

## MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON DECEMBER 16, 1977

W. G. BRAAKHEKKE (*Centrum voor Agrobiologisch Onderzoek – C.A.B.O., Wageningen*)

Stable equilibria between grassland plants

P. J. VAN LOENHOUD (*Universiteit van Amsterdam*) and J. C. P. M. VAN DE SANDE (*Laboratorium voor Aquatische Oecologie, Katholieke Universiteit, Nijmegen*)

Application of the “Braun-Blanquet” approach to a study of the rocky shore zonation in Aruba and Curaçao (Netherlands Antilles)

The rocky shore is an outstanding example of an environmental gradient (“wet-dry”; “salt water-fresh water”; “stable-dynamic”-gradient). The biocoenoses on it usually present themselves in the form of different zones perpendicular to this gradient.

Several schemes for this zonation have been proposed in the past, most recently one by VAN LOENHOUD & VAN DE SANDE (1977). In their scheme, three major units of the zonation are distinguished: The sublittoral region, the littoral region, and the maritime region. The littoral region, constituting the actual border zone between the sea and the land, is divided into infralittoral margin, the lower eulittoral, the upper eulittoral, and the supralittoral margin.

In the investigation reported here, the zonation of the littoral region in six stations in Aruba and Curaçao was investigated by means of methods of the “Braun-Blanquet” school. In the past, only limited use of this approach has been made in ecological rocky shore research; noteworthy exceptions are the papers by DEN HARTOG (1959) and by MOLINIER (1960), who also included sedentary animals in his investigations.

In each station, for the zones discernible on the shore the numbers of all larger animals were counted or estimated in a 2 m broad transect perpendicular to the zonation. In addition, in each zone 5 to 10 pieces of rock (5–50 cm<sup>2</sup> in size), with the adhering vegetation and small animals, were cut out. Of each piece of rock a single relevé including algal vegetation and sedentary animals was taken with the aid of a dissecting microscope.

When processing the data obtained, it was found impossible to follow the usual “Braun-Blanquet” method of classifying the relevés, because of the limited number of observations and the lack of reference works. The zones discerned at the six stations were instead classified in the scheme of zonation mentioned above on the basis of the distribution of a few species previously reported to be characteristic of the different units of the littoral region. Synoptic tables of the relevés were made for this classification, from which it appeared that a large number of algae, and both sedentary and mobile animals had a characteristic distribution over the units of the littoral region, and that groups of algae and animals could be designated with corresponding distributions, thus confirming the classification made. For details, see VAN LOENHOUD & VAN DE SANDE (1977).

On account of these results it is supposed that after adequate investigations, it will be possible to designate syntaxa for the biocoenoses of the littoral region on these rocky shores by means of differential and character-species of algae and animals. It is concluded that the “Braun-Blanquet” approach is a useful tool for an investigation of the complete biotic communities on rocky shores, and that even mobile animals, e.g., certain fishes and crabs, may have sufficiently specific distributions on the shore to be useful as character species.

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LOENHOUD, P. J. VAN & J. C. P. M. VAN DE SANDE (1977): Rocky shore zonation in Aruba and Curaçao (Netherlands Antilles), with the introduction of a new general scheme of zonation. *Proc. Kon. Ned. Ak. Wet., ser. C* 80: 437–474.

MOLINIER, R. (1960): Études des biocénoses marines du Cap Corse. I; II. *Vegetatio* 9: 121–192; 217–312.

S. VAN DER WERF (*Rijksinstituut voor Natuurbeheer – R.I.N., Leersum*)

The occurrence of *Fagus* and *Tilia*, with regards to toponymy

A. BARENDREGT (*Rijksuniversiteit, Utrecht*)

Vegetation of the “Zwanewater” in the dunes near Callantsoog (North Holland)

J. ROZEMA (*Biologisch Laboratorium, Afd. Ecologie, Vrije Universiteit, Amsterdam*)

Descriptive and experimental investigations on vegetation zonation at a coastal plain at Schiermonnikoog, The Netherlands

The vegetation along height gradients at small dunes (height 1.5–2.0 m) appears to be zoned. *Festuca rubra* ssp. *arenaria*, *Juncus articulatus* and *J. alpino-articulatus* ssp. *atricapillus* occur at the upper parts, *Festuca rubra* ssp. *litoralis*, *Juncus gerardii* and *J. maritimus* inhabit the lower zones. The latter sites are more saline than the higher zones because of accumulation of sodium chloride in the surface layer of the sandy soil due to capillary ascent of brackish soil water, which is impossible at the higher central parts. Secondly, gravitational sink of rain water builds up a fresh water lens in the centre of the dunes, with a height difference of the soil water of up to 15 cm as compared to the lower and saline parts of the dune.

Laboratory experiments showed that salt reduced germination of all species to the same extent (ROZEMA 1975). However, the growth of *Juncus maritimus*, *J. gerardii* and *Festuca rubra* ssp. *litoralis* was less reduced by salt in hydroculture as compared to the growth of *J. alpino-articulatus* ssp. *atricapillus* and *Festuca rubra* ssp. *arenaria* (ROZEMA 1976).

Chemical analyses showed that exclusion of sodium chloride may be an important salt tolerance mechanism. This is supported by analysis of plant material collected in the field. Salt exclusion means avoidance of toxic salt levels within plant tissues. Cytoplasmic enzymes extracted from all species were sensitive to salt (ROZEMA & BLOM 1977). However, only salt tolerant members of the Juncaceae demonstrated an accumulation of proline at increasing salinity. This organic solute might function as a non-toxic osmoticum within the cytoplasm, as high concentrations did not inhibit enzyme activity.

The rate of succession of the vegetation is low due to the low nitrogen status of the sandy soil (ROZEMA et al. 1977) and to the mechanical destruction of the superficial soil layer. Nevertheless, aerial photographs show a gradual increase of the rapidly growing species *Agrostis stolonifera* and *Juncus gerardii* and to a lesser extent *Festuca rubra* ssp. *litoralis*, which apparently exploit the frequently occurring less saline periods.

ROZEMA, J. (1975): The influence of salinity, inundation and temperature on the germination of some halophytes and non-halophytes. *Oecol. Plant.* 10: 317–329.

— (1976): An eco-physiological study on the response to salt of four halophytic and glycophytic *Juncus* species. *Flora* 165: 197–209.

— & B. BLOM (1977): Effects of salinity and inundation on the growth of *Agrostis stolonifera* and *Juncus gerardii*. *J. Ecol.* 65: 213–222.

—, H. J. M. NELISSEN, M. VAN DER KROFT & W. H. O. ERNST (1977): Nitrogen mineralization in sandy saline marsh soils in the Netherlands. *Z. Pflanzenernährung Bodenk.* 140: 707–717.

# MEETING OF THE SECTION FOR VEGETATION RESEARCH ON SEPTEMBER 27, 1978

M. J. M. L. OOMES (*Centrum voor Agrobiologisch Onderzoek – C.A.B.O., Wageningen*)

## Production of marginal grasslands in relation to the mode of exploitation

S. E. VAN WIEREN and A. VREUGDENHIL (*Laboratorium voor Plantenecologie, Haren (Groningen)*)

Heathland regeneration from grassland by means of grazing, mowing and cutting of sods

This research is aimed to investigate if it is possible to transform former grassland into heathland by simple management practices. To achieve this goal three kinds of experiments are performed all aimed at the removal of nutrients. By lowering the chemical soil fertility a suitable habitat for heathland species will be created.

The experimental grassland borders the upstream of the small river Drentsche A in the northeast of the Netherlands. At the start vegetation and soil characteristics showed only small spatial differences. The experiments include the management practices (sheep) grazing, mowing and removing of grass-sods, listed in order of supposedly increasing depletion of soil nutrients. On the grazed area small fenced plots are used for comparison. The results cover the period 1973–1978.

Yearly determination of the peak-standing crop and determination of the net removal of nutrients, in particular nitrogen and phosphorus, are used as estimates for changes in the chemical soil fertility.

Peak-standing crop was highest for grazing, lowest for removal of sods, while mowing gave intermediate values although a regular decrease was found. Since 1976 differences between mowing and removal of sods were not significant.

In 1975 the net removal of nitrogen and phosphorus was measured. In the grazed area the amount of nitrogen and phosphorus removed as well as the amount returned in the dung was measured.

It was found that by grazing 2.2 g N/m<sup>2</sup> and 0.6 g P/m<sup>2</sup> was removed; values for mowing were 1.0 g N/m<sup>2</sup> and 3.7 g P/m<sup>2</sup>. These figures fit rather well into the expected order of rate of nutrient removal as indicated above.

Grazing and mowing affected the botanical composition, studied in permanent plots, not dramatically during the 6 successive years of successive years of observations:

1. the total number of species slightly increased (with 2 and 4, respectively) by both grazing and mowing;
2. grazing affected positively *Lolium perenne*, *Agrostis stolonifera*, *Trifolium repens* and several rosette species, but negatively *Holcus lanatus*; and
3. mowing did not affect *Holcus lanatus*, *Trifolium repens* and several rosette species, but affected negatively *Lolium perenne* and *Agrostis stolonifera*.

Small scale barren spots created by removal of grass-sods were mainly colonized by species typical for relatively nutrient-poor habitats, especially *Holcus lanatus*, *Agrostis tenuis*, *Calluna vulgaris* and *Erica tetralix* were noticeable.

After 1976 peak-standing crop showed no differences as far as mowing and removal of sods are concerned, yet the botanical composition is obviously different. This may indicate that the prevailing conditions for germination and seedling growth play a much more important role relative to the level of chemical soil fertility. An identical conclusion may be drawn when the effects of grazing and mowing are compared. Associated effects of grazing, viz. trampling, selectivity etc., are much more important than the removal of nutrients.

The overall result of this investigation suggests that the creation of a heathland starting from a grassland strongly depends on a relatively low nutrient level of the soil and a temporarily barren soil, accepting that diaspores of heathland species are available.

After the establishment of a heathland-like vegetation, continued heathland regeneration will benefit by management practices like yearly mowing or grazing.

Some remarks on the relation between nitrogen mineralisation, groundwater table and standing crop in wet meadows

The effect of lowering the water table in two types of semi-natural grassland, bordering the small river Drentsche A, was studied. The soils differ in their nutrient status. Near Elp a vegetation on peat is found of which the nutrient status is denoted as low (WESTHOFF & DEN HELD 1969). The peat layer varies from 50–80 cm. The vegetation can be classified as the association *Cirsio-Molinietum* (Siss. et DE VRIES 1942), although it also has elements of the association *Parnassio-Caricetum pulicaris* (GÖRS 1965). Characteristic species are *Carex dioica*, *Carex pulicaris*, *Carex hostiana*, *Carex panicea*, *Cirsium dissectum* and *Parnassia palustris*. As a result of a change in the hydrology in 1963 and 1971, the botanical composition changed dramatically, leading towards the disappearance of rare species, such as *Parnassia palustris* and *Carex dioica*.

Downstream, near Anderen, a vegetation on peat with a relatively high soil fertility, is situated. The peat layer varies from 2–3 meters. The vegetation is related to the association *Lolio – Cynosuretum* (Tx. 1937) but also has elements of the association *Senecioni-Brometum racemosi* (Tx. et Preising 1951). Characteristic species are *Cynosurus cristatus*, *Caltha palustris*, *Lychnis flos-cuculi* and *Festuca pratensis*. The effects of lowering the groundwater table on N-mineralisation, species composition and standing crop were traced since 1976. The year of 1976 was extremely dry (560 mm/yr) and 1977 was moderately dry (750 mm/yr). Average rainfall is 810 mm/yr.

Soil samples were taken from 5–15 cm and incubated for 6 weeks in the same layer in polyethylene bags (ENO 1960; GERLACH 1973). Net N-mineralisation of the *Cirsio-Molinietum* stand, with artificially lowered groundwater level, was 250 kg N/ha/yr in 1976 and 220 kg N/ha/yr in 1977. The regular increase of  $\text{NO}_3^-$ -content up to 180 mg N/kg in the period April–September 1976 was striking. A similar observation was made in 1977, although these results were less pronounced. No such increase was registered when the groundwater table was not artificially changed. Net N-mineralisation was 100 kg N/ha/yr in 1977. LEON (1968) reported comparable values for low-moors with *Junco-Molinia* vegetations. ELLENBERG (1977), however, listed much lower values.

In the peat soil with a high nutrient status, lowering of the winter groundwater table from 10 up to 60 cm below surface, resulted in a net N-mineralisation of 500 kg N/ha/yr in 1976 and 250 kg/ha/yr in 1977. For both years it was observed that the  $\text{NO}_3^-$  content was low in spring and early summer, but increased very rapidly after mowing in summer (up to 80 kg N/kg). In unaffected (wet) areas net N-mineralisation was 50 kg N/ha/yr in 1976 and 20 kg N/ha/yr in 1977. The  $\text{NO}_3^-$ -content was approximately zero.

Further research into some abiotic factors of the soils of the two types of vegetation revealed that:

1. total nitrogen content varied slightly (2.2 and 2.7% respectively),
2. total phosphate content (after destruction) was considerably higher in the *Lolio-Cynosuretum* vegetation,
3. exchangeable potassium content was slightly higher in the *Lolio-Cynosuretum* vegetation.

Measurements of the standing crop indicated that the vegetation belonging to the association *Cirsio-Molinietum* reacted on lowering the groundwater table in two ways: some areas showed a slight increase (from 2.5 to 3.5 ton/ha) while others showed a decrease (from 2.5 to 1 ton/ha). Despite the high  $\text{NO}_3^-$ -content of the soil, plant growth was nevertheless very much restricted. This may point to other limiting factors, such as water supply and/or available phosphate.

Lowering of the groundwater table in the *Lolio-Cynosuretum* vegetation resulted in a strong increase of the standing crop (from 3 to 6.5 tons/ha). This increase is related to the increase of N-mineralisation.

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GERLACH, A. (1973): Methodische Untersuchungen zur Bestimmung der Stickstoffnetto-mineralisation. *Scripta Geobot.* **5**: 1–115.

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- Projektgroep Ruilverkavelingen R.U.G. (1978). Ecologische effecten van de Ruilverkaveling Rolde/Anloo. Gestencild rapport.
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J. H. WILLEMS (*Vakgroep Vegetatiekunde en Botanische Oecologie, Rijksuniversiteit Utrecht*)

# Experiments on the relation between species diversity and above ground plant biomass in chalk grassland

To test some hypotheses concerning plant community development as well as the relation between species diversity and above ground plant biomass, some experiments have been started in the extreme South of The Netherlands. Starting from the hypothesis that increasing above ground plant biomass has a lowering plant diversity as a result, and vice versa, the experiments were focused on the increase of this biomass on the one hand and on the decrease of it on the other hand.

In 1971 thirty permanent plots were established in an abandoned grassland on calcareous soil. In this year a number of species characteristic for heavy fertilized grassland showed the influence of former land use. At the same time a number of species were found which are mainly growing on poor calcareous soils. In half of the plots the nutrient rich upper layer of the soil was removed by cutting sods. Independently a part of the plots, both with or without cutted sods, were treated with artificial fertilizer (N.P.K.). Control plots received no fertilizer. All plots were mown in autumn every year.

During the first two years of the experiment the effect of sod cutting in 1971 dominated the effect of fertilizer treatment. In 1973, fertilizer treatment had become the dominant factor for both above ground biomass and plant diversity. The dry weight plant biomass in all plots with fertilizer treatment varied from 520–680 g/m<sup>2</sup>, the species number of phanerogams varied from 17–23 per m<sup>2</sup>. The dry weight of the biomass in the control plots was about 300 g/m<sup>2</sup> and the number of phanerogams varied from 24–33 per m<sup>2</sup>.

Up to 1977 the difference between the fertilized plots and the control plots has increased. The number of species in the fertilized plots became less than 20 per m<sup>2</sup>, whereas this number increased to 30 or more per m<sup>2</sup> in the control plots. The dry weight of the above ground plant biomass in the fertilized plots varied from 600–800 g/m<sup>2</sup>, and the biomass in the control plots has slightly decreased and varied from 180–320 g/m<sup>2</sup>.

On the fertilized plots the greater part of the above ground plant biomass was formed by a yearly decreasing number of species. These species included grasses as well as herbs. All these species may be considered as trivial ones. The expected correlation between species diversity and above ground plant biomass was strongly negative as was predicted by the hypothesis. Yet it is necessary to collect data about vegetation development during a longer period, amongst others in order to distinguish the influence of the yearly varying climatic conditions. Further investigations can also throw more light on the question whether higher plant diversity is positively correlated with stability. Stability can be seen as an absence of fluctuation both in species composition and in structure of vegetation. For this reason the study of the experimental plots will be continued the forthcoming years.

D. C. P. THALEN (*Internationaal Instituut voor Luchtkaartering en Aardkunde, afdeling Vegetatiekunde, Enschede*)

# Estimation of production for mapping and evaluation of vegetations

# MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY ON OCTOBER 26, 1978

C. J. VENVERLOO (*Botanisch Laboratorium, Leiden*)

## Cell division and differentiation in the epidermis of *Nautilocalyx* leaves

Explants of *Nautilocalyx* (Gesneriaceae) leaves, containing the epidermis and some collenchyma layers, were grown on agar media. They were able to regenerate roots and shoots, both originating in the epidermis (cf. VENVERLOO 1973).

Methods were developed for direct Nomarski microscope observation of the dividing epidermis cells during the growth of the explant. First mitoses took place in all cells after about 3 days. During the first, second and third day changes in the position of the nucleus and organelles, increase in nucleolar volume and changes in the pattern of cytoplasmic strands were observed. Nearly all first divisions were periclinal, second divisions were often anticlinal. Mitotic stages could be best observed in anticlinal divisions, whereas cell plate formation and growth of the phragmoplast were visible in both types of divisions.

Cytoplasmic configurations resembling the phragmosome (in the sense of Sinnott and Bloch) were visible from about one hour before the disappearance of the nucleolus, until some time after mitosis. Electron micrographs of 3-day-old cells seem to corroborate the presence of locally extremely thin, flattened strands in the presumed plane of the new cell wall. By using a combination of Nomarski observations and electron micrographs of the observed cell(s), we will try to obtain further information about the structure of the phragmosome in these big vacuolate cells. The study will be extended to a search for organelles, such as microtubules, during and before phragmosome formation in order to obtain more information about the regulation of the plane of cell division in these cells.

VENVERLOO, C. J. (1973): The formation of adventitious organs. A comparison of root and shoot formation on *Nautilocalyx* explants. *Acta Bot. Neerl.* 22: 310–322.

H. J. PLUYMAEKERS (*Botanisch Laboratorium, Katholieke Universiteit, Nijmegen*)

## Morphology and arrangement of microfibrils in cell walls of root hairs

In an electron microscopical investigation of root hairs of *Triticum monococcum*, the arrangement of cellulose microfibrils in the cell wall was studied and their size measured. To remove non-cellulosic wall material, roots of seedlings were boiled for 1 hour in a mixture of equal parts of glacial acetic acid and hydrogen peroxide (30%). The roots were then rinsed several times with distilled water, dehydrated in an ethanol series and embedded in butylmethacrylate. Ultrathin sections of root hairs were post stained for 30 min with 3% aqueous potassium permanganate, thoroughly washed with distilled water and viewed in the electron microscope. This method is modified from the procedure of DESHPANDE (1976) and clearly reveals a positive staining of cellulose microfibrils.

The cell wall of a root hair consists of an outer primary wall of irregularly arranged microfibrils and an inner secondary wall composed of several lamellae of axially oriented microfibrils, which is in accordance with a previous study using shadow casting techniques (PLUYMAEKERS 1977). It seems that in the extreme tip of growing root hairs only the primary wall is present. In very long hairs and sometimes in shorter ones, underneath the primary wall of the tip, lamellae are visible consisting of microfibrils, the orientation of which differs in each lamella. Since these lamellae originate from the secondary wall, the suggestion is made that after cessation of cell growth a secondary wall is also being synthesized in the tip.

The microfibrils of both the primary and secondary walls are about 7.8 nm wide with a clear centre of about 2.0 nm. This is especially obvious in cross sections of microfibrils. According to Preston (1974) the electron transparent centre is presumably the central crystallite of the microfibril. The question arises whether the stainable cortex represents cellulose or some remaining non-cellulosic material that surrounds the microfibril as an integral part of it.

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- PLUYMAEKERS, H. J. (1977): Zellwandaufbau in Wurzelhaaren von *Triticum monococcum*. *Ber. Deutsch. Bot. Ges.* **90**: 167–172.
- PRESTON, R. D. (1974): *The physical biology of plant cell walls*. Chapman and Hall, London.

M. T. M. WILLEMSE and S. A. REZNICKOVA (*Botanisch Laboratorium, landbouwhogeschool Wageningen*)

### Function of the tapetum in relation to pollen formation

From early physiological results it appears that in the anther of *Lilium* distinct periods of production of lipids, amylum and other carbohydrates can be distinguished. An ultrastructural study of localization of some of these and other products was undertaken to increase our understanding of the function of the tapetum in relation to pollen formation.

After an inventarisation of the cytological changes of the different tissues in the anther some processes can be revealed. In the cells of the middle layer the storage of amylum shows an increase during the early stages in microspore development till the vacuolisation of the microspore. Thereafter a decrease starts. Only in the outer layers a second increase can be noted just before the division of the microspore. The cells of the middle layer contain few spherosomes compared to the tapetal cell and the first cell layer next to the tapetal cell.

The tapetal cell is separated from the middle layer cells by a peritapetal cell wall. In these cells the formation of carotenoids takes place in the plastids. This product is mixed with the products of the spherosomes and forms the typhine on the pollen wall.

The plasma membrane undulates, and sharp folds are places of addition of electron dense material. These places remain visible as the so-called membrane-like lamellae. The ubisch bodies are formed in relation to these lamellae. They become separated by a newly formed tapetal wall.

During the increase in pollen volume the pollen wall exine has a constant thickness. By measurement of the increase of the volume of the foot layer it appears that a discontinuous process of thickening takes place. This becomes clear when observing the openings of the foot layer in the periods of small accretion of wall products and the closed foot layer during the period of a higher accretion of wall products. The foot layer is formed from products originating from the loculus as well as from the pollen. After the mitosis the pollen shows an accumulation of spherosomes and amylum, but the latter is probably partly used for the formation of the intine.

In the loculus some periods of an increase of proteins, free lipids and glucose were determined. In combination with the cytological data the hypothesis is proposed that during the formation of the microspore and pollen the carbohydrates originating from the middle layer are the main sources. The spherosomes, present in the tapetal cells and first middle layer, probably containing a combination of lipids, glycolipids and lipoproteins, are a second source. Both components play a role in the formation of the pollen and its wall.

R. D. WOITTIEZ (*Botanisch Laboratorium, Landbouwhogeschool, Wageningen*)

### Sticking of Pollen on Stigmas

Using a physical approach based on experiments with different types of pollen on wet stigmas of *Petunia spec.* and on dry stigmas of *Begonia spec.* the factors involved in sticking of pollen to stigmas were studied. The following factors, listed in order of importance are involved: the surface tension force, the wind force, the electrostatic force, the electrodynamical force, the gravity, and the inertial force. Based on physical formulas and some data on smooth, spherical pollen grains, taking into account mass, load as well as the adhesion factor, the following forces can be calculated: the surface tension force =  $3 \times 10^{-7} \text{N}$ ; the wind force =  $4 \times 10^{-8} \text{N}$ ; the electrostatic force =  $3 \times 10^{-9} \text{N}$ ; the gravity =  $6 \times 10^{-11} \text{N}$ ; the electrodynamical force =  $8 \times 10^{-12} \text{N}$ ; and the inertial force =  $6 \times 10^{-12} \text{N}$ .

The wetness of pollen or stigma mainly determines the sticking. During the aging of the styles the

wetness changes and this can cause the pollen to fall off, depending on wind force and the mass of the grains. The sculpture of the pollen wall hardly functions in sticking.

L. H. BATENBURG (*Afdeling Plantensystematiek, Biologisch Centrum, Haren (Groningen)*)

**The anatomy of the Carboniferous species *Sphenophyllum emarginatum* (Brongniart) Brongniart**

A previous publication (BATENBURG 1977) dealt with a detailed morphological study of the remains attributable to *Sphenophyllum* Brongniart found in sediments of latest Westphalian D age exposed southeast of Holz (Saarland, Federal Republic of Germany). It was shown that the material should be classed with *S. zwickaviense* Storch, *S. saxonicum* Remy et Remy, and *S. emarginatum*. These species were critically reviewed. The new specimens necessitated emendation of the diagnoses of *S. zwickaviense* and *S. emarginatum*. *S. cornutum* Lesquereux and *S. sp. n.?* Storch 1966 were shown to be heterotypic synonyms of *S. emarginatum*. A contribution towards a better reconstruction of the latter was offered; a clinging, ascending to climbing nature was suggested.

The anatomy of the "compression species" of *Sphenophyllum*, including the ones discussed here, is not or insufficiently known. Knowledge of the anatomy is a prerequisite for comparison, and possible correlation, with "coal ball species".

The anatomical data concerning *S. emarginatum* were obtained by means of so-called "transfers" and transverse stem-thinsections (light microscopy), and latex rubber casts of impressions (S.E.M.). The following parts and properties could be distinguished:

In stems and twigs: walls, outlines and arrangements of epidermal cells (including those of hairs or emergences) and fibres; a pitted wall fragment; probable relative thicknesses of cortex, xylary body, and primary xylem.

In leaves: walls, outlines and arrangements of epidermal, mesophyll, and fibrous cells; distribution, orientation and organization of stomata; a piece of cuticle; a pitted wall fragment.

The idea, that the heterophylly as occurring in several *Sphenophyllum* species would indicate a half-submerged way of life, is contradicted by the fact that in *S. emarginatum* the same heterophylly does not coincide with such a lifeform, witness the occurrence of stomata in the abaxial surfaces of all leaves. Anatomical study confirms that the hooks, formed by the protruding veins of the linear and deeply lobed leaves, have had a mechanical rather than, e.g. a hydathodical function; thus strengthening the concept of *S. emarginatum* as a clinging, ascending to climbing plant (BATENBURG 1977).

The known anatomical characteristics of the other "compression species" of *Sphenophyllum* do not differ considerably from those of *S. emarginatum*. The anatomy of the "compression species", as far as is known, does not importantly vary from that of the "coal ball species", except that in the latter all shoots thicker than some mm show (active) decortication and defoliation, whereas in the former even the thickest stems do not. This difference, together with the different sedimentary environments, might indicate that the "compression species" on the one hand, and the "coal ball species" on the other, represent (perhaps ecologically rather than phylogenetically) different groups within the genus. The position of *S. emarginatum* within the "compression group" is not yet clear. Hooks, entirely comparable with those of *S. emarginatum*, have been found in two other heterophyllous "compression species", viz., *S. laciniatum* Storch and *S. oblongifolium* (Germar et Kaulfuss) Unger. A more detailed publication is in preparation, and will probably appear in the *Rev. Palaeobot. Palynol.*

BATENBURG, L. H. (1977): The *Sphenophyllum* species in the Carboniferous flora of Holz (Westphalian D, Saar Basin, Germany). *Rev. Palaeobot. Palynol.* **24** (2): 69-99.



# MEETING OF THE SECTION FOR PLANT TAXONOMY AND -GEOGRAPHY ON NOVEMBER 3, 1978

J. WIEGERS, in cooperation with N. C. L. M. BEENTJES (*Hugo de Vries-Laboratorium, Amsterdam*)

## Aronia Medik. in the Netherlands: distribution and classification

The occurrence and distribution of *Aronia* (Chokeberry) in the Netherlands were investigated. The identification of the Dutch specimens with one of the three species recognised in most of the regional floras proved to be difficult. After an examination of material of North American origin it was concluded that the Dutch plants resemble *A. prunifolia* (Marsh.) Rehd. more closely than *A. melanocarpa* (Michx.) Ell. or *A. arbutifolia* (L.) Pers. They also approach some of the forms described by Rehder (1940), but the similarities are never conclusive. The possibility remains that the plants originally imported as ornamentals were already hybrids or cultivars, which subsequently spread from nurseries mainly ornithochorously. It has been suggested that *A. prunifolia* is a hybrid between *A. arbutifolia* and *A. melanocarpa*, and there is factual evidence that *A. melanocarpa* and *A. prunifolia* can hybridise. In the Netherlands Chokeberries were collected for the first time at Domburg in 1875. In 1950 they were known from six 5 km<sup>2</sup>-squares (Hour frequency class 2). Lately they were recorded from as many as 71 km<sup>2</sup>-squares (325 km<sup>2</sup>-squares, Hour frequency class 4). The Chokeberries occur in semi-natural, acid and oligotrophic wetland vegetation in the Haf-district. They also grow on wet and acid sandy soils, but there is strong evidence that at such sites they were originally escapes from cultivation. The Chokeberries thrive vigorously in vegetation types belonging to the *Caricion curtonigrae* and the *Alnion glutinosae*, and spread rapidly by lateral underground runners. The density of the stand may rise to 80 stems per square metre, so that the local vegetation cover is almost completely ousted. They may also penetrate into *Salicion cinereae* vegetation and the *Erico-Sphagnion*. On account of its competitive capacity the chosen common name in Dutch ("Appelbes") has locally been replaced by "Veenpest" ("marsh pest").

H. STEGENA (*Vakgroep Plantensystematiek, Vrije Universiteit, Amsterdam*)

## Systematics of Acrochaetiaceae (Rhodophyta)

Culture studies on a number of acrochaetioid algae indicate that many species in this group have sexual life histories of alternating gametophyte and tetrasporophyte, usually including, but sometimes without an intercalated carposporophytic stage. Phases may be nearly isomorphic to strongly heteromorphic.

In most species sexual reproduction takes place under special conditions of light and temperature, usually gametangia being formed under other conditions than tetrasporangia. Asexual reproduction by means of monospores occurs under a much wider variety of circumstances. Relating these data to field observations is far from simple, since sexual reproductive structures are not often found on field-collected plants.

The type of life history, combined with some morphological characters (chloroplast morphology, type of spore germination, and carposporophyte morphology) provides the base for a division of the Acrochaetiaceae into five genera. Proposed genera are *Chromastrum*, *Acrochaetium*, *Kylinia*, *Audouinella* and *Rhodochorton*. Species exhibiting only asexual reproduction, are presumed to represent reduced types, and their systematic position should be determined by morphological similarities to species with complete life histories; they should not be united into separate genera.

The presented system differs from other recent studies, which have led some authors to acknowledge only one genus in the family: *Audouinella*; on the other hand it resembles older systems, based on chloroplast morphology.

Within the family, the genus *Chromastrum* has been studied in more detail: Species can be distinguished on size of plants, cell- and sporangial dimensions, and morphology of spore germination in the tetrasporophyte; variability in culture of branching pattern and morphology of the prostrate part indicates that these characters are of limited use for this purpose. Critical examination

of type specimens of the c. 60 species of this genus, combined with the fact that phases of one life history have been described as separate entities, might considerably reduce the number of true species.

J. VAN DER VEER (*Afdeling Plantensystematiek, Biologisch Centrum, Haren (Groningen)*)

Relationship between the classes of the flagellates, based on their ultrastructure

A. J. M. LEEUWENBERG (*Laboratorium voor Plantensystematiek en -geografie, Wageningen*)

The sections of Buddlejia (Loganiaceae)

## MEETING OF THE SECTION FOR PLANT PATHOLOGY ON NOVEMBER 14, 1978

R. RABBINGE and P. H. VEREIJKEN (*Vakgroep Theoretische Teeltkunde, Landbouwhogeschool, Wageningen; Instituut voor plantenziektkundig Onderzoek, Wageningen*)

The effect of aphids and honeydew on the yield of winterwheat

In order to analyse and explain the complex relations of the cereal aphid *Sitobion avenae* and its host winterwheat a simplified crop growth model of wheat based on a detailed explanatory model for assimilation, respiration and transpiration of canopy surfaces is coupled to a population model of the cereal aphid. By comparison of the results of model calculations and the behaviour of the real system the insight in the system grows. It appeared that the primary effects of the aphid by sucking damage and saliva are negligible in comparison with the secondary effects due to honeydew and the promotion of fungal growth by this aphid excretion product. Experiments in which fungicides were applied confirmed these calculations since the effect of the aphids was much less severe in these treatments than when no fungicides were sprayed. These secondary effects seem to account for more than 60% of the yield losses.

The crop model calculates dry matter accumulation of shoot, root and head in relation to such changing abiotic conditions as daylength, incoming radiation, temperature and humidity. It utilizes leaf area index as a driving variable. The pest model simulates the development of the aphid population, calculating both absolute numbers and age distribution, in relation to abiotic conditions like temperature and humidity and considers emigration, immigration and the different antagonists such as predators, parasites and *Entomophthora* spp. The interrelations of aphid and host plant comprise data on the sucking rate, the honeydew production, the place of sucking, the effect of nitrogen level and the resulting decrease in gas exchange, leaf area duration etc.

Although the results of the preliminary calculations and experimental results are promising, a complete explanation of the whole relationship cannot be given. More detailed studies on the secondary effects of the aphid on its host plant and the effect of the fungal competition on the plant are therefore needed.

INGRID RIPHAGEN<sup>1</sup>, N. J. FOKKEMA<sup>1</sup>, W. J. KASTELEIN<sup>1</sup>, P. H. VEREIJKEN<sup>2</sup>  
(<sup>1</sup> *Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn;* <sup>2</sup> *Instituut voor Plantenziektenkundig Onderzoek, Wageningen*)

Effect of aphid honeydew on saprophytic and pathogenic fungi of wheat leaves under controlled environmental conditions

Honeydew from *Sitobion avenae* feeding on wheat leaves was collected from the walls of perspex leaf cages by washing in water and freeze-drying.

Wheat flag leaves were inoculated with a suspension of cells and conidia of the dominant phyllosphere fungi of wheat (viz.: *Cryptococcus laurentii* var. *flavescens*, *Sporobolomyces roseus*, *Aureobasidium pullulans* and *Cladosporium cladosporioides*) with and without addition of 15% honeydew. Under 70% r.h. during the light period and 95% r.h. during the dark period at 18°C, the total

population reached stationary levels within 5 days at a population density of  $2 \times 10^5$  propagules/cm<sup>2</sup> leaf when honeydew was added, and of  $2 \times 10^4$  propagules/cm<sup>2</sup> on the control leaves. Honeydew stimulated growth of all fungal groups to a similar extent.

When honeydew 15% was added to the inoculum of *Septoria nodorum* or *Cochliobolus sativus*, the infection of wheat leaves by these pathogens was enhanced considerably. A natural honeydew deposit, however, did not increase *Septoria* infection suggesting antagonistic interference by common phyllosphere fungi, which was further investigated. The infection stimulated by honeydew added to the inoculum was only reduced by simultaneously added saprophytes if their population density was very high. Honeydew never stimulated infection when applied to the leaves 4–5 days before inoculation during which period a dense population of naturally occurring phyllosphere saprophytes developed.

Under natural conditions, the saprophytic phyllosphere fungi will compete successfully for the nutrients in the honeydew before pathogens can take advantage of them, which is a good example of naturally occurring biological control.

J. VAN DER SPEK (*Instituut voor Plantenziektenkundig Onderzoek, Wageningen*)

### Stalk rot of maize in The Netherlands

Fodder maize growing for ensiling has rapidly increased in the Netherlands from 6400 ha in 1970 to 118500 ha in 1978. The crop is mainly grown on sandy soils. Because of its large acreage and a short rotation—sometimes even in monoculture—attention has to be paid to the incidence of fungal diseases, in particular to the occurrence of *Fusarium* stalk rot from 1975 onwards.

Stalk rot was not very prevalent on our farm fields in this period. It was only found to a certain extent if the crop was harvested late. This is remarkable because *Fusarium* spp. are very common in maize every year. These fungi develop in the phyllosphere in pollen and extruded anthers lying on the leaves, in leaf axils and behind the leaf sheaths. Also, it appeared that developing axillary buds, consisting of pale membranous structures, are often infected by *Fusarium* spp. These diseased parts can give rise to a slight attack of some nodes and the joint parts of the pith. An infection of leaf sheaths always remained local. Soil infection was seldom found. These findings show that stalk rot can be caused by wind-borne *Fusarium* spores, as was already stated by MENSAH & ZWATZ (1975). Identification of the isolated *Fusarium* spp. gave the following result: *F. culmorum* and *F. graminearum* were most common, whereas *F. equisetii*, *F. avenaceum*, *F. poae*, and *F. oxysporum* were also involved. *F. moniliforme* often mentioned in connection with the stalk rot complex, was not isolated up till now, however, the isolates made in 1978 must still be identified. Non-pathogenic microorganisms are involved in the decay of the stem. No infection at all occurred in our first variety trials of 1976 and 1977. The variety trial of 1978, however, was attacked by *Fusarium* spp. to such an extent that varietal differences in resistance against stalk rot could be readily observed. It was evident that this infection was soil-borne.

In 1978 soil-borne infection was also observed in an experiment that was carried out by the two institutions IPO and SVP at Wageningen.\*

Thus stalk rot is a soil-borne disease, as stated by RINTELEN (1966), also in the Netherlands. Now the factors influencing the incidence of the disease are being studied. MORTIMOR & WARD (1964) proved that *Fusarium*-stalk rot is a low sugar disease. Because of this discovery we create stress situations for maize in climate chamber experiments in order to find out which circumstances are favourable. We need this knowledge in connection with disease control and in connection with the creation of a method for testing the resistance of hybrids and inbred lines to stalk rot.

MENSAH, R. A. & B. G. ZWATZ (1975): *Pflanzenschutzberichte* XLIV, 11/12: 161–194.

MORTIMOR, C. G. & G. M. WARD (1964): *Can. J. Pl. Sci.* 44: 451–457.

RINTELEN, J. (1966): Diss. Landw. Hochschule Hohenheim.

\* This experiment is a part of the joint European FAO trial on *Fusarium* resistance in maize.

K. VERHOEFF<sup>1</sup>, F. WARNAAR<sup>2</sup> and F. J. P. SCHIJVEN<sup>1</sup> (<sup>1</sup> *Phytopathologisch Laboratorium "Willie Commelin Scholten"*, Baarn; <sup>2</sup> *Botanisch Laboratorium, Utrecht*)

### Infection of young tomato fruits by *Botrytis cinerea*

When conidia of *Botrytis cinerea* germinate on the surface of young tomato fruits, direct penetration of germ tubes into epidermal cells occurs. As observed with a scanning electron microscope penetration through the cuticle seems to be an enzymatic process. To obtain more information on this, cutin from young tomato fruits was analyzed, using GLC techniques. Cutin consists of hydroxy fatty acids, with 10, 16 dihydroxy palmitic acid as the main component.

After addition of the protein fraction of ungerminated ground conidia of *B. cinerea* to this cutin, fatty acids were detected in the reaction mixture, among them palmitic acid.

Therefore, in addition to endo polygalacturonase (VERHOEFF & LIEM 1978), conidia of *B. cinerea* contain an enzyme system which breaks down the tomato fruit cutin, and it is likely, that the whole penetration process is enzymatic.

VERHOEFF, K. & J. I. LIEM (1978): *Phytopath. Z.* 91: 110–115.

J. A. GLAZENER (*Phytopathologisch Laboratorium "Willie Commelin Scholten"*, Baarn)

### A defense mechanism in tomato fruit after infection by *Botrytis cinerea*

After infection of young tomato fruit, conidia of *Botrytis cinerea*, cause small necrotic spots, sometimes surrounded by a white halo. Microscopic examination of these spots showed a dark coloration of the cell walls surrounding the penetrating hyphae in the cell layers under the epidermis.

Earlier work has indicated a lignification of these cell walls. In the present work it was found that the total phenol content increased considerably in the infected tissue. Among the lignin precursors p-coumaric acid and caffeic acid accumulated to several times their concentration in healthy tissue. The phenolic acids bound to the cell walls seem to be the site of initial polymerisation. A detailed study of this lignin-like material is in progress. It has also been shown in a few other host-pathogen combinations that lignification could be an effective defense mechanism of the host.

J. W. VEENSTRA (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

### The cross-inoculation technique: A new technique for field experiments to study the combined effect of two diseases

In order to analyse the ecophysiological aspects of damage an experiment was performed to assess the separate and combined effects of the pathogens *Puccinia recondita* and *Septoria nodorum* on the yield of wheat (winterwheat, cv. Nautica). A cross-inoculation technique was used. Line sources of *P. recondita* and *S. nodorum* were created so that for each pathogen a gradient of severity developed. The *P. recondita* gradient and the *S. nodorum* gradient were perpendicular to each other. The cross-inoculation technique and the subsequent development of gradients lead to the result that every imaginable combination of severities for *P. recondita* and *S. nodorum* can be obtained in 4 replications within a limited area, e.g. 200 m<sup>2</sup>.

Table 1 shows the disease severity on July 15, 1978 and the yield in metric tons per hectare (MT.ha<sup>-1</sup>) *S. nodorum* shows a gradient in two directions, one due to inoculation (distance from the source), and the other probably due to preinfection by *P. recondita* (following the *P. recondita* gradient). This observation suggests some degree of interaction between *P. recondita* and *S. nodorum* in 1978. The higher disease severities led to a reduced kernel weight and thus to reduced yield.

Unsolved problems of the cross-inoculation technique are:

- The dispersal of the disease. When the severity is high, there is a risk missing the effect of a single pathogen on yield.
- The statistical procedure. An analysis of variance is less desirable because several variables are dependent.

Table 1. Relation between disease severity and distance from the line source on July 15, 1978. Entries are in pairs of which the first number is the severity (percentage of foliar surface attacked) of *P. recondita*, the second that of *S. nodorum*.

		<i>S. nodorum</i> distance from the source in m				mean yield in MT.ha <sup>-1</sup>
		1.2-2.4	2.4-3.6	3.6-4.8	4.8-6.0	
<i>P. recondita</i>  distance from the source in m	0. -1.5	10-10		10-15		6.6
	1.5-3.0	5-5	8-5		5-3	6.8
	3.0-4.5				5-3	6.8
	4.5-6.0			3-3		6.8
	6.0-7.5		3-1		3-1	6.9
	7.5-9.0	3-3		3-3		6.8

P. C. SCHEEPENS and J. C. J. VAN ZON (*Centrum voor Agrobiologisch Onderzoek, Wageningen*)

#### Perspectives for the control of some weeds in The Netherlands with pathogenic microorganisms

Biological control of weeds might be an alternative for chemical control in situations where herbicides are not available or undesirable.

Pathogenic microorganisms can be used in two possible ways:

- i) They can be released in an area where the weed was formerly introduced presumably without its pathogens. This classical approach has successfully been used by entomologists and recently by plant pathologists as well. In this way, the weed is not completely eradicated, but will be established at a lower density. *Prunus serotina* which was introduced into The Netherlands from N.-America and *Cirsium arvense* are likely candidates to be controlled in this way.
- ii) Endemic pathogens might be applied as a type of biological herbicide. The perspectives of this tactic are being investigated for four weed species: *Alopecurus myosuroides*, *Chenopodium album*, *Echinochloa crus-galli* and *Elytrigia repens*. The potential use of *Ascochyta atriplicis* and *Cercospora chenopodii* which are both pathogenic to *Chenopodium* and *Atriplex* species is being evaluated. For the other three weed species the search for specific pathogens is still going on.

H. P. MAAS GEESTERANUS (*Instituut voor Plantenziektenkundig Onderzoek, Wageningen*)

#### Where to find resistance for Fire blight in *Crataegus monogyna*

After the introduction of Fire blight caused by the bacterium *Erwinia amylovora* in the wild hawthorn population in The Netherlands the possibility of natural resistance to this disease in this morphologically variable genus became of urgent interest.

Field observations showed that heavily infected *Crataegus monogyna* shrubs could frequently be found adjacent to non-infected ones. This might be due to the morphological heterogenous species. Moreover there could also be differences in susceptibility in the various populations in the rather large geographic distribution area.

Recent studies have been concentrated on methods of expressing the degrees of susceptibility and to use them to demonstrate differences in susceptibility of populations with distinct morphological characters as well as differences in susceptibility of populations from different geographic origin. Actively growing plants were inoculated by submerging shoots in a bacterial suspension of *E. amylovora* and by cutting off the tops of the three youngest leaves. Wilting developed from the top of the shoot downwards. The size of the wilted shoot in relation to the total length was used as a degree of susceptibility.

Results of experiments using this inoculation method have so far been unable to show that specimens with special morphological characters were less susceptible than others. No differences in susceptibility could be demonstrated between populations from North-, East-, South- or West-Europe.

H. J. MILLER (*Plantenziektenkundige Dienst, Wageningen*)

#### Recent developments in the identification of *Agrobacterium radiobacter* subsp. *tumefaciens*

Although crown gall, caused by *Agrobacterium radiobacter* subsp. *tumefaciens* (E.F.Sm. & Town.) Keane et al. (1970), is found throughout the whole world, many countries demand that imported plant material should be free from this disease. Until recently biochemical identification procedures have failed to provide a clear differentiation between *A. radiobacter* subsp. *tumefaciens* and the saprophyte *A. radiobacter* subsp. *radiobacter* commonly found in soil. Pathogenicity testing with such host plants as tomato and *Kalanchoe* has therefore been the common practice but this method is very time consuming.

During the last decade considerable advances have been made in molecular biology. It appeared that the bacterium *A. radiobacter* subsp. *tumefaciens* possesses a plasmid which is associated with pathogenicity. The transferable portion of this plasmid which is responsible for crown gall development is able to govern the synthesis within the host plant of the arginine derivatives octopine or nopaline. However, on the same plasmid, genes are also situated that are responsible for the breakdown of octopine or nopaline.

In octopine experiments in this laboratory, 20 of the 22 *A. radiobacter* subsp. *tumefaciens* strains isolated in The Netherlands were able to break down octopine within three days. All strains of *A. radiobacter* subsp. *radiobacter* tested were negative. This suggests therefore, that in many cases a positive identification of the crown gall bacterium may be obtained within one week instead of the usual 2–8 weeks.

At the present time three biotypes are distinguished among these bacteria (KEANE et al. 1970). This part of the identification procedure is especially important in connection with the biological control of the disease (NEW & KERR 1972; KERR & HTAY 1974; KERR & PANAGOPOULOS 1977). However, no correlation could be found between the biotypes in octopine experiments, although only two of the six biotype 3 strains were able to break down octopine.

KEANE, P. J., A. KERR & P. B. NEW (1970): Crown gall of stone fruit. II. Identification and nomenclature of *Agrobacterium* isolates. *Aust. J. biol. Sci.* **23**: 585–595.

KERR, A. & K. HTAY (1974): Biological control of crown gall through bacteriocin production. *Physiol. Pl. Path.* **4**: 37–44.

— & C. G. PANAGOPOULOS (1977): Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. *Phytopath. Z.* **90**: 172–179.

NEW, P. B. & A. KERR (1972): Biological control of crown gall: field measurement and glasshouse experiments. *J. appl. bact.* **35**: 279–287.

G. T. N. DE LEEUW<sup>1</sup>, A. A. POLAK-VOGELZANG<sup>2</sup>, A. M. HAGENAARS<sup>2</sup> and P. A. STEERENBERG<sup>2</sup> (<sup>1</sup> *Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*; <sup>2</sup> *Rijks Instituut voor de Volksgezondheid, Bilthoven*)

#### ELISA as a diagnostic tool for plant mycoplasmas

A microplate method of the enzyme-linked immunosorbent assay (ELISA) was used for the detection and assay of spiroplasmas and MLO's in plants. By using peroxidase-conjugated specific anti-*Spiroplasma citri*  $\gamma$  globulin a positive reaction could still be obtained with a 1:640 dilution of crude sap expressed from *Vinca rosea* plants affected by *Spiroplasma citri*. The MLO's in the *Vinca* plants studied with ELISA did not show a serological relationship to *S. citri* or to *Acholeplasma laidlawii*. However, former results obtained with immunofluorescence and with immunohistochemical techniques (DE LEEUW et al. 1977; DE LEEUW & POLAK-VOGELZANG 1978) indicated a possible serological relationship

to *A. laidlawii* of some of the MLO's studied. These contradictory results render a more detailed comparison of the sensitivity of the different methods used necessary.

- DE LEEUW, G. T. N., J. R. F. TH. GROESBEEK & A. R. SCHADEWIJK (1977): Immunofluorescence and immunohistochemistry of plant mycoplasmas in situ. (Abstr.) *Acta Bot. Neerl.* **26**: 272.  
 — & A. A. POLAK-VOGELZANG (1978): Attempts at serological characterization of plant mycoplasmas with immunofluorescence, immunohistochemistry and the microplate method of the enzyme-linked immunosorbent assay (ELISA). *Zentbl. Bakt. ParasitKde (Abt.A)* **241**: 230.

M. B. PONSEN (*Laboratorium voor Virologie, Landbouwhogeschool, Wageningen*)

# Replication of Adoxophyes nuclear polyhedrosis virus in the midgut of Adoxophyes orana (Lepidoptera: Tortricidae)

First-instar larvae of the summer-fruit tortrix moth, *Adoxophyes orana* F.v.R., were fed on broad bean leaves (*Vicia faba* L.) previously coated with a suspension of polyhedra. After 1–4 days, the midgut of a number of larvae was taken out and fixed for electron microscopy.

The polyhedra (0.7–1.8  $\mu$ m) consist of a cubic, crystalline mass of protein, in which single virus rods (250  $\times$  75 nm) were embedded at random. The rods were composed of a helical structure surrounding a fibrillar or rod-shaped structure. The nature of the two types of structure was not determined but may be DNA and protein. Both structures are enclosed within two membranes, namely a protein (inner) membrane and a unit (outer envelope) membrane. The midgut epithelium consisted of three types of cells; columnar, goblet, and regenerative. The goblet cells had an internal cavity opening into the lumen of the midgut. The cells were lined with a peritrophic membrane. The midgut lumen contained both "normal" polyhedra and partly dissolved ones together with digested leaf. Some rods disintegrating into the lumen, between the peritrophic membrane and microvillar system, and in goblet cavities. Some rods were attached to microvilli and the attached ones seem to be completely intact, including their unit membrane.

After fusion of the unit membrane with the microvillar membrane, the unenveloped virus entered the cell, then migrating to the nucleus or presumably directly to the basal cell membrane. The unenveloped rods attach to the nuclear pores after which the DNA penetrates the nucleus (SUMMERS 1971). One day after inoculation, chromatin of the nuclei of both columnar and goblet cells dispersed to the periphery, forming virogenic stroma; virus particles were in various stages of replication, and there were helical fibrillar masses. Membrane-like structures were present between the single rods, presumably serving to envelope the rods, of which some were enveloped, partly enveloped or unenveloped. There were also bundles of virus rods arranged in a crystalline pattern, from which rods separate to be subsequently enveloped by the membrane-like structures which originated from the helical fibrillar masses. The nuclei of both cell types of non-inoculated larvae had dense chromatin, dispersed in clumps.

After disintegration of the nuclear membrane, enveloped and unenveloped rods entered the cytoplasm where they were engulfed in vesicles to accumulate finally in the basal region of the cell. Engulfed, unenveloped virus rods may not originate from the nucleus after replication but from dissolved polyhedra in the gut lumen. Virus is transported to the haemocoel by fusion of the vesicle or envelope with the basal cell membrane, or by budding (TANADA & HESS 1976).

SUMMERS, M. D. (1971): Electron microscopic observations on granulosis virus entry, uncoating and replication processes during infection of the midgut cells of *Trichoplusia ni*. *J. Ultrastruct. Res.* **35**: 606–625.

TANADA, Y. & R. T. HESS (1976): Development of a nuclear polyhedrosis virus in midgut cells and penetration of the virus into the hemocoel of the armyworm, *Pseudaletia unipuncta*. *J. Invertebr. Pathol.* **28**: 67–76.

J. J. VAN DER PUT (*Laboratorium voor Virologie, Landbouwhogeschool, Wageningen*)

#### Latent virus infection in parsnip (*Pastinaca sativa*)

In our efforts to isolate a virus with filamentous particles from parsnip with yellow mottle we found two latent viruses with isometric particles.

One of them infected 22 out of 28 plant species and cultivars. This virus had a particle diameter of 25–31 nm, a sedimentation coefficient of 116–119 S, a buoyant density of 1.391 g/cm<sup>3</sup>, an iso-electric point of 4.5, a dilution end-point of 10<sup>-6</sup>–10<sup>-7</sup>, a thermal inactivation point of 95 °C and a longevity in vitro of 18 days. The virus reacted positively with antisera to tobacco necrosis virus. The molecular weight of its RNA was 1.38 × 10<sup>6</sup> D. From these results it was clear that this virus was identical to tobacco necrosis virus.

The other isometric virus infected only 4 out of 32 plant species and cultivars. Dip preparations for the electron microscope made from systemically infected leaves of *Chenopodium quinoa* plants revealed the presence of virus particles, most of them enclosed in tubular structures. In ultrathin sections these particles were also found either in tubular structures (in the cell wall) or freely distributed (in the cytoplasm, vacuole and cell wall). The virus had a particle diameter of 25–29 nm, a sedimentation coefficient of 125–131 S, a buoyant density of 1.446 g/cm<sup>3</sup>, a dilution end-point of 10<sup>-5</sup>–10<sup>-6</sup>, a thermal inactivation point of 60 °C and a longevity in vitro of 8 days. In epidermal cells of systemically infected leaves we found crystal-like structures reminiscent of those caused by NEPO-viruses. From these results we concluded that this latent virus may be identical to strawberry latent ringspot virus.

A. FUCHS and T. HIJWEGEN (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

#### Specificity in degradation of isoflavonoid phytoalexins

See *Acta Bot. Neerl.* 28 (2/3), 227–229 (this issue).

J. D. PAXTON (*University of Illinois, U.S.A., presently at Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

#### The possible breakdown of glyceollin and medicarpin by symbiotic microorganisms of soybean and lucern, respectively

Phytoalexins are thought to play an important role in the resistance of plants to diseases. And because of the toxicity of phytoalexins to many microorganisms, the ability to degrade phytoalexins is thought to play an important role in the virulence of plant pathogens.

Three isomers of glyceollin have been identified as phytoalexins of soybean which may be important in disease resistance. Since these isomers of glyceollin are toxic to *Phytophthora megasperma* var. *sojae* (a pathogen of soybean plants) *in vitro* at a concentration of 50 µg/ml, the ability to degrade or avoid these compounds may be important for virulence of this fungus.

Therefore the ability of *Phytophthora megasperma* var. *sojae* and *Rhizobium japonicum* (which nodulates soybeans) to degrade glyceollin isomers will be studied. Similar microorganisms on lucern (*Phytophthora megasperma* var. *megasperma* and *Rhizobium meliloti*) will also be contrasted for their ability to degrade medicarpin (a phytoalexin from lucern) and glyceollin.

L. C. DAVIDSE (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

#### Preliminary studies on the host-parasite combination lucern-*Phytophthora megasperma*

Root rot of lucern (*Medicago sativa*) caused by *Phytophthora megasperma* (Pm) is an economically important disease of this crop and is found world wide. The disease becomes apparent under wet soil conditions and causes damping-off of seedlings. Roots of older plants are affected which causes a



reduced productivity. The causal organism is morphologically similar to *Phytophthora megasperma* var. *sojae* (Pms), an important root pathogen of soybean (*Glycine max*). Lucern isolates of Pm are not pathogenic to soybeans nor are soybean isolates of Pms pathogenic to lucern.

Our research is focused on the physiology of the differential pathogenicity of both types of this fungus and the mechanism of resistance of some lucern cultivars to Pm. Since phytoalexins are thought to play a role in resistance of plants to fungi, the ability of Pm and Pms to induce the accumulation of medicarpin in lucern was investigated. Inoculation of 4- to 6-day old seedlings with zoospores of both types resulted in the accumulation of medicarpin. Up to c. 10 µg medicarpin/gram fresh weight accumulated in seedlings of the Pm-susceptible cultivar Vernal, inoculated with zoospores of Pm during a 48 h. incubation period.

Medicarpin, for use in toxicity and degradation studies, was isolated from wounded and Pm inoculated cotyledons of jack beans (*Canavalia ensiformis*). In liquid V8 medium (clarified and neutralized 10% V8 juice) growth of Pms was visibly affected at 23 µg medicarpin/ml, whereas this became apparent at 88 µg/ml with Pm. In the same medium after a 6-day incubation period mycelium of Pms was killed by medicarpin at a concentration between 114 and 175 µg/ml. At 350 µg/ml, the highest concentration studied, medicarpin was not lethal to Pm.

The differential sensitivity of Pms and Pm to medicarpin was not due to a differential breakdown of the compound. 60–80% of the medicarpin initially added to the medium could be recovered after the 6-day incubation period irrespective of whether Pms or Pm was growing or not.

## MEETING OF THE SECTION FOR VEGETATION RESEARCH ON MARCH 23, 1979

No abstracts of the papers read at this – hundredth – meeting of the section will be published in *Acta Botanica Neerlandica*; the papers have been collected in:

M. J. A. WERGER (ed.): *The Study of Vegetation, a review of the developments in the various branches of vegetation science with special attention to the Dutch contributions, compiled to celebrate the one-hundredth meeting of the Commission for the Study of the Vegetation of the Royal Botanical Society of the Netherlands*. W. Junk, 's-Gravenhage, 1979, XII + 317 p. ISBN 906193. f 115,-.