fiber* **14**: 104–113.

STANT, M. Y. (1953): Variations in reed structure in relation to thatching. *Kew Bull.* **2**: 231–239.

WILCOX, W. W. (1970): Anatomical changes in wood cell walls attacked by fungi and bacteria. *Bot. Rev.* **36**: 1–28.

W. VERKERKE (*Hugo de Vries Laboratorium, Plantage Middenlaan 2a, 1018 DD Amsterdam*)

Reduction of the seed coat in Xanthophyllum and other Polygalaceae.

The seed coat development of *Xanthophyllum* Roxb. is described. On account of a different post-fertilization development, the variation in seed coat morphology is considerable. The seed coat is either thin and strongly adherent to the endocarp, or provided with a hard endotesta and a thick mesophyll layer. Within the Polygalaceae several genera have indehiscent fruits containing seeds with complex seed coats, but in other genera the endotesta is reduced. This reduction is illustrated by *Carpolobia alba* G. Don and *Monnina wrightii* A. Gray in which the endotesta is disrupted by the post-fertilization growth of the ovule. Vestigial endotestas can be homologized with well-developed ones. This facilitates the interpretation of vestigial seed coats in other families.

C. J. KEIJZER (*Vakgroep Plantencytologie en -morfologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)

Pollen dispersal and the function of the orbicules

An investigation of the anther development of *Gasteria verrucosa* revealed some processes that presumably have a function in pollen dispersal.

The locule wall of *Gasteria* consists of four cell layers: the exothecium, the endothecium, one middle layer and the tapetum. By means of electron microscopy the following observations were made.

During meiosis the orinigal pectin-cellulose walls of the tapetum and the meiocytes disappear completely. Moreover, in the later stages of development the walls of the middle layer and the endothecium partly dissolve. On the contrary, sporopollenine structures appear i.e. the pollen walls on one hand and the tapetal membranes with attached orbicules on the other. After all these changes the border of the locule wall versus the pollen grains finally consists of the hydrophobic substances sporopollenine and pollenkit instead of the original hydrophylic substances.

These findings support HESLOP-HARRISONS (1968) suggestion that the function of the orbicules is the formation of a non-wettable surface from which the pollen grains may be easily removed by their vectors. We tested this hypothesis as follows. Firstly we removed the pollen grains with most of the pollenkitt from a dehisced anther. Next the orbicules were removed by micromanipulation and the pollen grains were reattached to the thus created wettable surface. Given this situation the pollen grains could be easily removed by wetting the anther, wheras wetting an intact dehisced anther leads to the loss of just a small part of the pollen. In natural circumstances this means that the lack of orbicules leads to removal of the pollen grains by rain, being not the suitable pollen vector for these species with functional pollenkitt.

HESLOP-HARRISON, J. (1968): Pollen wall development. *Science* **161**: 230–237.

M. T. M. WILLEMSE (*Vakgroep Plantencytologie en -morfologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)

Light absorption of pollen grains

Absorption spectra between 380 to 750 nm wave-length of different pollen grains were measured and expressed in the absorption percentage of a constant volume of about $6 \times 10^3 \mu m^3$, containing cytoplasma and pollen wall.

Based on measurements of 15 different pollen grains three types of spectra can be distinguished. White pollen as *Digitalis* and *Delphinium* containing few carotenoids as pigment show a decreasing slope from 380 to 500 nm. Yellow and orange pollen as *Chrysanthemum* and *Potentilla*, containing carotenoids are characterized by a maximal absorption between 400 to 500 nm. Some yellow pollen as *Cedrus* and *Colchicum* show a nearly constant or a very slow decreasing absorption percentage over the whole spectrum. During pollen development, the microspores of *Gasteria, Lilium* and *Physostegia* show an increase in absorption just after tetrade stage. Thereafter the absorption percentage decreases but the shape of the absorption spectrum does not alter. This means that the absorption characteristics of the cytoplasm and the wall hardly change, only the absorption percentage diminishes by the change in the stretching pollen wall and the density of the cytoplasm in the measured volume. From measurements of empty pollen, pollen sacs of *Pinus* or male sterile pollen of *Impatiens* the pollen wall shows an absorption from 380 to 500 nm wave length. After chemical treatment of *Gasteria* pollen with ethanol the absorption percentage decreases, with chloroform the absorption spectrum of the pollen wall appears.

Because different pigments, as flavonoids with a maximum mostly between 200 to 300 nm wave length have not been detected and only some carotenoids with their maximum between about 360 to about 500 nm wave length in general are involved in these absorption spectra, the spectra show a general pattern. Nevertheless, each pollen has its own individual characteristic spectrum. The pollen wall absorption seems to take place from mainly 380 to 500 nm wave length, the cytoplasm mostly from 500 upto 750 nm wave length. The influence of the pollenkit seems to be almost negligible.

J. L. VAN WENT (*Vakgroep Plantencytologie en -morfologie, Arboretumlaan 4, 6703 BD Wageningen*)

Unequal distribution of plastids during generative cell formation in Impatiens

Plastids of angiosperm plant species are known to be inherited either biparental (through both male and female gametes) or uniparental, maternal. In the latter only the egg cell transfers plastids to the zygote, while the sperm cell does not transfer its plastids or does not contain them. Absence of plastids in sperm cells can result from various processes. In one of these, the so-called *Lycopersicon*-type, already the generative cell in the pollen grain (from which the sperm cells derives) does not contain plastids *ab-initio*.

In young microspores of *Impatiens walleriana* and *I. glandulifera* there is a gradual change in organization of the cytoplasm and distribution of the organelles. This includes the formation of vacuoles, the movement of the nucleus to a position near the microspore wall in the central part of the cell, and the accumulation of the plastids near the wall at the opposite side of the cell.

The generative cell is formed by an unequal division of the microspore, in that part of the cell in which no plastids are present. As a result the generative cell does not contain plastids *ab-initio*, and *Impatiens walleriana* and *I. glandulifera* have uniparental, maternal plastid inheritance. The absence of plastids in the generative cell is the result of a specific cell organization which is achieved before mitosis, and does not result from events during mitosis as e.g. mechanical exclusion of the plastids by the spindle fibres.

F. D. BOESEWINKEL (*Hugo de Vries-Laboratorium, Universiteit van Amsterdam, Plantage Middenlaan 2a, 1018 DD Amsterdam*)

Seed development and relations of Cneorum tricoccon

Published in:
F. D. BOESEWINKEL (1984): Development of ovule and seed coat in Cneorum tricoccon L. (Cneoraceae). *Acta Bot. Neerl.* 33: 61–70.

MEETING OF THE SECTION FOR THE PLANT TAXONOMY AND PHYTOGEOGRAPHY ON 18 NOVEMBER 1983

J. KUIPER (*Vakgroep Biosystematiek, de Boelelaan 1087, 1081 HV Amsterdam*)

An endophytic Chromastrum species (Rhodophyta, Acrochaetiaceae)

The life history of an endophytic *Chromastrum* species has been investigated, both in the field and in culture. Living plants were isolated from several substrates (mostly other Florideophyceae), collected at the west coast of France (Britany), England, Ireland and Scotland. In cultures all isolates grew well in the absence of any natural substrate. The plants were also able to penetrate 'natural' substrates offered in culture (e.g. rhizoids of *Polysiphonia urceolata*) especially when the cultures were shaken to simulate wave action.

Sexual reproductive structures (spermatangia and carpogonia) and tetrasporangia were found on different plants, which were morphologically similar. Chromosome counts indicated that the gametophytes were haploid, the tetrasporophytes diploid (2n = c. 22). The life history is of the triphasic, dimorphic type.

Both generations reproduced asexually by monospores. Spore germination patterns showed a remarkable variation, irrespective of the ploidy level: aseptate germination with or (as in most cases) without loss of contents of the original spore, or septate germination, resulting in a two-celled structure. This variation, probably due to the endophytism of this species, appears to be unique within *Chromastrum*: all other – epiphytic! – species studied thus far, showed an alternation of a gametophyte with a unicellular base (= original spore) and a tetrasporophyte with a septately dividing spore.

When the results obtained were compared with original species descriptions the impression was received that a number of species within the Acrochaetiaceae might be considered conspecific. The type material of several species will be studied in order to try to solve some taxonomical problems within the complex of endophytic *Chromastrum* species.

H. J. M. SIPMAN (*Botanischer Garten und Botanisches Museum, Königin-Luise-Strasse 6-8, D-1000 Berlin 33, B.R.D.*)

Distribution patterns in the lichen family Megalosporaceae

The lichen genus *Megalospora* used to be characterised by its large, bicellular ascospores, and its distribution was supposed to be mainly tropical with extensions into some temperate areas, mainly Australia and New Zealand. A revision by the author (*Biblioth. Lichenol.* **18**, 1983) yielded a number of new characters concerning anatomy, ascocarp ontogeny and chemistry. These necessitated a redefinition of the genus *Megalospora* and of the family Megalosporaceae. Consequently new distribution patterns became apparent. The centre of diversity, where all genera of this family and most of the variation in ascospore type occur, is situated in southeast Australia and in New Zealand. For the genus *Megalospora* itself the highest species numbers, of mostly endemics, occur in New Zealand, Australia and New Guinea, whereas New Caledonia and Africa equally show a remarkable endemism. In most of the tropics widespread species predominate.

The presence of many species with small distribution ranges and the very uniform ecology throughout the family suggest that it concerns a conservative group whose distribution may largely reflect the movements of the continental shields in the geological past. Therefore the following hypothesis on the evolution and dispersal of the Megalosporaceae has been made. Much of its present variation, i.c. all present genera and most spore types, seems to have developed during the mesozoic era on the north coast of eastern Gondwanaland. Subsequent northward movement of that shield and corresponding climatic changes have lead to a southward movement of Megalosporacea along the east coast into their present habitats in New Zealand and southeastern Australia. Part of the genus *Megalospora* apparently escaped the climatic change by adapting itself to tropical mountain habitats, and thus became able to spread through the tropics. This happened most probably in the late Cretaceous, when the main continental shields were still close to each other. The resulting

migration seems to have reached Africa first, where it led to the development of endemic species. Later it reached Central and South America, where the endemism is less pronounced. An even later migration seems to have happened along the north coast of the Thetis sea into North America and Japan, and has led to populations which are differentiated below species level. An important early Tertiary migration seems to have occurred from the east coast of Gondwanaland via the antarctic coast into southern South America, and a late Tertiary migration from New Guinea into Malaysia has probably created the Malaysian-New Guinean ranges of certain species.

W. GAMS (*Centraalbureau voor Schimmelcultures, Postbus 273, 3740 AG Baarn*)

Taxonomic work on the Hyphomycetes at the Centraalbureau voor Schimmelcultures

A general survey of taxonomic research on the Hyphomycetes was presented with an outline of basic ideas. Teleomorph-anamorph connections are the most important means of verifying a preliminary classification. Some problems with the approach were pointed out, for example several teleomorphs connected with indistinguishable anamorphs or vice-versa. In some cases the anamorph may present important clues to teleomorph classification. Particularly in morphologically little differentiated Hyphomycetes, chemical and DNA analyses may further improve the classification; however, a morphological classification must be built up first.

S. R. GRADSTEIN (*Instituut voor Systematische Plantkunde, Postbus 80.102, 3508 TC Utrecht*)

Transoceanic Hepaticae of Tropical America

In the early 19th century there was a common belief that bryophyte species distribution was often world-wide. Influenced by the evolution theory, the idea that bryophytes are normally restricted to single continents or smaller regions became more widely accepted and initially resulted in an enormous increase in the number of described species. Recent monographic studies have shown that this 'geographic species concept' was ill-founded and the true ranges of the species are now gradually becoming known. Thus, among the Hepaticae of Tropical America (c. 1000–1500 species in 150–200 genera, of which about 15% studied critically) an increasingly large number of widely distributed, transoceanic species is emerging from recent literature. Mostly these patterns result from new synonymy although recent floristic discoveries have expanded our knowledge too.

Transoceanic liverworts of Tropical America include species of tropical origin (lowland, montane and alpine element) or of temperate origin (mainly alpine): southern-subantarctic or northern-holarctic. In the lowland forests – where members of the large family Lejeuneaceae prevail – transoceanic species are more common than in the mossy forests of the montane zone, which probably relates to the relatively young age of the main mountain ranges (e.g. the Andes).

As to dispersability, it appears that transoceanic Lejeuneaceae are mainly autoecious and possess relatively large spores (40–60 μ), while most other transoceanic species have smaller spores, more suitable for air transport, but are predominantly dioecious (the more usual sexual condition in liverworts). Interpretation of liverwort dispersability is still hampered by lack of data on spore viability (as contrary to mosses), although studies, by Dr. van Zanten (University of Groningen), are now in progress. It is postulated that transoceanic species ranges in most cases should have arisen from succesful long-range dispersal, whereas generic disjunctions and species vicariance might be the result of ancient land connections, viz. evolution following the dissection of Gondwanaland.

B. O. VANZANTEN (*Biologisch Centrum, Afdeling Bryologie, Postbus 14, 9750 AA Haren (Gn)*)

Some considerations on the feasibility of long-distance transport in Bryophytes

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