Acta Bot. Neerl. 30 (3), May 1981, p. 239–256.

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT PHYSIOLOGY ON SEPTEMBER 14, 1979

H. F. BIENFAIT and M. L. VAN DEN BRIEL (Laboratorium voor Plantenfysiologie, Universiteit van Amsterdam, Kruislaan 318, 1098 SM Amsterdam)

Ascorbate mobilizes ferritin iron in the presence of oxygen

Plant ferritin is generally assumed to release its iron in green or greening plastids. We studied ferritin iron mobilization under "illuminated stroma" conditions: 50mM ascorbate, pH 8.0 and oxygen. Under those conditions ferritin releases its iron, after a lag of about 4 hours, at a rate of 8 % per hour at 25°C. 10^{-5} M Cu^{++} decreases the lag to 1 hour, and increases the rate to 20 % per hour; oxygen is necessary for the reaction, and catalase inhibits strongly.

We conclude that an oxidation product of ascorbate, or an intermediate produced by its interaction with ferritin and oxygen, reduces ferritin iron. This compound is unstable or has a high affinity for ferritin iron.

J. SNEL, H. B. A. PRINS and R. J. HELDER (*Afdeling voor Plantenfysiologie, Biologisch Centrum, postbus 14, 9750 AA, Haren (Gr)*)

Some aspects of the photosynthetic use of bicarbonate by aquatic angiosperms

Use of HCO_3^- as carbon source for photosynthesis by aquatic species leads to the production of one OH^- for every molecule CO_2 fixed. The OH^- is subsequently released to the medium. In a number of HCO_3^- using aquatics with so-called polar leaves (a.o. *Elodea* and *Potamogeton*) the site of HCO_3^- uptake and OH^- release are spatially separated. HCO_3^- is taken up at the morphologically lower side of the leaf while OH^- is released at the upper side. This flux of negative ions from the lower to the upper side is balanced by a kation flux in the same direction.

The uptake of HCO_3^- and kations at the lower side is accompanied by a pH drop here. The release of OH^- at the upper side causes a sharp raise of the pH here. The pH changes at both sides and the kation (in our experiments K^+) concentrations at the leaf surfaces can be measured with miniature pH and K^+ sensitive electrodes which touch the leaf surface. From this the CO_2 (including H_2CO_3), HCO_3^- and $CO_3^=$ concentrations near the leaf surface are calculated. After the onset of light following a light period the pH at the upper and lower side increases at both sides at the same rate. As both sides behave in the same way during this initial phase we call this the apolar phase. During the apolar phase there is no K^+ transport through the leaf. Experiments at different ambient pH's and comparison with aquatic plants which can only use CO_2 (and not HCO_3^-) indicate that this initial pH raise results from "normal" CO_2 fixation. After 5–10 minutes the polar phase starts. At the lower side the pH and the K^+ concentration drop; at the upper side the pH and the K^+ concentration increase markedly.

Calculation of the CO_2 concentration at the lower side of a leaf of *Potamogeton lucens* showed that during the apolar phase the concentration of CO_2 decreased from 4×10^{-5} mol.l^{-1} ($\sim$ 1200 ppm) in the dark to 0.7×10^{-5} mol.l^{-1} ($\sim$ 200 ppm) whereafter in the polar phase the CO_2 concentration increased to 40×10^{-5} mol.l^{-1} ($\sim$ 12000 ppm)! Thus we have the paradoxical situation of a rapidly photosynthesizing leaf increasing the CO_2 concentration in the medium. This sharp increase of CO_2

may be explained either by CO_2 diffusion from the leaf cells previously taken up as HCO_3^- or by proton pump activity at the lower epidermis causing extra cellular conversion of HCO_3^- into CO_2.

J. VAN BREDERODE[1], G. VAN NIGTEVECHT[1] and G. J. NIEMANN[2] ([1]*Vakgroep Populatie- en Evolutiebiologie, Padualaan 8, 3584 CH Utrecht* [2]*Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

The significance of Silene alba flavonoids in the plant/environment interaction

Silene alba populations from different localities in Europe are rather variable in morphology and chemical constitution. Especially in the glycosylation pattern of the flavone 6-C-glucoside isovitexin, present in all plants, there is a remarkable geographical variation. In western Europe isovitexin 7-0-glucoside (7 Glc) is the only isovitexin glycoside found. The formation of this compound is governed by gene g^G. The frequency of this gene diminishes in eastern direction. In eastern Europe isovitexin 6″-rhamnosides (6 Rha) and isovitexin 6″-rhamnosides, 7-glucosides (6 Rha, 7 Glc) are found. The rhamnosylation of isovitexin is governed by gene gl^R, the frequency of which is normally one in eastern European populations but zero in western ones (VAN NIGTEVECHT & VAN BREDERODE 1972).

From climatological experiments with *S. alba* plants with a homogenous genetic background (F 2 of the cross $\frac{g^G}{g^G}\frac{gl}{gl} \times \frac{g}{g}\frac{gl^R}{gl^R}$), it appears that:

A: plants containing isovitexin have strongly deformed petals and leaves and stay behind in growth.
B: plants with 6 Rha in a cold/wet climate differ in leaf-form from 7 Glc and 6 Rha, 7 Glc plants.
C: 6 Rha plants stay behind in total leaf growth in a cold/wet climate when compared with 7 Glc and 6 Rha, 7 Glc. In a hot/dry climate a significant recovery in growth of 6 Rha plants occurs after a previous delay.

These results provide a possible explanation for the distribution of *S. alba* flavonoid types over Europe, in which 6 Rha plants were found to be almost absent in the western sea climate.

G. VAN NIGTEVECHT; J. VAN BREDERODE (1972): Flavonoid-glycosylation genes in European populations of Melandrium album and Melandrium dioicum. Genen Phaenen **15**: 9–13.

H. H. VAN GENDEREN, W. J. BAAS and G. J. NIEMANN (*Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Phenols and triterpenes of Hoya australis related to leaf development

Hoya australis leaves contain chlorogenic acid, isochlorogenic acid, glucosyl-*p*-coumarate and low concentrations of the flavones apigenin-7-glucoside, -7-rutinoside and -7(ferulylglucoside) and chrysoeriol-7- rutinoside. In the leaf wax the triterpenols β-amyrin and lupeol were found.

In the course of leaf development the concentration of the phenolic depsides decreases rapidly to reach a more or less stable, low concentration in just fully expanded leaves. At this stage the cyanidin glucosides, found in very young leaves, could no longer be detected. On the other hand, a large accumulation of lupeol starts just after the disappearance of the anthocyanins and the decrease in phenolic depside concentration. The results suggest the possibility of some physiological interaction.

I. STULEN, H. LAMBERS, F. POSTHUMUS, L. LANTING, S. VAN DE DIJK and R. HOFSTRA (*Afdeling voor Plantenfysiologie, Biologisch centrum, Postbus 14, 9750 AA Haren (Gr)*)

Energy and nitrogen metabolism of Plantago lanceolata as dependent on the supply of nutrients

The current investigation with *Plantago lanceolata* is the first in a series of experiments on the adaptation of species from various habitats to the supply and to a sudden change in the supply of nutrients.

Seedlings of *Plantago lanceolata* were initially grown on either a high nutrient solution (1/4 strength Hoagland) or a low nutrient solution (2% of the high nutrient solution). After 29 days half of the high nutrient plants were switched to low nutrient conditions (100% → 2% plants) while the other half was kept in a high nutrient solution (100% plants). Likewise, half of the low nutrient plants were switched to high nutrient conditions (2% → 100% plants), while the other half remained on the low nutrient solution (2% plants). From one week before to two weeks after the switch various processes related to energy and nitrogen metabolism were regularly determined: Growth, photosynthesis, dark respiration of the shoot, root respiration (including the activity of the alternative chain), nitrate uptake, nitrate reductase (NR), glutamate dehydrogenase (GDH) and glutamine synthetase (GS). Sugar and starch content, free nitrate content and total nitrogen content (soluble and insoluble) were determined in the same material.

Transfer of the plants from 100% → 2% immediately affected shoot growth, while root growth was not affected. This resulted in a decreased shoot/root ratio. Relative growth rate of both roots and shoots of the 100% plants was approximately constant. A switch from 2% → 100% decreased the rate of dry matter production in the roots while shoot growth rate was increased after a couple of days. The relative growth rate of both shoots and roots of the 2% plants decreased constantly.

The growth data revealed that growth of the 100% → 2% plants occurred at the expense of stored ions. This was confirmed by data obtained for the nitrate content of both roots and shoots, which showed that the free nitrate content rapidly declined after the switch from 100% → 2%. It is very likely that the osmotic function of the ions was replaced by sugars. The sugar content of the roots increased immediately after the switch. At the same time an increase in photosynthetic rate was observed.

The experiments showed a close relationship between growth, photosynthesis and respiration. In the 100% plants photosynthesis gradually decreased with increasing age of the plants while growth was not hampered. The decrease in photosynthesis appeared to be compensated by an increase in the efficiency of root respiration due to a decrease in the non-phosphorylative alternative chain. The data obtained for the 2% and the switched plants also showed that the alternative chain enables *Plantago lanceolata* to react very flexibly upon a sudden change in the nutrient conditions.

The enzymes of nitrogen metabolism studied (NR, GDH and GS) all responded to a change in the nutrient supply. Not only the adaptive enzyme NR but GDH and GS as well increased upon a switch from 2% → 100% and decreased upon a switch from 100% → 2%. NR activity in the roots, as measured by an intact tissue assay, decreased with increasing age of the seedlings, as did root respiration. When plotted against each other a linear relationship was obtained. This strongly points to the fact that the reducing power for nitrate reductase in the roots is derived from glycolysis. Total nitrogen content of both shoots and roots was correlated with the supply of nutrients in the root environment. Nitrogen content changed rapidly after a switch from either 100% → 2% or 2% → 100%.

The data presented here clearly show that *Plantago laceolata* can adapt various processes related to energy and nitrogen metabolism after a sudden switch in the nutrient supply.

This experiment is part of a comparative study with species from various habitats. Whether the fact that a species can react flexibly is of ecological significance cannot be concluded until results from similar experiments with other species are known.

Grassland Species Research Group, Publ. no. 21.

G. E. DE VRIES, P. A. IN 'T VELD and J. W. KIJNE (*Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden*)

Enzyme induction in Pisum root nodules as a result of hypoxia

Nitrogen fixation takes place in root nodules under low oxygen concentration, due to increased respiratory activity in infected root nodule cells, when compared to ordinary root cells.

Activity of alcohol dehydrogenase was found in crude extracts of roots with a Km = 23 mM towards acetaldehyde. Increased activities were detected in root nodule cell extracts with higher affinity towards acetaldehyde (Km = 1.3 mM). This parallels the reaction of *Pisum* roots to a state of

hypoxia caused by waterlogging. Increased activities of PEP-carboxylase and malate dehydrogenase were also found in root nodule cells. These enzymes form an alternative pathway under oxygen stress, and effect malate production. Synthesis of malate by CO_2 dark fixation via PEP carboxylase was reported for broad bean root nodules.

It is postulated that a PEP carboxylase – malate dehydrogenase pathway, leading to malate accumulation, is induced in *Pisum* root nodules by a state of oxygen shortage, or hypoxia.

Earlier results from our laboratory showed that acetylene reduction and oxygen uptake by isolated bacteroids was stimulated by succinate, fumarate and malate while various common sugars did not show these responses.

It is suggested that malate, or other derived organic acids, could serve as primary carbon and energy source for the bacteroids if accumulated within the peribacteroid membrane. This mechanism would parallel the accumulation of malate in the vacuole of root cells under hypoxia in various species.

J. W. KIJNE, C. A. M. VAN DER SCHAAL and G. E. DE VRIES (*Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden*)

Pea lectins and the recognition of Rhizobium leguminosarum

The two pea "Rondo" seed lectins were isolated by affinity chromatography on Sephadex G-100 and chromatography on DEAE-cellulose DE 32. Both lectins agglutinated human A erythrocytes, and *Rhizobium leguminosarum*. Agglutination by both lectins of both types of cells could be inhibited by the same haptens, (α-bound) sugars with unsubstituted C_4- and C_6-hydroxyls in the same stereochemical position as with glucose or mannose. GLC-MS analysis indicated that one lectin is the glycosylated form of the other lectin, with mannose as the most probable and only neutral sugar present (5%w/w).

Pea root slime contains at least two glucose-binding proteins, one of which is a red cell and *Rhizobium leguminosarum* agglutinating lectin with the same electrophoretic mobility as the glycosylated seed lectin. Both glucose-binding proteins are co-purified with a carbohydrate fraction of glucan-nature. Neutral sugar analysis suggests that pea root slime is pectine in nature, although poor in rhamnogalacturonate. It is hypothesized that *Rhizobium* is recognized in leguminous root slime by a lectin which is normally engaged in glycan metabolism.

W. L. HOMAN (*Plantenfysiologisch Laboratorium, Universiteit van Amsterdam, Kruislaan 318, 1098 SM Amsterdam*)

Sexual cell recognition in Chlamydomonas eugametos

Sexual reproduction in *Chlamydomonas eugametos* takes place by mating-type specific adhesion of the flagella, which eventually leads to cell fusion and zygote formation. The aim is to determine which of the eight discernable complex carbohydrate fractions present in the flagellar membrane is involved in this adhesion process. We have found that membrane vesicles (isoagglutinins) rapidly lose their biological activity (isoagglutination) by sonification. The active component(s) are solubilized but can be recombined with the membrane fraction with restoration of biological activity. By gel filtration on Biogel H 150-M evidence is obtained that biological activity is associated with only one high-molecular weight glycoprotein.

Analysis of isoagglutinins of mating type (+) and (–) indicates that only lipids and glycoconjugates are present, and the most striking difference between both mating types is in the carbohydrate composition. Results of these analyses related to the reconstitution experiments are presented.

P. F. LENS (*Plantenfysiologisch Laboratorium, Universiteit van Amsterdam, Kruislaan 318, 1098 SM Amsterdam*)

Sex-specific glycoproteins in flagella of Chlamydomonas eugametos

To identify mating-type specific glycoprotein(s) present in flagellar membranes of *Chlamydomonas eugametos*, antibodies were raised against purified flagella. The Ig fractions were tested for mating-type specificity by Ouchterlony double immunodiffusion, agglutination of live gametes, indirect immunofluorescence; crossed immunoelectrophoresis and incubation of SDS-gels of flagellar proteins with the Ig fraction of antiserum and subsequent staining with peroxidase labelled goat anti-rabbit IgG.

The IgG fraction exhibited partial mating-type specificity in the immunofluorescence, the agglutination of gametes, and in the immunoelectrophoresis test. No specificity was shown in the immunodiffusion and in the SDS-gels of flagellar proteins. The specificity could be made absolute by absorption with flagella of the opposite mating type. On immunoelectrophoresis it then appeared that one component near the origin was mating-type specific, consisting of two glycoprotein fractions, which both possessed mating-type specific antigenicity as was confirmed by the peroxidase staining of SDS-gels of flagellar material incubated with absorbed Ig fractions. Since these two glycoproteins exhibit sex specificity they could well be involved in sexual agglutination.

H. LUBBERDING, H. MATTHIJS, and M. SCHOLTS (*Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Energy metabolism in bluegreen algae

The energy for growth of bluegreen algae is derived from light and in some cases from sugars. For the investigation of the membrane properties in the light and in the dark and the ATPases, cell fractions are made by lysozyme treatment.

The heterotrophic species *Plectonema boryanum* is used to study the mechanistic interaction in the light and in the dark of electron transport and the localization of both processes in the cell. When cell membranes are separated on sucrose gradients, solely photosystem I activity can be detected. Under certain circumstances of growth the thylakoid membranes degenerate, resulting in yellow cells which still consume O_2. It seems however, that the cytoplasmic membranes do not contain cytochromes.

The uptake of glucose and ribose is an active process. The energetization of transport is related to both energy conserving processes in the cell.

In a study of the effect of temperature on membrane composition and functioning, particularly on ATPase, two closely related photoautotrophic bluegreen algae, *Synechococcus lividus* (thermophilic) and *Coccochloris peniocystis* (mesophilic) have been studied.

From ATPase activation experiments and the effect of inhibitors it has been concluded that the ATPases from the bluegreen algae are more related to those of chloroplasts than to those of mitochondria and most bacteria. The properties of the soluble coupling factor AF1 of both organisms were compared with those of the membrane-bound ATPases and of cytoplasmic enzymes such as glucose-6-phosphate dehydrogenase. The cytoplasmic enzymes of the thermophilic bluegreen algae have a reduced affinity for their substrates. It is likely that properties of enzyme proteins as well as membrane characteristics make thermophilic life possible. Reconstitution of mesophilic AF1 in thermophilic thylakoids and vice versa should give more information about this ambiguity.

L. H. W. VAN DER PLAS and M. J. WAGNER (*Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Changes in mitochondrial characteristics during the induction of potato tuber callus

Incubation of potato tuber discs on agar nutrient medium with hormones (a cytokinin and a

synthetic auxin) leads to callus induction and a doubling of fresh weight in about 2–3 weeks. During this incubation mitochondrial cytochrome oxidase increases steadily. Mitochondrial cyanide-resistant alternative oxidase is not detectable in freshly sliced tissue, but it is induced during callus formation. Four days after the start of the incubation, alternative oxidase activity is about 40 % of the uninhibited respiration and this percentage remains stable afterwards.

Incubation of potato tuber tissue discs on agar nutrient medium without hormones does not lead to callus induction or changes in the fresh weight. Mitochondrial cytochrome oxidase activity of these discs only increases during the first week of incubation and remains stable afterwards. When freshly harvested potatoes are used some induction of alternative oxidase activity occurs in these slices but this activity stabilizes at a value of about 10 % of the uninhibited respiration. In slices from potatoes, stored for more than six months after harvest, the induction of mitochondrial alternative oxidase activity occurs despite the absence of hormones in the agar, to about the same level as in callus-forming slices. This is possibly due to an increase in endogenous auxin or to a decrease in substances with anti-auxin properties during storage.

Investigations on the effect of chloramphenicol, added to the nutrient medium during callus induction, were started. This inhibitor of mitochondrial protein synthesis inhibits callus formation and induces a decrease in cytochrome oxidase activity. Preliminary conclusions are, that this decrease is not accompanied by an increase in alternative oxidase activity. It possibly leads to an increase in alcoholic fermentation in the slices.

G. J. VAN HOLST and F. M. KLIS (*Laboratorium voor Plantenfysiologie, Universiteit van Amsterdam, Kruislaan 318, 1098 SM Amsterdam*)

Isolation and partial characterisation of a cell wall precursor from stem tissue of bean seedlings (Phaseolus vulgaris L.)

Sonical disruption of a crude organelle fraction obtained by differential centrifugation liberated some hydroxyproline-containing material. Pulse-chase experiments demonstrated that the hydroxyproline-containing substance(s) were chaseable indicating that they are indeed cell wall precursors. We purified the hydroxyproline-containing material by a two-step procedure, consisting of gel filtration over Sephadex G-150 followed by isoelectrofocusing. The purified material had an apparent molecular weight of 120,000 daltons and an isoelectric point of about 2. The protein-carbohydrate ratio was 1:8. The carbohydrate moiety was rich in galactose, arabinose and uronic acid; the protein portion was rich in hydroxyproline (24 mole %), serine (15 mole %), alanine (13 mole %) and glycine (11 mole %). All the evidence available suggests that we have isolated a so-called arabinogalactan protein, that is on the way to the cell-wall.

F. M. KLIS and P. VAN EGMOND (*Laboratorium voor Plantenfysiologie, Universiteit van Amsterdam, Kruislaan 318, 1098 SM Amsterdam*)

Loosely-bound hydroxyproline-containing proteins in walls of suspended bean cells (Phaseolus vulgaris L.)

The amount of protein released from isolated cell walls during sonical treatment depended on sonication time and on the composition of the extraction solution. Although sodium dodecyl sulphate (2 %, w/v) was more effective than sodium chloride (1M) in liberating protein from cell walls, an equal amount of hydroxyproline-containing proteins was released in both cases. Sodium chloride extractable wall proteins were run on sodium dodecyl sulphate polyacrylamide gels under reducing conditions. Staining with Coomassie Brilliant Blue revealed two major bands and several minor ones. When cells were labelled with ^{14}C-proline for 30 min, most but not all bands became radioactively labelled. Radioactive hydroxyproline was detectable in three bands and in high molecular weight material incapable of penetrating the gel. The results suggest that isolated cell walls contain several loosely-bound hydroxyproline-containing proteins.

F. VAN IREN and P. BOERS- VAN DER SLUIJS (*Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden*)

Rb^+ transport in barley roots: epidermis (and hypodermis) provide for primary absorption

When plasmolyzed with mannitol or sucrose excised barley roots have a reduced uptake capacity. Kinetics indicate that the reduction largely coincides with incipient plasmolysis. Localization by means of precipitative freeze dissolution and autoratiography reveals that epidermis (and hypodermis) have taken up normal amounts of Rb^+ while cortical uptake has become low to negligible. So the cortical plasmalemmas have little or no uptake capacity and the cortex normally receives the ions by symplasmic transport from the outermost cell layer(s). The cortical free space is not involved in ion uptake, at least for Rb^+ in the concentration range of mechanism I.

An extensive report will appear in: *Planta*, 1980.

A. J. E. VAN BEL and C. VAN DER SCHOOT (*Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Proton co-transport during the uptake of amino acids by xylem parenchyma cells.

The existence of co-transport in the amino acid uptake was investigated in xylem parenchyma cells of tomato internodes. In the light, concentrations of K^+, lower than about 5 mM, stimulated the uptake of alpha-aminoisobutyric acid and alanine, whereas higher K^+-concentrations (up to 50 mM) increasingly inhibited it. At all concentrations of K^+, the uptake was in the dark 30% lower than in the light. It was assumed that light hyperpolarized the membranes (the cells contained chloroplasts) and that high K^+-concentrations caused depolarization. The stimulation of the uptake by low K^+-concentrations was attributed to K^+-co-transport.

The apparent presence of co-transport through the xylem parenchyma cell membranes raised the question, whether proton co-transport is also involved in the xylem-to-phloem transfer. High K^+-concentrations in the xylem vessel fluid of plants without roots drastically decreased the xylem-to-phloem transfer.

Introduction of CCCP into the xylem vessels evoked a similar effect. Both observations indicate that co-transport mechanisms may play a role in the transfer of materials from the xylem to the phloem.

P. WOLSWINKEL and G. DE BEER (*Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Efflux of solutes from the sieve tube system and from parenchymatous cells in stem parts of Vicia faba

By washing out solutes from the free space of stem segments of *Vicia faba*, the release of solutes by stem tissues can be measured. In stem parts parasitised by *Cuscuta* an enhanced release of solutes like sucrose and K^+ can be demonstrated.

In a new series of experiments we have started to study the influence of the composition of the washing solution on efflux phenomena. In experiments in which ^{14}C could be expected to be mainly present in phloem cells during the washout experiment, addition of several buffers to the washing solution resulted in a strongly enhanced release of ^{14}C-solutes during the last hours of the experiment. When ^{14}C was present in parenchyma cells, addition of the same buffers did not clearly influence the efflux phenomena. The phloem cells (sieve tube elements will be most important in this context) seem to be much more sensitive to external factors than cells of parenchymatous tissues of the stem. Possibly, the absence of a tonoplast in sieve tube elements, as reported in literature, is related with this phenomenon.

P. M. SCHILDWACHT (*Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Cell elongation, leaf water potential and turgor in Zea mays

P. MIEDEMA (*Stichting voor Plantenveredeling, Droevendaalse steeg 1, 6708 PB Wageningen*)

The effect of low temperature on germination and growth of seedling in Zea mays

H. L. KRAAK (*Laboratorium voor Plantenfysiologisch Onderzoek, Gen. Foulkesweg 72, 6703 BW Wageningen*)

The influence of the phytochrome system on development and characteristics of etioplasts

H. VEEN (*CABO, Bornsesteeg 65, 6708 PD Wageningen*)

Antiethylene action of silver salts

J. BRUINSMA and J. M. FRANSSEN (*Botanisch Laboratorium, Arboretumlaan 4, 6703 BD Wageningen*)

Phototropism as a phenomenon of inhibition

A. VARGA and J. BRUINSMA (*Botanisch Laboratorium, Arboretumlaan 4, 6703 BD Wageningen*)

Tomato fruit development in vitro as a tool of fundamental research

C. M. KARSSEN (*Botanisch Laboratorium, Arboretumlaan 4, 6703 BD Wageningen*)

Levels of ABA during primary and secundary dormancy in seeds of weedy species

P. J. C. KUIPER (*Afdeling voor Plantenfysiologie, Biologisch Centrum, Postbus 14, 9750 AA Haren (Gn)*)

A physiological study of Plantago species from different habitats: growth reaction of the plant, ATPases and lipids in roots in relation with the mineral nutrition of the plant

C. E. E. STUIVER, L. ERDEI and P. J. C. KUIPER (*Afdeling voor Plantenfysiologie, Biologisch Centrum, Postbus 14, 9750 AA Haren (Gn)*)

Lipid composition of Plantago species in relation with salt resistance

P. R. VAN HASSELT (*Afdeling voor Plantenfysiologie, Biologisch Centrum, Postbus 14, 9750 AA Haren (Gn)*)

The inhibition of photosynthesis of gherkin leaf discs by light at low temperature

R. KRAAYENHOF, A. L. J. PETERS and J. J. SCHUURMANS (*Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

The mechanism of photosynthetic energy conservation

T. TIETEMA and F. VAN DER AA (*Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Xylem transport in rhizomes of Carex arenaria

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON SEPTEMBER 24, 1980

I. S. ZONNEVELD (*I.T.C., Boulevard 1945, 7500 AA Enschede*)

General aspects of the vegetation in semi-arid areas

L. LINSEN and W. GIESSEN (*Afdeling Gebotanie, Toernooiveld, 6525 ED Nijmegen*)

Composition and structure of semi-arid vegetation-types in the area of Mt. Kenya and S. of Isiolo

H. VAN GILS[1] and F. VAN DER MEULEN[2] ([1]*International Institute for Aerial Survey and Earth Sciences, Enschede;* [2]*Duinwaterleiding, Den Haag*)

Savannas in Southern Africa

Four stations were selected for detailed description because of the availability of appropriate data. The selected locations are: Matsheng village area, Kalahari, Rep. Botswana; Kuhtse, Kalahari, Rep. Botswana; Nietverdiend, Western Transvaal, Rep. South Africa; Nylsvley Northern Transvaal, Rep. South Africa.

Data were supplied on the following landscape features:

(i) *climate*: type, precipitation, temperature,
(ii) *physiography:* altitude, relief, soil type, soil pH, drainage pattern,
(iii) *vegetation*: structure, dominant plant species, growth form composition, phytomass, C.P., grazing capacity,
(iv) *large herbivores*: domestic livestock and wildlife species,
(v) *land utilization types*: arable agriculture, grazing, hunting-gathering, nature conservation, urban.

Some conclusion drawn after a comparion of the data are:

- The similarity of the vegetation (structure, floristic composition, growth form composition) over such a long distance is remarkable.
  For example in all cases the ground layer consists mainly of perennial grasses contrary to the west-african savannas.
- The gradient in the amount of precipitation along the transect is not reflected in the vegetation structure as far as mesophyllous savannas and microphyllous thorn savannas are compared. However, the height of the trees is positively correlated with increasing precipitation.
- The most striking differences between the four stations are the land utilization types ranging from nature conservation/traditional hunting-gathering (Kuthse) over communal cattle/goat grazing/hunting till commercial fenced cattle ranches subdivided in paddocks (including dry farming of fodder crops).
- The differences in land utilization types along the transect are explained by the accessibility of drinking water and colonisation in the last century by white pastoralists who knew how to drill wells and black pasturalists without this knowledge.
- The current trend in the Kalahari of Botswana is to replace wildlife grazing areas by commercial cattle ranches. It is argued that this change in land use is not profitable.

Instead of cattle ranching some improved wildlife utilization types are presented.

C.A.R.A.P (1980): *Countrywide animal and range assessment Project, Botswana.* DHV/ITC, Amersfoort: 7 vols. + maps.

F. VAN DER MEULEN, (1979): Phytosociology of the western Transvaal Bushveld. *Diss. Bot.* Bd. **49**. Cramer, Lehre.

E. WESTINGA (*Euroconsult, Arnhem*)

Vegetation and landscape mapping of the Rada district, Yemen Arab Republic

The vegetation of Yemen is diverse, because of the many different habitats present. Yemen has a flora with many African, but also Oriental elements. On the high plateaux and mountains elements from northern and colder regions are found.

Yemen can be divided geographically in three main areas. First by the Tihama, a coastal plain, which is a low lying, hot sandy desert, between the Red Sea and the excarpment, that lies almost parallel to the coast.

Second by the excarpment and the mountains, which have a rich flora, with numerous species of African affinity. The enormous amount of cultivated terraces dominates the landscape. And third by the montane plains and mountains at a general altitude of 2000-2500 m. In this third area the Rada district is situated.

A survey and problem analysis was carried out in the Rangelands of the Rada district, as a part of a development project of the Netherlands carried out by Euroconsult. During this study a vegetation and landscape map (1:100000) was produced with the help of aerial photographs (1:62500).

The rada district was geologically divided into six main landscape types: 1. Eastern gneiss and granite hills, 2. Northern gneiss mountains, 3. Tawilah sandstone plateau, 4. Tertiair volcanic hills and mountains, 5. Quartair volcanic plateaus and volcanoes, 6. Extensive saline and cultivated areas.

The main landscape types were subdivided on geomorphological grounds in 23 sublandscapes. These sublandscapes formed the map units. Per sublandscape the vegetation in the different terrain units (wadi, valley, slope, plateau) was investigated floristically for the perennials only.

The vegetation of the Rada district is very poor and scanty. Almost the whole surface is covered with loose stones (hammada), rocky outcrops or bare soil. The vegetation samples were classified floristically and 23 vegetation types could be distinguished, which were matched with the different map units.

The main factors influencing the vegetation were 1. the type of terrain unit, 2. the altitude and 3. the influence of man (cutting of trees and grazing by the animals). The first two factors determine the availability of water which in semi-arid areas is very important.

The survey and the map have provided a better understanding of the rangelands as well as of the food supply of the animals. Measures are currently being undertaken for improvement (re-afforestation efforts with local species).

H, DOING (*Afdeling Vegetatiekunde en Plantenoecologie, De Dreyen 11, 6703 BC Wageningen*)

The vegetation of semi-arid regions in Australia

M. J. A. WERGER (*Vakgroep Vegetatiekunde en Botanische Oecologie, Heidelbergerlaan 2, 3584 CS Utrecht*)

Form and function of plants in arid regions of southern Africa (Karoo and Kalahari)

MEETING OF THE SECTION FOR PLANT PATHOLOGY ON OCTOBER 30, 1980

P. J. G. M. DE WIT, E. KODDE, G. MULDER and I. P. RIETSTRA (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhavenweg 9, 6709 PD Wageningen*)

Structure and specificity of glycoprotein elicitors from culture filtrates and cell walls of Cladosporium fulvum (syn. Fulvia fulva)

Cell-free culture filtrates and mycelial walls of *Cladosporium fulvum* (syn. *Fulvia fulva*) contain high-molecular weight components which elicited accumulation of rishitin in tomato fruits and also polyacetylenic phytoalexins in fruits as well as leaves. Elicitors from cell walls (CWE) appeared to be 5 tot 10 times more active than those isolated from culture filtrates (CFE). The chemical composition of CFE is dependent on the composition of the growth medium and the age of the culture. CFE of young cultures contains nearly only glucose and is not a very active elicitor of rishitin accumulation. At the end of the growth cycle *Cladosporium fulvum* produces CFE that contains in addition to glucose a high percentage of mannose and galactose. CFE of old cultures is very likely derived from the cell wall and very similar to CWE.

CWE is a water soluble peptido galactoglucomannan that can be extracted from the cell walls by water at 100°C. The carbohydrate moiety of the peptido galactoglucomannan has a mannose: galactose ratio of 1.2:1 and contains traces of glucose. Methylation analysis showed mannose but mainly galactose as non-reducing end groups and chains with 1→2 linked mannose and 1→6 linked galactose. There are many branch points in the structure.

The protein moiety is rich in alanine, asparagine/aspartic acid, glutamine/glutamic acid, proline, serine and threonine. The carbohydrate of the peptido galactoglucomannan is O-glycosidically linked to serine and threonine as was shown by β-elimination.

There is a strong positive correlation between the mannose and galactose content of the peptido galactoglucomannan and its rishitin- and necrosis-inducing activity. The necrosis and rishitin induction by the peptido galactoglucomannan is likely mediated by binding of its terminal mannose and/or galactose residues to the host cell.

The peptido galactoglucomannan appeared to be neither race- nor cultivar-specific with respect to the accumulation of phytoalexins in tomato leaves and fruits.

O. KAMOEN (*Rijksstation voor Plantenziekten, Merelbeke, België*)

Elicitors of phytoalexins from Botrytis cinerea.

Exopolysaccharides from *B. cinerea* can be fractionated in glucan, mannan and heteropolysaccharides. The separation was carried out by fractional precipitation with ethanol, by separation on Sepharose 6 B, Sephacryl S 300, DEAE Sepharose CL-6B and Concanavalin A.

The methods and procedures for elicitor testing have already been described (KAMOEN et al. 1980).

Both the glucan and heteropolysaccharide fractions contain thermostable elicitors. Elicitation of phaseolin was possible on the epidermis and in the intercellular spaces of bean leaves (*Phaseolus vulgaris*).

Mannan was not tested as an elicitor because contamination with α-methyl-D-mannoside (an inhibitor for elicitors) from the Con A was found.

It is difficult to obtain polysaccharides which are completely free from proteins. Some polysaccharides also show a stron mutual affinity.

The affinity of fungal polysaccharides with proteins (lectins) or with the polysaccharides of the plant may explain their mode of action. Incubation of leaf pieces in polysaccharide solutions induce morphological changes in the cells (KAMOEN et al. 1978) These can be related to structural disturbances at the cell surface, due to affinity phenomena.

KAMOEN, O., G. JAMART, H. DECLERQ & D. DUBOURDIEU (1980): Des éliciteurs de phytoalexines chez le Botrytis cinerea. *Ann. Phytopath., Paris* (in press)

KAMOEN, O., G. JAMART, R. MOERMANS, L. VANDEPUTTE & D. DUBOURDIEU (1978): Comparative study of phytotoxic secretions of Botrytis cinerea Pers. ex. Fr. *Med. Fac. Landbouww. Rijksuniv. Gent* **43** (2): 847–857.

H. W. M. FUCHS, J. L. M. VAN DER LUBBE and A. FUCHS (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 6709 PD Wageningen*)

The effect of cold storage, plant age and pod size on the ability of pea pods to accumulate pisatin

Various factors are known to influence the capacity of pea pods (*Pisum sativum* L.) to accumulate pisatin. For instance, CRUICKSHANK & PERRIN (1963) found pod maturity and storage conditions (4° versus 20 °C; aerated versus 'sealed') to have a pronounced effect on the ability of pea pods to form pisatin following inoculation with *Monilinia fructicola*. Similar effects of cold storage were observed with chemically unrelated phytoalexins such as rishitin and lubimin induced in potato tuber discs upon treatment with Hg-acetate and u.v. light (CHEEMA & HAARD 1978).

For experiments on pisatin degradation (cf. Fuchs et al. 1980) large amounts of pisatin were needed and, therefore, appropriate numbers of pea plants, cv. Gloire de Quimper, were grown under climate room conditions. Pea pods of maturity class 1–2 according to CRUICKSHANK & PERRIN (1963) were harvested at varying time intervals from about 6 weeks after sowing, with two distinct peaks in pods produced after c. 7 and 10 weeks. All pods were stored at 4 °C until use. Pisatin accumulation was induced by incubating pea pod halves, without seeds, in an aerated Hg-acetate-containing mineral solution. After 1, 2, 3, and 4 days pisatin was extracted and quantitated spectrophotometrically as described by CRUICKSHANK & PERRIN (1961).

Preliminary observations showed the amounts of pisatin induced to increase, for up to 4 weeks, almost linearly with the duration of cold storage. Plant age at the time of harvest – that is the time elapsed from sowing the seeds until harvesting the pods – hardly influenced the amount of pisatin accumulated, with an only very slight tendency to increase with time.

To substantiate these preliminary observations experiments were initiated in which the effect of cold storage and plant age were studied independently of each other. To this end, the two 'peak' harvests, representing two age classes, were stored at 4 °C, and from these lots samples were taken after known storage periods and further treated as described above. In addition, other samples were harvested weekly and stored for one week only, and then processed. With increasing plant age the size of pods of similar maturity appeared to decrease; hence, it was virtually impossible to clearly separate the effects of plant age and pod size. Again, the amount of pisatin accumulated after induction with Hg-acetate increased almost linearly with time, until after 4 weeks of cold storage a level was attained about 3 times that of pea pods stored for 1 week only. Upon longer storage the amount decreased again to zero after another 3 weeks. Pisatin accumulation also seemed to increase with plant age. However, since concomitantly average pod size decreased to about a third of that of the first harvested pods, this increase in pisatinforming capacity should rather be ascribed to decreasing pod size.

Apparently, post-harvest storage conditions and plant age/pod size strongly influence the pods' ability to form pisatin. These effects should be taken into account not only when large amounts of pisatin are needed for experimental work, but also in any discussion on the role of pisatin in disease resistance.

CHEEMA, A. S. & N. F. HAARD (1978). Induction of rishitin and lubimin in potato tuber discs by non-specific elicitors and the influence of storage conditions. *Physiol. Pl. Path.* **13**: 233–240.

CRUICKSHANK, I. A. M. & D. R. PERRIN (1961). Studies on phytoalexins. III. The isolation, assay and general properties of a phytoalexin from Pisum sativum L. *Aust. J. biol. Sci.* **14**: 336–348.

— & — (1963). Studies on phytoalexins. VI. Pisatin: the effect of some factors on its formation in Pisum sativum L., and the significance of pisatin in disease resistance. *Aust. J. biol. Sci.* **16**: 111–128.

FUCHS, A., F. W. DE VRIES & M. PLATERO SANZ (1980). The mechanism of pisatin degradation by Fusarium oxysporum f. sp. pisi. *Physiol. Pl. Path.* **16**: 119–133.

J. C. M. BEIJERSBERGEN and CAROLA TH. C. VAN DER HULST (*Laboratorium voor Bloembollenonderzoek, Vennestraat 22, 2160 AB Lisse*)

Influence of the cultivar on the detection of lily symptomless virus (LSV) in bulb tissue of lily by means of ELISA.

BEIJERSBERGEN, J. C. M. & Carola Th. C. VAN DER HULST (1980): *Acta Horticulturae* **109**: 487–494.

R. J. SCHEFFER and D. M. ELGERSMA (*Phytopathologisch Laboratorium 'Willie Commelin Scholten', Javalaan 20, 3742 CP Baarn*)

An ELISA for demonstrating a phytotoxic glycopeptide produced by *Ophiostoma ulmi* in inoculated elms

A phytotoxic glycopeptide was isolated from liquid cultures of *Ophiostoma ulmi* (Buisman) Nannf., the causal agent of Dutch elm disease and antiserum was prepared against a pure fraction of this material. This antiserum was used for setting-up an ELISA which showed a sensitivity of approx. 0.3 /µg/ml of the pure glycopeptide (SCHEFFER & ELGERSMA, 1981).

In field plots susceptible and resistant elms were inoculated with the aggressive strain H6 of *O.ulmi*. After 5 to 40 days the inoculated branches were harvested. The bark was peeled off and the woodsap was collected by means of hydraulic press.

Positive ELISA results indicating the presence of antigen were obtained with the susceptible *Ulmus hollandica* 'Belgica', with *U. americana*, and, to a lesser extend, with *U. glabra*. Pure glycopeptide added to these woodsaps could be traced back quantitatively, indicating that there was no important interaction between the glycopeptide and the woodsap.

In the moderately resistant clone '390' no different ELISA results between inoculated and control plants were observed, although the fungus spreads throughout the tree (ELGERSMA & HEYBROEK, 1979).

With increasing time after inoculation, however, the added pure glycopeptide could only be traced back partly. Apparently the antigenic sites of the glycopeptide were altered.

We suppose that a substance is produced in the inoculated tree that reacts with the fungal glycopeptide. Such a reaction may play a role in the mechanism of resistance against Dutch elm disease.

Research on this phenomenon therefore will be continued.

EIGERSMA, D. M. & H. M. HEYBROEK (1979): *Neth. J. Plant Path.* **85**: 235–240.
SCHEFFER, R. J. & D. M. ELGERSMA (1980). *Physiol. Plant Path.* **18**: 27–32.

J. W. M. VAN LENT[1]), J. J. M. VAN GRONINGEN[2]), E. EGBERTS[2]) and D. PETERS[1])
([1]*Laboratorium voor Virologie, Binnenhaven 11, 6709 PD Wageningen;* [2]*Laboratorium voor experimentele diermorfologie en celbiologie, Landbouwhogeschool, Marijkeweg 40, 6709 PG Wageningen*)

The production of monoclonal antibodies against cowpea mosaic virus

Hybrid cell lines secreting monospecific antibodies against the plantvirus cowpea mosaic virus (CPMV) were established by a polyethylene glycol (PEG, MW 1000) mediated fusion of mouse myeloma cells and mouse spleen cells (MILSTEIN 1980).

Mice were stimulated by an intraperitoneal injection of 1 µg CPMV (in 0,3 ml PBS) followed 6 weeks later by an intravenous booster injection of 100 µg CPMV (in 0,1 ml PBS). Three days after the last injection, the spleens of two mice were minced and spleen cells were fused with P3-NSI/1-Ag4-1 myeloma cells, which are defective for the enzyme hypoxanthine phosphoribosyl transferase (HG-PRT). These cells synthesize but do not secrete the κ light chain of mouse immunoglobulin (Ig). The fused cellsuspension was divided over two 96-well microculture plates. Hybrids were selected in a medium containing hypoxanthine/aminopterin/thymidine (HAT). In this medium only the hybrids

will grow. The parental myeloma cells die and the spleen cells do not survive in culture for more than a few days.

Supernatants of the hybrid cultures were tested for specific murine antibodies against CPMV using a double-sandwich-ELISA.

58 hybrids were obtained of which 42 (= 72%) secreted specific antibodies against CPMV. Out of these 42, 16 good producing hybrids were selected for further characterization. During maintenance of these hybrid cultures, 4 lost the capacity of producing specific antibodies. Of the 12 remaining, 5 hybrids synthesize mouse immunoglobulins of the IgC_{2a} subclass, 6 synthesize IgG_{2b} and 1 hybrid produces Ig of the IgM class.

MILSTEIN, C., 1980: Monoclonal antibodies. *Scientific American* **243** (4): 57–64.

H. RATTINK (*Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, 6709 PD Wageningen/gestat. Proefstation voor de Bloemisterij, Aalsmeer*)

Studies on variation and specific pathogenicity of Phytophthora nicotianae- and P. cryptogea- isolates

Neth. J. P. Path. 1981.

A. BURGERS and F. J. GOMMERS (*Laboratorium voor Nematologie, Landbouwhogeschool Postbus 8123, 6700 ES Wageningen*)

Photochemical aspects of some nematicidal thiophenes

The discovery of the nematicidal principles 2,2′-5,2″-terthienyl and 5-(3-buten-1-ynyl)-2,2′-dithienyl from roots of marigolds (*Tagetes* sp.) led to synthesis of nematicidal thiophene derivatives. Several dithienyls and dithienylethenes were nematicidal *in vitro*, but exerted no or only weak nematicidal activity when mixed with the soil.

-Terthienyl appeared to be a powerful singlet oxygen ($^1O_2$) sensitizer when irradiated. It has been demonstrated that 5-methyl-2,2′-dithienyl, 5,5′-dichloro-2,2′-dithienyl, 5-benzene-2,2′-dithienyl, trans-1-(2-thienyl)-2-(5-chloro-2-thienyl)-ethene, and trans-1,2-di-(5-chloro-2-thienyl)-ethene also act as singlet oxygen sensitizers upon irradiation with proper wavelengths. This system was studied by following the inhibition of glucose-6-phosphate dehydrogenase activity by the photoactivated compounds and by examining the protection of the enzyme activity in the absence of oxygen and by various additions. Addition of mannitol, benzoate, superoxide dismutase or catalase did nog have any effect. This excludes $OH\cdot$, O_2^- and H_2O_2 as the reactive oxygen species involved. $^1O_2$ quenchers such as histidine, methionine, tryptophan and azide protected the enzyme.

Inactivation of the enzyme was about three times faster in D_2O than in H_2O.

Direct evidence for the production of $^1O_2$ by photoactivated compounds was obtained by irradiation in CH_2Cl_2-solution in the presence of the olefin adamantylideneadamantane. With $^1O_2$ this compound forms a stable dioxetane which decomposes to adamantanone when heated above its melting point.

Apparently, these compounds exert nematicidal activity only upon photoactivation, thus explaining low activity when mixed with soil where light is absent. In field conditions these compounds may be effective against organisms aboveground that take up these chemicals and are transparent to ultraviolet light.

IDA BLOK (*Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, 6709 PD Wageningen*)

Playing with oospores of Bremia lactucae

Since it has been shown that *B. lactucae* is heterothallic, it is possible to make crossings between physiologic races of the fungus which belong to different compatibility types. Both types occur in the Netherlands.

From the oospores resulting from crossings we were able to obtain progeny. The arising isolates were identified on a differential set of lettuce varieties. We have not tried to make a quantitative assessment of the different types (races) of the isolates.

From the first crossing experiment two different races came forward. These showed a marked loss in virulence compared with the parents, which indicates that virulence is recessive. One of these races was back-crossed with both parental races. As in both cases oospores developed, it was concluded that in the F_1 both compatibility types were present. Each of the back-crossings reslulted in about 25 isolates, belonging to only two different races, which were identical for both crossings. They were different from the existing Dutch races. One of these races could also attack some lettuce varieties that were not attacked by the parents.

With the present knowledge of the possible genes for virulence in the *Bremia* races and genes for resistance in the lettuce varieties, only part of the results can be explained.

G. WESTSTEIJN and P. VINK (*Laboratorium voor Bloembollenonderzoek, Vennestraat 22, 2160 AB Lisse*)

Possibilities for non-fungicidal control of Pythium root rot in ornamental bulb crops

Root rot caused by *Pythium* spp., is a serious problem in the cultivation of ornamental bulbs in the field (especially hyacinth, crocus and iris) as well as in flower production under glass (mainly tulip and lily). Under commercial forcing conditions indications were obtained that the disease can sometimes be controlled without the use of fungicides. This raised the question as to whether biological, physical, or chemical soil factors are responsible for the control of the pathogen in such cases.

With *P. ultimum* as pathogen and the tulip as host plant, steam sterilization of the soil prior to artificial inoculation enhanced root rot considerably.

Because soil treatment with benomyl had a similar effect, it was concluded that the observed control was most probably due to a biological factor.

Enrichment of the potting soil with composted tree bark gave a striking improvement of the growth of tulips and crocuses in soil heavily infested with *P. ultimum*. In crocus the root rot index was reduced from 5.0 in the non-supplemented soil to 1.8 in the soil supplemented with bark, whereas in tulip the corresponding values were 4.8 and 3.7. Although root infection was not entirely eliminated, it seems likely that it can be reduced in certain crops to such a degree as to insure an adequate yield of cut flowers of good quality.

G. JAGER (*Instituut voor Bodemvruchtbaarheid, Oosterweg 92, 9751 PD Haren*)

Rhizoctonia suppressiveness in potato fields in the northern part of the Netherlands

The existence of properties that suppress *Rhizoctonia solani* in a potato field near Baflo, in the northern part of the province of Groningen, was reported by VAN EMDEN in 1967. We could confirm his finding, which led to a search for fields with similar properties.

Of 125 experimental plots studied in 1978 and 1979 (JAGER & VELVIS 1980), only one proved strongly suppressive, three were suppressive and another five were moderately suppressive. The estimation of suppressiveness was made by comparing the average number of sclerotia on the seed potatoes with that on the crop(tubers). As several uncertainties exist with regard to the quality of the sclerotia on the seed (dead or alive, healthy or infected with antagonists, pathogenic or saprophytic strains of *R. solani*), differences must be large to justifiably distinguish between suppressive and non-suppressive soils.

With regard to the behaviour of *R. solani* from the soil and/or the seed potato towards the potato plant and the crop, three types of soils can be distinguished:

A. Conducive soils with pathogenic strains that damage stems and stolons, and with saprophytic

strains that cause little or no damage. Both types of strains contribute to the formation of sclerotia on the tubers. The number of sclerotia on the crop from clean or contaminated seed potatoes is about the same.

B. Soils in which clean seed potatoes give a (nearly) clean crop, but in which contaminated seed potatoes cause damage to stems and stolons and produce a crop with many sclerotia. These soils presumably have a weak suppressive ability.

C. Suppressive soils, producing a (nearly) clean crop from clean seed potatoes, and an almost clean crop from contaminated seed potatoes. Suppressive soils were often found to be sandy soils.

Suppressiveness appears to depend not only on soil properties, but also on the origin of the seed potato and its 'load' of antagonists.

EMDEN, J. H. VAN (1967): *Meded. Dir. Tuinbouw* **30**: 248–256.
JAGER, G. & H. VELVIS (1980): *Inst. Bodemvruchtbaarheid, Rep.* **1**–80: 62 pp.

R. FROSSARD[1], N. J. FOKKEMA[1] and T. TIETEMA[2] ([1]*Phytopathologisch Laboratorium 'Willie Commelin Scholten', Javalaan 20, 3742 CP Baarn;* [2]*Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Influence of *Sporobolomyces* and *Cladosporium* on the leaching of ^{14}C-labelled assimilates from wheat leaves

In order to investigate possible detrimental effects of saprophytic phyllosphere fungi to the plant, their influence on the leaching of assimilates from leaves was assessed. High population densities of *Sporobolomyces roseus* or *Cladosporium cladosporioides* on flag leaves of potted spring wheat were obtained by spraying with suspensions of cells or conidia and nutrients, followed by appropriate incubation in a controlled environment. After c. five days the population of the fungi had reached a stationary phase, indicating that nutrients had become limiting. The leaves were then labelled with $^{14}CO_2$ applied to the tips for 1 h. After 2 h the middle part of the flag leaf was cut off, the ends were sealed, and the leaf was shaken in a solution of 0.005 % Tween 80 in tap water for 2.5 h. Flag leaves from non-inoculated plants with very low microbial population densities were used as controls. The radioactivity of the shaking fluids (leachates plus micro-organisms) was periodically measured.

The population density of the fungi was determined by culturing techniques.

The amount of label found in the shaking fluids, expressed as percentage of the total radioactivity present in leaves, was independent from the saprophytic colonization. Still, determination of radioactivity of membrane filters on which the fungi were collected revealed that ^{14}C-label had been taken up by the fungi.

The data suggest that these phyllosphere micro-organisms are not able to enhance the leaching of assimilates from leaves.

Acknowledgement. This work was partly made possible by a grant of the Swiss National Science Foundation to R. Frossard.

H. J. MILLER (*Plantenziektenkundige Dienst, Geertjesweg 15, Postbus 9102, 6700 HC Wageningen*)

Phytobacteriological nomenclature: Problems and prospectives

International codes of nomenclature are not penal codes but codes that have been developed to meet the requirements of taxonomy. They merely regulate the selection and give precision to the use of names employed after taxonomic decisions have been made. Although we should not forget that nomenclature should be our servant and not our master, adherence to the rules of the code is essential for uniformity and the avoidance of confusion.

Until 1930 bacteriologists had mostly used the Botanical Code, although some followed the Zoological Code. However, it became evident that even the Botanical Code did not fit too well the needs of the bacteriologist and in 1939 it was approved that a recognized Bacteriological Code be developed. The Code emerged in 1958 as the International Code of Nomenclature of Bacteria and

Viruses. In 1966 the virologists decided to prepare their own rules of nomenclature and the revised Bacteriological Code was approved in 1973 by the Judicial Commission of the International Committee on Systematic Bacteriology and the Plenary Session of the First International Congress of Bacteriology. This Bacteriological Code has been in effect since January 1, 1976 and supersedes all previous editions.

The original starting point used by botanists is May 1, 1753 but later for certain plant groups other starting points were conceived. Mycologists also follow the Botanical Code and use 1801 and 1821 when naming fungi. May 1, 1753 has been used by bacteriologists but throughout the years, mostly due to the many differences in taxonomical opinion, a great degree of confusion has been created in bacterial nomenclauture. As a means of eliminating the thousands of forgotten and useless names a new starting date was implemented on January 1, 1980. In conjunction with this new date a list of approved names of bacteria has been published (SKERMAN et al. 1980).

In the preparation of the approved lists, Dr. D. W. Dye of New Zealand was chiefly involved with the selection of the names of phytopathogenic bacteria which had to meet the requirements of the Judicial Commission. YOUNG et al. (1978) proposed that most of these bacteria should be reduced to the infrasubspecific level of pathovar which should have the same status under the Bacteriological Code as species or subspecies. Due to a failure in making a compromise for the Judicial Commission and the fact that the Judicial Commission was not prepared to change the Bacteriological Code to include pathovar in it rules, the marjority of phytopathogenic bacteria belonging to the genera *Pseudomonas* and *Xanthomonas* have now no official standing in nomenclature.

In an attempt to establish the naming of pathovars of phytopathogenic bacteria, DYE et al. (1980), as the ISPP Committe on Taxonomy of Phytopathogenic Bacteria, published a statement on behalf of the Executive Committee of the International Society for Plant Pathology. However, it should be pointed out that the pathovar names as presented in this publication are illegitimate combinations as well as being not validly published according to Rule 27 of the Bacteriological Code.

Even without discussing the merits or pitfalls of the taxonomic division as proposed by YOUNG et al. (1978) and DYE et al. (1980), plant bacteriologists as well as all those concerned with plant pathology are now faced with the dilemma of the lost names. It is urged that the names published in the International Journal of Systematic Bacteriology according to the Bacteriological Code should be used.

The author recommends that those names of phytopathogenic bacteria which have not been included in the Approved Lists of Bacterial Names (1980) and which have not been listed as *Nomina rejicienda* by the Judical Commission be provisionally used until they can be validly published as legal combinations. By taking this path we will remain as close as possible to the intentions of the Code and cause less confusion among the many workers in plant pathology.

DYE, D. W., J. F. BRADBURY, M. GOTO, A. C. HAYWARD, R. A. LELLIOTT & M. N. SCHROTH (1980): International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Rev. Pl. Path.* **59**: 153–168.

LAPAGE, S. P., P. H. A. SNEATH, E. F. LESSEL, V. B. D. SKERMAN, H. P. R. SEELIGER & W. A. CLARK (1975): *International Code of Nomenclature of Bacteria. Bacteriological Code 1976 Revision.* Am. Soc. Microbiol., Washington, D.C.

SKERMAN, V. B. D., V. MCGOWAN & P. H. A. SNEATH (1980): Approved list of bacterial names. *Int. J. Syst. Bacteriol.* **30**: 225–420.

YOUNG, J. M., D. W. DYE, J. F. BRADBURY, C. G. PANAGOPOULOS & C. F. ROBBS (1978): A proposed nomenclature and classification for plant pathogenic bacteria. *N.Z. Jl. agric. Res.* **21**: 153–177.

J. D. JANSE (*Plantenziektenkundige Dienst, Geertjesweg 15, 6706 EA Wageningen*)

Observations on the pathogenesis of the so-called bacterial canker of ash, caused by *Pseudomonas savastanoi* subsp. *fraxini*

The bacterial disease of common ash (*Fraxinus excelsior* L.), caused by *Pseudomonas savastanoi* subsp. *fraxini* (Brown)Dowson may be confused with other diseases and with insect attacks on ash (JANSE 1980). Therefore light- and electron-microscopic studies were made to investigate bacterial invasion of

the bark tissues and the host response.

It was shown that *P. savastanoi* subsp. *fraxini* is a necrotrophic parasite, which induces much less cellular division than *P. savastanoi* in olive. Following intercellular colonization and break-down of middle lamellae and cell walls by the bacteria, cavities are formed, which are surrounded by corklayers formed by the host. The bacteria regularly escape around the cork layers, so that slow growing wart-like excrescences develop.

The results will be published in detail in the *Eur. J. For. Pathol.*

JANSE, J. D. (1980): Symptoms on common ash caused by Pseudomonas savastanoi and several other organisms. *Acta Bot. Neerl.* **29**: 214.

D. H. WIERINGA-BRANTS (*Phytopathologisch Laboratorium 'Willie Commelin Scholten', Javalaan 20, 3742 CP Baarn*)

The rôle of the epidermis in virus-induced local lesions on cowpea and tobacco leaves

Removal of the lower epidermis from virus-inoculated cowpea and tobacco leaves resulted in a reduction of local lesion numbers in the mesophyll when Tobacco Mosaic Virus (TMV) or Tobacco Necrosis Virus (TNV) was used. Darkening of the plants 24 h prior to inoculation greatly influenced this reduction.

The contact period between mesophyll and epidermis after inoculation, required for lesion formation, differed markedly in darkened and non-darkened plants.

In the combination cowpea-TNV this period was 1 and 6 h, respectively, and for cowpea-TMV $1\frac{1}{2}$ and 8 h, respectively. For Xanthi nc tobacco inoculated with TNV the period was 1 and 6 h, respectively, and in the tobacco-TMV system 1 and $8\frac{1}{2}$ h, respectively.

In darkened cowpea leaves four times more plasmodesmata could be demonstrated in the cell walls between mesophyll and epidermis than in non-darkened leaves.

In darkened tobacco leaves about five times as much plasmodesmata occurred as in non-darkened leaves. A better exchange between the cells could be the reason of the rapid passage of infectious virus units from the epidermis into the mesophyll.

E. DEN BELDER, J. M. VLAK and D. PETERS (*Laboratorium voor Virologie, Binnenhaven 11, 6709 PD Wageningen*)

The control of the beet armyworm Spodoptera exigua (Lepidoptera: Noctuidae) in glasshouses with a nuclear polyhedrosis virus

The beet armyworm was introduced with cuttings of chrysanthemum plants from Florida in Dutch glasshouses. The larvae which are rather resistent to lannate and permetrin compounds, can cause great economic losses in chrysanthemum and other cultures grown in glasshouses in The Netherlands.

Studies were conducted on the control of these larvae with a nuclear polyhedrosis virus isolated from some dead larvae found in Aalsmeer. This virus proved to be highly infectious. The LD50 for the second, third and fourth instar larvae was 410, 960 and 4740 polyhedra, respectively.

The efficacy of this virus in the control of this moth was tested in two glasshouse experiments. In one experiment solutions of polyhedra were applied in such a way that 1 cm^2 leaf surface was covered by 10^6, 10^5 and 10^4 polyhedra. The polyhedra in these densities caused the death of 99, 91 and 55 % of the second instar larvae infesting the chrysanthemum plants. In the other experiment plots of chrysanthemum were infested with second, third and fourth instar larvae and treated with 10^6 polyhedra/cm^2, that is about 10^{13} polyhedra/ha. In this experiment all the larvae which could be recovered after 10 days, were killed by the virus. We conclude that the prospects to control the beet armyworm with this virus are promising.

The isolate of the *Spodoptera exigua* nuclear polyhedrosis virus found in Aalsmeer differs with respect to its DNA composition from the viruses isolated in the USA and Egypt. It will be of great importance to compare the effect of these viruses in the control of this moth.