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Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON 22 SEPTEMBER 1988

PHYSIOLOGICAL DEVELOPMENTAL PROCESSES OF POLLEN FROM *NICOTIANA TABACUM*

J.A.M. SCHRAUWEN, P.F.M. DE GROOT, M.M.A. VAN HERPEN, TH. VAN DE LEE, W.H. REYNEN AND G.J. WULLEMS

Molecular Plant Physiology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

The formation of pollen from vegetative tissue passes through different phases: meiosis, development of the generative cell and vegetative nucleus, and synthesis of the pollen-wall and other pollen-specific compounds that have a function during germination and fertilization.

The production of these pollen-specific structures and compounds is caused by changes in the gene expression of the plant and are concomitant with a series of processes by which type-specific gene products, e.g. mRNA's, are formed. Analysis of the mRNA population in mature pollen from maize showed that 10% of this population represented pollen-specific mRNAs.

These mRNAs presumably, have a function during germination and pollen tube growth, which require protein synthesis but no transcription. Analyses of the mRNAs in microspores of *Nicotiana tabacum* during microsporogenesis may demonstrate the function of these mRNAs in relation to phase development of the microspores. Microspores of tobacco were separated from vegetative tissue, the RNA was extracted and translated in an *in vitro* system and the translation products were separated by two-dimensional gel electrophoresis.

The results demonstrate a correlation between the bud length and the morphological stage of the microspore as well as a 10-fold increase in the RNA content of the microspore during development. The qualitative composition of the translation products during the different stages of development of the microspore show a considerable change in the overall pattern and several specific proteins are present only during a certain phase of development. These results demonstrate a continuous change in the accumulation of transcription products, which may have a function in microspore development and germination.

The presence of these 'prefab' mRNAs in mature pollen may cause the typical reaction of pollen under stress during germination. Incubation of germinated pollen at 39°C for 30 min resulted, at room temperature, in a normal protein pattern and synthesis of a minor group of HS-proteins. These latter proteins appear to be related to the recovery of the pollen tube growth, which is retarded at the elevated temperature.

MICROSPORE CULTURE IN *BRASSICA OLERACEA* VEGETABLES

J. DUIJS, R.E. VOORRIPS AND J.B.M. CUSTERS

Institute for Horticultural Plant Breeding (IVT), P.O. Box 16, 6700 AA Wageningen, The Netherlands

Using the procedure described by Swamson *et al.* (*Plant Cell Reports* 1987, **6**: 94–97), isolated microspores were cultured from 52 genotypes belonging to five different *Brassica oleracea* types. Almost all the genotypes developed somatic embryos: 88% in Brussel sprouts, 90% in white

cabbage, 100% in savoy cabbage, 64% in kale, and 100% in broccoli. The numbers of embryos obtained showed a clear genotype effect: *c.* 0·5–50 embryos per flower bud. In addition, plant development from the embryos was different between genotypes; in certain genotypes the embryos developed directly into plants, in others plants could only be obtained via a secondary embryogenesis. Using a flow cytometer, the ploidy level of the microspore-derived plants was examined from four genotypes. With the Brussel sprouts 'Gower' and 'Lunet', the percentage of doubled haploids was 54 and 58, respectively, with accession numbers 84010 and 84022 of broccoli it was 43 and 90%, respectively. Because the procedure can save time in producing homozygous plant material, it is of great importance for the breeding of F_1 hybrid varieties in cole crops.

POSSIBILITY OF A POLLEN-MEDIATED TRANSFORMATION SYSTEM IN MAIZE

G. BOOY

Foundation for Agricultural Plant Breeding (SVP), P.O. Box 117, 6700 AC Wageningen, The Netherlands

Germinating pollen of maize was incubated with a mixture of carrier DNA and plasmid DNA carrying a gene that codes for kanamycin resistance. It was then used for *in vitro* pollination. None of the 830 embryos obtained showed kanamycin resistance.

It was demonstrated by electrophoresis that plasmid DNA was degraded by nucleases (which are released by germinating pollen) soon after it was mixed with the pollen. After using a washing procedure for the pollen of 5 or 10 min before mixing it with the DNA, a fraction of the nucleases appeared to be removed. However, considerable nuclease activity remained associated with the pollen and degraded the DNA completely. A pretreatment of the pollen with Denhardt's solution, which might coat the pollen and therefore decrease the release of nucleases, had no influence on the degradation of the DNA. Addition of EDTA ($\geqslant 1$ mM) (a possible inhibitor of the nuclease activity) to the pollen germination medium completely inhibited pollen germination. Varying the pH (4–6) of the pollen germination medium had no influence on the nuclease activity.

An alternative method by which problems concerning nuclease activity can be avoided was investigated. Ovaries with styles were placed *in vitro* and the style was then cut through. A small space was maintained between the two stylar parts. DNA can be added to this space and may be taken up by the passing pollen tubes after pollination of the upper part of the style. However, preliminary experiments showed that the pollen tubes were unable to bridge the space between the two stylar parts. This may be explained by the absence of a chemotropic gradient directed by the ovary.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 11 NOVEMBER 1988

STRUCTURE OF THE ACTIN AND TUBULIN CYTOSKELETONS DURING MEIOSIS IN *GASTERIA VERRUCOSA*

A.A.M. VAN LAMMEREN, M.T.M. WILLEMSE AND J. BEDNARA*

*Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands and *Department of Plant Anatomy and Cytology, Maria Curie Slodowska University, Akademicka 19, 20033 Lublin, Poland*

The three-dimensional structures of the microtubular and microfilamental cytoskeletons were investigated during male and female meiosis and sporogenesis of *Gasteria*. Microtubules were

observed using antibodies and FITC applied to sections of fixed plant material embedded in polyethylene glycol. Microfilaments were stained in unfixed and unembedded cells that were treated with Nonidet P-40, DMSO and incubated with rhodamine–phalloidin.

In microspore mother cells microtubules were predominantly arranged randomly throughout the cytoplasm. At the metaphase stage spindles were highly fluorescent indicating the presence of microtubules. After the telophase, microtubules radiated from the nuclear envelopes (Van Lammeren *et al., Planta* 1985, **165:** 1–11). Actin filaments were observed in the central cytoplasm during all the stages of pollen development. In meiotic spindles there was an apparent localization of large arrays of phalloidin-reactive material. After the telophase, microfilaments radiated from the nuclear envelopes. In the pollen conspicuous cortical arrays were present.

In megaspore mother cells microtubules were randomly oriented in the cytoplasm. Microtubules form meiotic spindles, radiate from nuclear envelopes after telophase I and II and disappear for the greater part at the binucleated coenocyte stage. The micropylar cell of the dyad exhibited most fluorescence and formed an oblique-oriented spindle at meiosis II (Willemse & Van Lammeren, *Sex. Plant Reprod.* 1988, **1:** 74–82; Bednara *et al. Sex. Plant Reprod.* 1988, **1:** 164–172). Microfilaments were always present in the cytoplasm. At meiosis they encaged the nuclear regions but were not found in the spindles. The future megaspore of the tetrad predominantly exhibited the F-actin cytoskeleton.

It can be concluded that tubulin and actin form cytoskeletal arrangements independently. Co-distributions occur but the example of spindle formation showed that male and female development varies.

CYTOSKELETON AND CELL WALL TEXTURE IN *URTICA DIOICA* ROOT HAIRS

A.N.M. VAN AMSTEL

Department of Experimental Botany, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

Since the discovery of cytoskeletal elements in plant cells, attempts have been made to relate these elements to morphogenesis and cellulose deposition. Based mainly on the observed co-alignment between the orientation of cortical microtubules and nascent cellulose microfibrils in many studies, it has been concluded that cortical microtubules orient nascent cellulose microfibrils. However, recent studies have shown that such a relationship does not exist in root hairs, including *Urtica* root hairs.

The main problem in determining co-alignment is quantitative. Contradictory results were found for both the cell wall texture of the secondary wall in *Urtica* root hairs (Sassen *et al. Cell walls '81*, 1981, WVG, Stuttgart) and the spatial organization of the cortical microtubules (Traas *et al. J. Cell Sci.* 1985, **76:** 303–320). To explain these discrepancies, several methods were used.

The microtubular skeleton was studied *in toto* using immunofluorescence and in detail by the use of Spurr-embedded thin-sectioned material. As determined from the pooled measurements on thin sectioned material, microtubules show orientations that are slightly diverse (10–20°) from the cells' axis length. However, if the mode of orientation of the microtubules is determined in individual root hairs, it becomes clear that root hairs show right- or left-hand helical orientations of microtubules. Similar helices are also observed in immunofluorescence preparations of whole root hairs.

To study the texture of the secondary walls, cells were extracted in hydrogen peroxide/glacial acetic acid. Root hairs were either broken in liquid nitrogen or dry-cleaved. Both types of preparation showed helical patterns of cellulose microfibrils. The results show no differences between the

methods used. Microfibrils are deposited in an 'S'-helix configuration with a 5–20° deviation from the cells' axis. A small fraction is deposited in a 'Z'-helix. This may be due to the measurement of older layers of microfibrils or bending of the new layers after extraction.

It must be concluded, therefore, that there is no co-alignment between cortical microtubules and nascent microfibrils and that microtubules do not direct cellulose microfibrils. Equally important are the results that show that measurements of microtubule orientation, based on only one method, might be unreliable and should be handled carefully.

IMMUNOGOLD LOCALIZATION OF LIPOPOLYSACCHARIDE OF *RHIZOBIUM LEGUMINOSARUM* BIOVAR *VICIAE* STRAIN 248 IN FREE-LIVING BACTERIA AND IN ROOT NODULES OF *VICIA SATIVA*

L. GOOSEN-DE ROO AND R.A. DE MAAGD

Department of Molecular Botany, University of Leiden, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands

Leguminosae infected by *Rhizobium* with altered lipopolysaccharide (LPS) in their outer membranes, often form abnormal nodules that do not fix nitrogen. This has led to the supposition that LPS plays a role in the symbiosis with leguminous plants.

In order to study the possible role of LPS and, in particular, the O antigen part of LPS, monoclonal antibodies were made. Three monoclonal antibodies to react with the O antigen of LPS were selected after immunization of mice with a peptidoglycan/outer membrane complex of free-living bacteria.

We have localized ultrastructurally the antigens recognized by these monoclonals in the free-living *Rhizobium leguminosarum* biovar *viciae* strain 248 and in bacteria in infection threads, and in bacteroids in root nodules of *Vicia sativa*, using indirect immunogold staining. We compared free-living bacteria and bacteria in the infection threads. Special attention was given to the infected cells in the young symbiotic zone of the root nodules. In these cells endocytosis of bacteria had just occurred and inter- and intracellular infection threads were present.

Immunogold staining was carried out on ultrathin sections, sectioned from specimen embedded in LR white, polymerized at 50°C for 16 h. Free-living cells (concentrated in gelatin) and root nodules were fixed in 1% glutaraldehyde and 1% para-formaldehyde in 0·1 M sodiumcacodylate buffer, pH 7·4, at room temperature. Only the root nodules were post-fixed in 1% osmiumtetroxide.

In free-living bacteria, immunogold labelling of the outer membrane occurred by using only two of the monoclonal antibodies. In the infection threads, labelling of the outer membrane of enclosed bacteria occurred with all three monoclonals. Immunogold labelling was present in very small quantities in the bacteroids, with all three monoclonals.

The results indicate that one epitope of the O antigen does not appear until the stage that it enters the infection thread, and that three epitopes are no longer expressed after the release of bacteria from the infection threads.

LOCALIZATION OF NODULIN TRANSCRIPTS IN *PISUM SATIVUM* BY IN-SITU HYBRIDIZATION

C.C.M. VAN DE WIEL

Departments of Molecular Biology and Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

The symbiotic interaction between certain leguminous plant species and bacteria of the genus *Rhizobium* leads to the formation of root nodules in which nitrogen fixation takes place. Plant genes

have been identified that are expressed exclusively in these nodules. These genes encode the so-called nodulins. Pea (*Pisum sativum*) nodules are of the indeterminate type, i.e. they have a persistent apical meristem. Consequently, in one nodule a graded developmental series is present from the top to the root attachment site. In the central tissue this comprises the differentiation of the infected cells (which includes, consecutively; penetration of infection threads, release of bacteria from the infection threads, and multiplication and differentiation of the bacteroids) and uninfected cells. This indeterminate character of the pea nodule makes it possible to study the developmental pattern of nodulin gene expression in sections of one nodule by specific labelling of transcripts with in-situ hybridization. For instance, in the case of leghaemoglobin, which has a role in the regulation of oxygen transport to the nitrogen fixing bacteroids, the first visible signal, from the meristem, is present in the cells where the release and multiplication of the bacteria takes place. Thereafter the signal reaches a maximum in the youngest cell layers that are fully packed with bacteroids. In older layers there is a gradual diminishing of the signal. This agrees with previous immuno-histochemical studies on the protein (Van de Wiel *et al. J. Plant Physiol.* 1988, **132:** 446–452). cDNA clones representing other nodulin genes that are expressed in earlier stages of root nodule development are available and will likewise be used in in-situ hybridization experiments to relate their expression pattern to development and, eventually, it is hoped, to their function in the nodulation process.

NUCLEAR PROTEINS IN EMBRYOGENIC CARROT CULTURES STUDIED BY IMMUNOFLUORESCENCE MICROSCOPY

M.A. SANCHEZ-PINA, H. KIEFT*, AND J.H.N. SCHEL*

*Centro de Investigaciones Biologicas, Madrid, Espana and *Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands*

Somatic embryogenesis in liquid cultures is an ideal system for studying development and differentiation in plants at the cellular level. One approach to characterize the nuclear events that take place during this process is to identify some of the proteins involved in processes such as replication, transcription and/or processing. Here we report on an immunofluorescence microscopical study during early somatic embryogenesis in the carrot. This study was performed using antibodies that detect two main types of nuclear protein: (i) the, so-called, small nuclear ribonucleoprotein (snRNP), and (ii) the nuclear matrix protein.

The material used was from somatic embryos of *Daucas carota* L. at 0, 1, 2 and 3 days after the induction of embryogenesis by auxin deprivation. They were fixed in 3% para-formaldehyde in PBS, cryoprotected with sucrose in PBS, cryofixed in liquid propane and cryosectioned at −80°C. The study used a FITC-conjugated second antibody.

SnRNPs are exclusively localized in the nucleus, in general with a speckled pattern; the nucleolus is negative at all the stages studied. Nuclear matrix proteins are localized exclusively in the nucleus with a speckled pattern, except for the nucleolus, in each one of the stages studied.

These results on a plant system correspond with those previously obtained with animal cells. The speckled pattern given by the snRNPs seems to indicate a localization of these proteins in specific nuclear regions where processing of RNA takes place; snRNPs have been proposed to participate in the splicing process. With respect to the localization of nuclear matrix proteins, which have been related to cell proliferation in animal cells, these could play a role in the nuclear activation processes that take place after triggering the embryogenic cultures to embryo formation.

DEVELOPMENT AND ULTRASTRUCTURE OF MUCILAGE CELLS OF *CINNAMOMUM*

M.E. BAKKER AND A.F. GERRITSEN

Rijksherbarium, University of Leiden, P.O. Box 9514, 2300 RA Leiden, The Netherlands

The ultrastructure of developing and mature mucilage idioblasts in the shoot apex and leaves of *Cinnamomum burmanni* and *Cinnamomum verum* (Lauraceae) has been studied and compared with that of oil cells found in the same species.

As in oil cells, all mucilage cells have a suberized layer in the outer cellulosic cell wall from a very early stage in development onwards.

Four stages have been distinguished in the development of these mucilage idioblasts.

(i) Cells that only differ from the surrounding cells by the presence of a suberized layer. These cells have a large, central vacuole. In this early stage future mucilage cells cannot be distinguished from future oil cells.

(ii) Cells in which the mucilage secretion has started and is located in a layer between the cell wall and the cytoplasm. These cells still possess a central vacuole.

(iii) Cells in which the mucilage deposition has forced the cytoplasm to move inward, leading to the disappearance of the central vacuole.

(iv) Cells in which the cytoplasm is degenerating and completely embedded in the mucilage. The cells approach full maturity.

These results are the first record of a suberized layer in mucilage cells revealed at the ultrastructural level. As the presence of a suberized layer in the cell walls of oil cells is well documented, our results support the hypothesis that oil cell and mucilage cell initials are identical or at least homologous.

AN ANATOMICAL STUDY OF THE *IN VITRO* BULBLET REGENERATION FROM FLOWERING STEM EXPLANTS OF *HIPPEASTRUM* CV. LIBERTY

P.C.H. SMITS, R.L.M. PIERIK* AND J.H.N. SCHEL

*Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands and *Department of Horticulture, Agricultural University, Wageningen, Haagsteeg 3, 6700 AA, Wageningen, The Netherlands*

Since 1985, a new method for the *in vitro* vegetative propagation of *Hippeastrum* hybrids has been developed that consists of the formation of adventitious bulblets on flowering stem explants. In order to estimate the chance that these bulblets differ genetically from their parent tissue, their origin and development during the first 3 weeks were investigated by light microscopy. A culture was started using the easily regenerating cultivar Liberty, and each 2–3 days explants were fixed and embedded in Technovit 7100. Sections were stained in toluidine blue.

From the results it is evident that most of the bulblets, particularly the early ones, originate from cell divisions in the epidermis and some adjacent inner cell layers of the parenchymatic ground tissue. As the origin of these bulblets is multicellular and no callus phase is involved in their development, there is little chance that these bulblets differ genetically from their parent tissue. Later, bulblets also originate, via the callus, from cell divisions in the phloem tissue and the parenchyma near vascular bundles. Because of the intervenient callus phase, the chance that these bulblets differ genetically from their parent tissue is thus greater.

MORPHOLOGICAL ASPECTS OF PETIOLE ELONGATION IN SUBMERGED *RUMEX* SPECIES

P.J.M. PERIK, L.A.C.J. VOESENEK, C.W.P.M. BLOM AND M.M.A. SASSEN

Department of Experimental Botany, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

Rumex species show a distinct zonation in river areas. This distribution is related to the flooding tolerance of the plant species. *Rumex crispus* and *R. palustris* occur in the low and frequently flooded parts of the flood plain and must have adapted to survival in these conditions. One of the adaptations in response to flooding is enhanced growth of the petioles. This elongation response, mediated by the gaseous plant hormone, ethylene, restores the contact between the leaves and the atmosphere. In these species the greatest elongation response was observed in the youngest petioles and those that developed during the flooding treatment. *Rumex acetosa*, a species that occurs on rarely flooded dykes and river levees, is unable to elongate its petioles in response to submergence and ethylene. In this species the growth of the youngest petioles is even inhibited by flooding. In *R. crispus* and *R. palustris* growth of the petiole under flooded conditions occurs over the whole length of the petiole. However, growth in the basal part of the petiole is mainly caused by cell elongation, whereas growth in the apical region is related to both cell expansion and increased cell division. In *R. acetosa* the inhibition of growth is manifest over the whole petiole. This restricted growth is related to inhibited cell elongation.

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON 12 JANUARY 1988

OZONE-INDUCED CHANGES IN THE SUSCEPTIBILITY OF BEAN TO *BOTRYTIS CINEREA*

C. LEONE, C.J. VAN DIJK, I. VAN DE LINDE AND A.E.G. TONNEIJCK

Research Institute for Plant Protection, P.O. Box 9060, 6700 GW Wageningen, The Netherlands

Young plants (21 or 25 days old) of four *Phaseolus vulgaris* cultivars differing in O_3 sensitivity, were exposed to 0, 120, 180 and 270 $\mu g\ O_3\ m^{-3}$ for 8 h. One day after the fumigations were started, leaves were inoculated with conidia of *Botrytis cinerea* suspended in water or in a 62·5 mM KH_2PO_4 solution (Pi). The conidia in the Pi-solution caused lesions on healthy leaves whereas conidia suspended in water did not. The primary leaves of the O_3-sensitive cultivar, Pros, showed the highest level of visible injury after exposure to O_3, whereas those of the O_3-tolerant, Groffy, were much less affected. The trifoliate leaves of all cultivars were less sensitive to O_3 than the primary leaves. Visible injury increased with increased O_3 concentrations. In the presence of O_3-induced symptoms, the leaves of all cultivars showed lesions after inoculation with water-suspended conidia of *B. cinerea*. The increase in the number of lesions depended on the level of O_3 injury. The number of lesions on the primary leaves of cultivar Pros and of the slightly less O_3-sensitive cultivars, Stratego and Lit, also increased with increasing O_3 concentrations when these leaves were inoculated with conidia in Pi-solution. However, the primary leaves of the O_3-tolerant cultivar, Groffy, showed a decrease in Pi-stimulated infection after exposures to the lower O_3 concentrations. A similar decrease in infection was obtained on the O_3-tolerant trifoliate leaves. The results indicate that O_3 can influence the susceptibility of bean plants to *B. cinerea*. Both a stimulation and a reduction in the number

of lesions can be found depending on the cultivar, type of leaf, inoculation method and O_3 concentration.

BOTRYTIS CINEREA ON GERBERA FLOWERS: INTERACTIONS AND POSSIBILITIES FOR RESISTANCE BREEDING

J. SALINAS

Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, The Netherlands

In the last few years, spotting of ray florets of gerbera flowers caused by *Botrytis cinerea* has become an important problem. This type of small necrotic lesion can appear during the growth of the flowers in greenhouses or later during storage or transport of the cut flowers. In the latter case the export of gerbera flowers may be affected directly.

Inoculation with *B. cinerea* induced the formation of typical necrotic lesions only if dry conidia were dusted on the flowers and the inoculated flowers were kept at 100% r.h. for a short period (at least 5 h) at 18–25°C.

Inoculation of flowers with conidial suspensions in ultrapure water gave poor results. When nutrients were added rotting was the most common symptom.

Germination of conidia and lesion formation occurred between 4 and 25°C at 100% r.h.; at 30°C germination and lesion formation did not occur. Between 18 and 25°C many lesions become visible within 1 day; at 4°C it takes 2–3 days before lesions appear. If kept dry, conidia of *B. cinerea* remained ungerminated on ray florets of gerbera flowers.

Lesions appeared to consist of one to several necrotic, usually epidermal, cells. A single conidium gives rise to a lesion. The infection of ray florets from single conidia was followed using a microscope. At 18–25°C germ tubes penetrated the cuticle only and one or two epidermal cells became brown and collapsed. Even after incubation for 14 days, most lesions still consisted of two necrotic cells. At 4°C, the fungus penetrated the cuticle and an epidermal cell, but later it also invaded neighbouring cells, thus giving rise to a spreading lesion.

From the results it is suggested that the ability of *B. cinerea* to penetrate the cuticle is a key factor in inducing the formation of lesions in gerbera flowers. Validation of this hypothesis may offer interesting possibilities for disease control, e.g. plant resistance; breeders may search for varieties that are able to resist cuticular penetration.

A HYPOTHESIS ON THE GENETICS OF RESISTANCE TO *PHYTOPHTHORA FRAGARIAE* IN STRAWBERRY

W.E. VAN DE WEG

Institute for Horticultural Plant Breeding, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Little is known, to date, of the genetics of resistance to *P. fragariae* in strawberry. It has been assumed for more than 30 years that resistance is inherited quantitatively although it is known to be race-specific. Cultivar:race interactions, however, have never been interpreted on the basis of the gene-for-gene hypothesis. Interpretation on this basis showed that many of the compatible and incompatible interactions mentioned in the literature can be described by a gene-for-gene model with five resistance and virulence genes (see Table 1). The validity and completeness of this model has yet to be proved. The argument is hampered by the fact that results are inconsistent when testing the same cultivar:race combination.

Table 1. Compatible (+) and incompatible (−) interactions between seven American races (A1–A6 and A10) and one British isolate (B1) of *P. fragariae* and six differentials of strawberry together with the suggested genotypes of races and differentials

Race	Virulence genes	Differentials and their resistance genes					
		Blakemore −	MD 683 1	Climax 2	Perle de Prague 3	Del Norte 4	Yaquina A 5
A1	2	+	−	+	−	−	−
A2	1 4	+	+	−	−	+	−
A3	23	+	−	+	+	−	−
A4	4	+	−	−	−	+	−
A5	123	+	+	+	+	−	−
A6	234	+	−	+	+	+	−
A10	45	+	−	?	?	+	+
B1	34	+	−	−	+	+	?

INDUCED RESISTANCE AGAINST *FUSARIUM OXYSPORUM* F.SP. *GLADIOLI* IN GLADIOLUS

E.J.A. ROEBROECK

Bulb Research Centre, P.O. Box 85, 2160 AB Lisse, The Netherlands

An isolate of *Fusarium oxysporum* f.sp. *gladioli* Snyd & Hans. (LBO-G5) appeared to be pathogenic on Gladiolus ramosus cv. 'Robinetta', G. colvillii cv. 'Alba' and G. nanus cv. 'Nymph' but not on gladiolus hybrid cv. 'Peter Pears'. Another isolate of *F. oxysporum* (LBO-G2) was pathogenic on all cultivars mentioned.

When corms of cv. 'Peter Pears' were inoculated with isolate LBO-G5, by dipping in a spore suspension of 10^6 spores ml^{-1}, and incubated for 0–2 days at 20°C under moist conditions, before they were planted in soil infested with isolate LBO-G2, the disease incidence and severity scored 5 weeks after planting were lower than in control corms not inoculated with isolate LBO-G5.

Disease reduction was more pronounced when the length of the incubation period between inoculation with isolate LBO-G5 and planting increased from 0 to 2 days.

When corms, inoculated with isolate LBO-G5 and incubated for 2 days, were disinfected in 2% formaldehyde for 30 min and then planted in infested soil, disease reduction was not affected.

This finding indicates that the mechanism of disease reduction acts through the defence response of the host tissue rather than through antagonism between the non-pathogenic isolate and the pathogenic isolate.

EVALUATION OF LIGNIFICATION AND PHYTOALEXIN ACCUMULATION IN CARNATION STEMS BY PYROLYSIS-MASS SPECTROMETRY

R.P. BAAYEN, J.J. BOON* AND G.J. NIEMANN

*Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, and *FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands*

The response of resistant carnations to infection with *Fusarium oxysporum* f.sp. *dianthi*, among others, includes changes in the lignification of cell walls and accumulation of phytoalexins (Baayen

Can. J. Bot. 1988, **66**: 784–792; Niemann & Baayen *Neth. J. Pl. Pathol.* 1988, **94**: 289–301). By means of pyrolysis (gas chromatography) mass spectrometry, minute pieces of fresh tissue from the stems of both healthy and infected carnations were analysed for lignification (lignin/cellulose ratios), lignin composition and, as far as possible, for phytoalexin accumulation.

In healthy carnations the epidermis and cortex of young and old stems were almost unlignified. Pyrolysis mass spectra (PYMS) only showed fragments from polysaccharides and pectin. Pith parenchyma was only lignified in mature and senescing tissues. The type of medullary lignin differed from that in the adjacent xylem and in the sclerenchyma, however. Lignification in the xylem had already started in very young tissues with the formation of a guaiacyl-type lignin, which gradually changed into a mixed guaiacyl–syringyl lignin in older tissues. The sclerenchyma was more strongly lignified than the xylem located in the same transverse section of the stem. For all the tissues the contribution of protein fragments in the PYMS spectra was very low.

Preliminary investigations of the xylem of infected plants showed that inoculation with *F. oxysporum* f.sp. *dianthi* affects the polysaccharide composition. Furthermore, one of the accumulated phytoalexins, dianthalexin (Niemann & Baayen *Neth. J. Pl. Pathol.* 1988, **94**: 289–301), showed up in the PYMS spectra by way of its molecular ion. In the resistant cultivar Novada dianthalexin could only be detected in occluded parts of the xylem, but not in adjacent, seemingly unaffected parts of the xylem, or in adjacent pith or phloem tissue. In the susceptible cultivar Lena dianthalexin appeared to be absent in the diseased part of the xylem.

SELECTION FOR RESISTANCE TO BACTERIAL CANKER IN TISSUE CULTURES OF TOMATO

R.W. VAN DEN BULK AND H.J.M. LÖFFLER

Institute for Horticultural Plant Breeding, P.O. Box 16, 6700 AA Wageningen, The Netherlands

A promising way to obtain disease-resistant plants is the use of *in vitro* selection with toxins produced by the pathogen as selective agents. We investigated the production of a toxin by *Clavibacter michiganensis* subsp. *michiganensis* (*Corynebacterium michiganense* subsp. *michiganense*), the causal agent of bacterial canker in tomato, and the possibilities of using this toxin as a selective agent in tissue cultures of tomato. Phytotoxic compound(s) were isolated from the culture filtrate of the bacterium using recognized methods. Phytotoxicity was expressed as wilt-inducing activity in a bioassay with plant cuttings. The activity was confined to a high molecular weight fraction.

The effects of this fraction at the cellular level were studied using leaf discs, cell suspension cultures and protoplasts. A decrease in the growth of cell suspension cells was shown for toxin concentrations over 1 mg ml^{-1}. Regeneration of cell suspension cells of tomato is not yet possible, however.

No inhibiting effect was found on the number of shoots produced on leaf discs of a bacterial canker susceptible and a (partially) resistant tomato genotype. On the protoplast level an inhibiting effect on the plating efficiency, according to a dose–response curve, was found if the toxin was added to protoplasts of two bacterial canker susceptible genotypes. However, this inhibiting effect was not found when protoplasts of a (partially) resistant genotype were used. At present, the effect on other susceptible and resistant genotypes is being investigated. Protoplasts are the most promising tissue culture system for use in *in vitro* selection for resistance to bacterial canker at the cellular level.

EFFECTS OF TEMPERATURE TREATMENT AND CUTTING ON THE DETECTION OF IRIS SEVERE MOSAIC VIRUS IN IRIS BULBS

C.I.M. VAN DER VLUGT

Bulb Research Centre, P.O. Box 85, 2160 AB Lisse. Agricultural University Department of Virology, P.O. Box 8045, 6700 EM Wageningen, The Netherlands

Detection of iris severe mosaic virus (ISMV) in ISMV infected iris bulbs during storage, by either ELISA or electron microscopy, has been problematic. We have applied different storage-temperature treatments and a cutting method, according to Stein *et al.* (*Ann. Appl. Biol.* 1986, **109:** 147–154), as possible procedures to enhance ISMV detection in stored bulbs cv. Professor Blaauw. While at lifting ISMV could not be detected in the bulbs, gradually the virus became detectable over a storage period at about 17° or 20°C.

Better results were obtained when the cutting method was employed (Van der Vlugt *et al. Acta Hortic.* 1989, **234:** (in press)). Using ELISA, a 100% score was obtained for infected bulbs that were stored at 20°C and cut 2 weeks prior to testing. These results offer good prospects for the development of a reliable detection method for ISMV in iris bulbs.

EXPRESSION AND REPLICATION OF THE GENOME OF TOBACCO RATTLE VIRUS

G.C. ANGENENT, E. POSTHUMUS AND J.F. BOL

Department of Biochemistry, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Tobacco rattle virus (TRV) is a RNA plant virus with a bipartite genome. RNA-1, the longer RNA with a length of about 6800 nucleotides, encodes for the viral replicate, a protein that is probably involved in cell-to-cell transport of the virus and a protein with a molecular weight of 16 000 (16 K). The function of this 16-K protein is unknown. RNA-2 of TRV varies in length between 1800 and 4000 nucleotides, depending on the strain. Part of this variability is due to differences in the length of the 3'-terminal sequence of RNA-2 that is identical to that of the corresponding RNA-1. The sequence that is unique to RNA-2 encodes the coat protein (CP).

The sequence of RNA-2 of TRV strain PLB (2196 nucleotides) was determined and showed a homology with the 3' end of PLB RNA-1 over a length of 820 nucleotides. Consequently, this strain is diploid for the 16-K gene. To permit an analysis of the expression of the 16-K gene in infected cells, an antiserum was raised against a synthetic peptide corresponding to the conserved C-terminus of the 16-K protein. TRV-infected tobacco protoplasts accumulated similar amounts of 16-K protein and CP, but in infected tobacco plants only the CP was detectable. The 16-K protein was incorporated in to a high molecular weight structure that was resistant to treatment with non-ionic detergents.

In order to study gene function and other properties of non-coding regions of the plant viral genome, recombinant RNA molecules are indispensable. Such recombinant RNA molecules can be obtained in *in vitro* transcription of full-length cDNA clones into biologically active transcripts. Transcription products of a cDNA clone of PLB RNA-2 were highly infectious in tobacco protoplasts and plants when inoculated together with RNA-1 of another TRV strain. Deletion mutants of PLB RNA-2 showed that at least 70% of this genome part can be deleted without a reduction of the replication efficiency. Sequences at the near 5' and 3' end are important for replication.

EFFECT OF NITROGEN AND POTASSIUM FERTILIZATION ON THE DEVELOPMENT OF *SPHAEROPSIS SAPINEA* IN *PINUS NIGRA*

M. DE KAM, C.M. VERSTEEGEN, B.C. VAN DAM, J. VAN DEN BURG AND D.C. VAN DER WERF

Research Institute for Forestry and Landscape Planning De Dorschkamp, P.O. Box 23, 6700 AA, Wageningen, The Netherlands

In the 1980s a serious epidemic of *Sphaeropsis sapinea* developed in *Pinus nigra* and *P. sylvestris* forest stands in The Netherlands. Field surveys revealed that the damage was most serious in forest stands on poor sandy soils in regions subjected to high depositions of ammonium originating from nearby intensive livestock farms. Seriously attacked trees had higher foliar nitrogen contents than weakly attacked or healthy trees. It was postulated that the trees had become more vulnerable to *Sphaeropsis* because of an excess of ammonium sulphate deposition. This was examined in the following experiment.

Two-year-old plants of *P. nigra* ssp. *maritima* were potted in buckets containing 12 kg of homogenized dry humuspodzol soil, developed in cover sand and placed in a greenhouse. During three subsequent vegetation periods they were fertilized with various amounts of ammonium sulphate (AS) and/or with potassium sulphate (PS). Thereafter, the stems of 87 of the then 5-year-old plants were wounded and inoculated with mycelium of *Sphaeropsis sapinea* grown in pure culture. The size of the bark necroses that developed as a result of the inoculations was measured 15, 21 and 26 days after inoculation. The average lengths of the necroses in the various treatments varied from 17 to 103 mm. It was found that *Sphaeropsis* caused longer necroses on plants fertilized with AS ($P=0{\cdot}012$ on log-scale), whereas fertilization with PS caused smaller necroses ($P<0{\cdot}001$ on log-scale). AS fertilization caused a threefold increase in the mean length of the necroses in some treatments. The mineral content of the pine needles was analysed periodically. The analyses showed that excess of AS disrupted the mineral balance of the plants. It is concluded that *P. nigra* becomes more susceptible to *Sphaeropsis* when grown in poor sandy soil and when fertilized with an excess of AS. It is therefore likely that high depositions of ammonium played a considerable role in the development of the epidemic of *Sphaeropsis sapinea* in The Netherlands in the 1980s.

DEVELOPMENT OF A METHOD FOR TESTING THE SUSCEPTIBILITY OF *SALIX ALBA* TO *ERWINIA SALICIS*

A.M. DINGJAN-VERSTEEGH AND M. DE KAM

Research Institute for Forestry and Landscape Planning De Dorschkamp, P.O. Box 23, 6700 AA, Wageningen, The Netherlands

Some of the results of a CEC-funded research project to develop a method for testing the susceptibility of *Salix alba* to *Erwinia salicis* are presented in this paper.

Successful artificial inoculation experiments are necessary for the selection and breeding of resistant clones. Many attempts have been made to inoculate willow trees artificially with *E. salicis*. All researchers report that artificial inoculations often produce variable results. Therefore, a reliable infection test needs to be developed. Part of the project was an inoculation experiment that was performed to ascertain whether there is a statistically significant correlation between plant water potential and susceptibility to artificial inoculation with *E. salicis*.

The data indicate that in *Salix alba*, as in other woody plants, plant water potential changes with the evaporative demand of the air and the water availability in the soil. Furthermore, trees inoculated when plant water potential was low showed a higher response to inoculation than trees

inoculated when plant water potential was high. These findings indicate that all artificial inoculation experiments must be performed in randomized plots, to exclude the influence of the plant water potential, which changes throughout the day, on the susceptibility of the willow trees.

The highest response to artificial inoculation with *E. salicis* is reached when the trees are inoculated when the plant water potential is low, i.e. when the evaporative demand of the air is high and the water availability in the soil is low. Thus the best time to inoculate willow trees is on hot sunny days after at least 1 week without rain.

EFFECT OF IRRIGATION ON COLONIZATION OF POTATO ROOTS BY PLANT GROWTH PROMOTING *PSEUDOMONAS PUTIDA* STRAIN WCS358

D.C.M. GLANDORF, B.J. DUIJFF, J.G. LAMERS*, P.A.H.M. BAKKER AND B. SCHIPPERS

*Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, and *Research Station for Arable, Farming and Field Production of Vegetables, P.O. Box 430, 8200 AK Lelystad, The Netherlands*

The colonization by a rifampicin-resistant strain of *Pseudomonas putida* WCS358 of potato roots, arising from seed tubers treated with this strain, was followed throughout the season. The possible effects were studied of the delivery of water and of the bacterium in irrigation water, applied every 2 weeks during the season. The experiments were performed at the Experimental Farm 'De Schreef' near Lelystad. From day 46 after seeding until the end of the season (day 131), irrigation gave rise to 2–100-fold higher mean population densities of WCS358 on roots 20–50 cm below the soil surface; dependent on the amount of rainfall. Application of WCS358 increased root colonization by this strain at a depth of 20–50 cm with a factor 10–100 from day 61 until day 131, compared to the irrigated treatment. Irrigation had no effect on the population densities of WCS358 on seed tubers, progeny tubers, underground stem parts and stolons. The stability of rifampicin resistance of WCS358 was studied by screening approximately 1200 fluorescent pseudomonads isolated from underground parts of potato plants for agglutination with WCS358 antiserum and growth on a medium supplemented with rifampicin. All tested pseudomonads that agglutinated with the antiserum grew on the selective medium, indicating that no loss of resistance had occurred under field conditions.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 18 JANUARY 1989

THE RELATION BETWEEN EXTREME WEATHER AND SUCCESSION IN GRASSLAND VEGETATION

L.F.M. FRESCO, J.P. BAKKER AND R. STRIJKSTRA

Department of Plant Ecology, Biological Centre, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

The effects of extreme weather conditions on the dynamics of species in grassland vegetation was studied in 57 permanent plots (recorded once each summer) in hay fields. These grasslands are situated in the valley of the Drentse A in the north of The Netherlands. Management aims to

decrease the macronutrient level. The vegetation has been monitored since 1972 (van Duuren, L., J.P. Bakker & L.F.M. Fresco, *Vegetation* 1981, **47**: 241–258).

The species coverage was corrected for the successional trend of the species by replacing the abundance values by the residuals with respect to a best-fit third-degree polynomium. Residuals of at least 5% coverage were used only.

Weather conditions over a 3-month period were regarded as being 'extreme' if they exceeded the mean (1972–1987) by more than one standard-deviation. In this paper two extreme periods are treated: the warm and dry summer of 1976 and the long wet period (summer, autumn and winter) of 1980/1981.

In both periods a relatively large number of species showed a relevant decrease in coverage during the extreme period. In the years following the extreme weather many species restored their coverage. Successional trends could both be delayed and accelerated in the extreme seasons. The dry summer of 1976 caused (as expected) a delay in the vegetation development in the drier plots and an acceleration in the wetter ones. In and after the long wet period of 1980/1981 the reverse could be observed.

The following conclusions can be drawn.

(i) To interpret the results of vegetation monitoring, it is essential to take extreme weather periods into consideration. (ii) Vegetation indicates the existence of extreme weather by a strong decline in the coverage of a number of species. This decline is restored in the next period in many cases. (iii) Delayed effects after an extreme weather period are no less important than the short-term effects. (iv) Successional trends can both be delayed and accelerated by extreme weather. (v) Studies such as the one presented here cannot answer the question as to whether the direction of succession can be affected by the climate. (vi) The search for a correlation between meteorological variables and the fluctuation of coverage values is best avoided. (vii) Because of the complex net of inter-relations between biotic and abiotic variables and direct and indirect, short-term and long-term effects, the effects of extreme weather on vegetation composition can only be approached (and predicted) using a systems-model.

PRODUCTION OF SALT-MARSH PLANT COMMUNITIES IN RELATION TO WEATHER CONDITIONS

J. DE. LEEUW

Delta Institute for Hydrobiological Research, Yerseke, The Netherlands

Although the production of salt-marsh plant communities has been studied intensively, little attention has so far been paid to year-to-year variation.

We analysed a 13-year dataset of peak standing crop estimates for six plant communities from the high salt marsh on the island of Schiermonnikoog, The Netherlands. Production of the six communities showed a synchronous pattern over the 13-year period. This synchrony indicates that a common external factor operating on the production of all plant communities was responsible for the observed year-to-year variation in the peak standing crop. A stepwise multiple regression analysis revealed that the rainfall deficit during the growing season was significantly correlated ($P<0{\cdot}05$) to peak standing crop for four out of six plant communities. Inundation frequency during the growing season did not contribute to the regression model. These results indicate that the production of higher salt-marsh vegetation is influenced by the weather conditions during the growing season. It would be interesting to investigate the sensitivity of the production of lower salt-marsh plant communities to variations in the rainfall deficit during the growing season.

EFFECTS OF DIFFERENT GRAZING INTENSITIES ON THE SALT-MARSH VEGETATION IN THE LEYBUCHT (GERMANY)

M. BRONGERS AND Y. DE VRIES

Department of Plant Ecology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

The Leybucht salt-marsh is located along the mainland coast of Lower-Saxony in Germany. Since 1980 the effects of different grazing intensities on the salt-marsh ecosystem have been studied. Four management regimes were imposed, namely abandoning, and grazing by 0·5, 1·0 and 2·0 cattle ha^{-1}. In 1988 vegetation characteristics were studied by vegetation mapping, point-quadrat analysis and measurements of the canopy height.

The greater part of the lower salt-marsh in all regimes is covered by a species-poor *Puccinellia maritima*-community. On the ungrazed part of the study area the community type of *Elymus* cf. *pycnanthus* dominated the higher salt-marsh.

With increasing grazing intensity, lower salt-marsh *Puccinellia maritima* types spread further into the higher salt-marsh zones. Thus the species-rich types dominated by *Festuca rubra, Lolium perenne* and *Elymus* cf. *pycnanthus* decreased. As grazing pressure increases, the height of the flowering *Aster tripolium* layer as well as the underlayer of the vegetation decrease and the soil is progressively bared, due to trampling, which results in a more open canopy. Species on the increase are the lower salt-marsh species and annuals, in particular, namely *Puccinellia maritima, Suaeda maritima* and *Spergularia salina.*

Investigation of the invertebrate fauna between 1980 and 1982 indicated that invertebrate groups of the lower salt-marsh expanded into the higher zones, and species diversity decreased with increasing grazing intensity (Irmler, U, and B. Heydemann *Natur. Landschaft. in Niedersachsen* 1986, **15:** Hannover). As abandonment will eventually result in a dominance of *Elymus* cf. *pycnanthus*, it can be expected that the diversity of the invertebrate fauna will decrease as a consequence.

We conclude that grazing with a low stocking rate (0·5 animals ha^{-1} or less) is the optimal management regime for both plant species and invertebrate fauna diversity of the Leybucht salt-marsh.

THE VEGETATION OF DITCH BANKS ON DAIRY FARMS IN PEAT AREAS IN THE NETHERLANDS IN RELATION TO ENVIRONMENTAL FACTORS

W. TWISK AND A. J. VAN STRIEN

Milieubiologie, University of Leiden, P.O. Box 9516, 2300 RA Leiden, The Netherlands

Ditch banks in the western peat areas with intensive dairy farming offer an extensive potential refuge for plant species. To find the best management strategy for these ditch banks the factors that govern their vegetation must be known.

The effects of environmental factors on different indices of floristic richness and on individual plant species were assessed by means of spatial comparisons of sampling plots, using analysis of variance and matched pairs analysis.

A higher ditch water table, a higher pH of the bank soil, a lower grazing intensity and a lower nitrogen supply on the adjacent grasslands favoured floristic richness. The floristic richness of the vegetation proved to be higher on south-facing banks than on north-facing banks, and on steep south-facing banks than on gentle south-facing banks. The floristic richness was also higher on

banks along ditches that were cleaned once every 2–3 years as compared to banks along ditches that were cleaned yearly or once every 4 years or less frequently. No difference in botanical composition could be assessed between cleaning methods, perhaps due to the variation existing within each method.

The results indicate several measures that enhance the conservation value of ditch banks. According to farmers the ones that seem to be compatible with intensive dairy farming are: Keeping the banks free from nitrogen input; liming banks with a low pH; and changing the frequency of ditch maintenance. Experiments have been carried out to study the botanical as well as agricultural effects of these measures.

ATMOSPHERIC DEPOSITION TO CHALK GRASSLAND AND ION TRANSPORT IN THE SOIL

D. VAN DAM, R. BOBBINK, G. W. HEIL AND B. HEIJNE

Department of Plant Ecology, Lange Nieuwstraat 106, 3512 PN Utrecht, The Netherlands

Atmospheric input by wet and dry deposition to chalk grassland in the Southern part of The Netherlands was studied over a period of 3 years. Techniques for measuring throughfall below grassland canopies were developed and applied to quantify the input to the soil. With one method the ion fluxes in throughfall were measured with ion exchange resins (van Dam, Heijne & Heil 1987: *Functional Ecol.* **1:** 423–427) and appeared to be strongly correlated with Leaf Area Indices. With another method (Heil & van Dam *Proceedings of the Seventh World Clean Air Congress* IUAPPA, 1985, Sydney, Australia 25–29 Aug., 1986; **5:** 16–21) throughfall was collected twice a month. In this case, the ion fluxes in throughfall showed clear seasonal patterns, coinciding with the structural dynamics of the grassland canopy. Leaching of K, Ca and Mg, buffering of H^+ ions within the canopy and foliar uptake of NH_4 were observed. Deposition velocities for SO_2 (derived from sulphate fluxes in throughfall minus bulk precipitation, and from SO_2 concentrations in the atmosphere) vary between 2 cm s^{-1} during the summer (at maximum standing crop with LAIs of 5–10) and values smaller than 0·5 cm s^{-1} during the winter and spring (LAI < 2).

Data for sulphur cycling in chalk grasslands were integrated within a model describing atmospheric input, above- and below-ground hydrology, ion uptake, mineralization, sulphate adsorption and leaching. Simulated and observed leaching of sulphate were similar to within 13%. On an annual basis the net retention of sulphate is almost absent; the output with drainage equals 78% and the output with harvest equals 13% of throughfall (2·22 keq ha^{-1} $year^{-1}$).

In contrast to sulphur, nitrogen (24 kg N ha^{-1} $year^{-1}$ in throughfall) almost exclusively leaves the ecosystem through harvest, because the leaching of nitrate is less than 2 kg N ha^{-1} $year^{-1}$.

The substantial atmospheric input and retention of nitrogen results in an increased dominance of *Brachypodium pinnatum* and a reduced species diversity.

EFFECTS OF NUTRIENT ENRICHMENT IN DUTCH CHALK GRASSLAND

R. BOBBINK

Department of Plant Ecology, Lange Nieuwstraat 106, 3512 PN Utrecht, The Netherlands

During the last decade an obvious increase in the dominance of *Brachypodium pinnatum* was observed in chalk grasslands. Enhanced nutrient input from the atmosphere, particularly of nitrogen, was thought to cause this increase (Bobbink & Willems *Biol. Cons.* 1987, **40:** 301–314). In order

to test this hypothesis, a 3-year experiment was carried out in which either potassium 10 g K m^{-2} $year^{-1}$), phosphorus (3 g P m^{-2} $year^{-1}$), or nitrogen (10 g N m^{-2} $year^{-1}$), as well as a complete fertilization (N + P + K), was applied.

After 3 years of fertilizer treatment in two Dutch chalk grassland sites, the peak standing crop increased significantly in the N and NPK series. Phytomass of legumes increased after P application, whereas the amount of *Brachypodium* more than doubled in the N series. However, NPK fertilization resulted in a sharp increase in weight of the other graminoids.

Analyses of N and P in the plant material revealed a very efficient acquisition and redistribution of nutrients in *Brachypodium*. In addition, and in contrast with the other grasses, it was shown that *Brachypodium* can produce more phytomass per unit time after N application, even without enhanced P uptake. Only *Brachypodium* is able to increase markedly the N:P ratio.

The vertical structure of the vegetation was affected by N- and NPK-fertilization and light penetration in the canopy was considerably reduced in these series. As a result of these changes, phanerogamic species density and diversity decreased tremendously in this vegetation.

It is concluded that *Brachypodium* benefits more from atmospheric N input than the other species in chalk grassland. This is a continuous threat for the highly estimated species richness of this ecosystem.

MOSSES AND HORSES IN DRIFTING SANDS

H.C. GREVEN

Institute for Nature Management, Postbox, Leersum, The Netherlands

Racomitrium lanuginosum (Hedw.) Brid. is a rare moss species in The Netherlands. *Racomitrium canescens* (Hedw.) Brid. is also rare, except in the dunes along the coast. The former is a typical species of *Ericetum tetralicis* plant communities in damp heathland and the latter is a pioneer species of *Koelerio–Corynephoretae* communities on dry, more or less basic, sandy or gravelly soil.

During an investigation into drifting sands in the Utrechtse Heuvelrug area (south of Amersfoort), both species were frequently found growing together as sandbinding pioneers.

As the species were not found in nature reserves, nor in sands with a strong recreative use, it is suggested that these species need some weak dynamics. These dynamics occur in sands where horsemen practise their sport. In those sites the species are found mostly along horse tracks were they are regularly covered by drifting sand.

EFFECTS OF EXPERIMENTAL FERTILIZATION ON FOREST UNDERGROWTH IN NORTHERN SWEDEN

H.F. VAN DOBBEN AND G.M. DIRKSE

Research Institute for Nature Management, P.O. Box 46, NL-3956 ZR Leersum, The Netherlands

In Sweden a number of forest fertilization experiments have been carried out to assess optimal nutrient levels for tree growth. The present study aims to record the impact of nutrient additions on the floristic composition of the undergrowth. Some preliminary results are reported here from the experiment at Norrliden, which consists of 102 plots measuring 30 × 30 m^2, situated in a 35-year-old *Pinus sylvestris* stand at 64°21'N, 19°46'E, *c.* 100 km NW of Umeaa in northern Sweden. The treatments, which commenced in 1971, are: addition of ammonium nitrate and urea (30, 60 and 90 kg N ha^{-1} $year^{-1}$), potassium, phosphorus and magnesium (40 kg P, 80 kg K and 50 kg Mg ha^{-1} every third year), acid (1, 2 and 3 kmol H^+ ha^{-1} $year^{-1}$, yearly from 1971 to 1976), lime (2000 kg Ca ha^{-1}, once), sulphur and micronutrients, and irrigation.

Vegetation records of the undergrowth in the inner 20 × 20 m^2 of the plots were made in 1988. The first ordering of the data was obtained by application of TWINSPAN. Nitrogen addition appeared to have a strong effect on the floristic composition of the plots, favouring such species as *Deschampsia flexuosa, Chamerion angustifolium*, and the moss *Brachythecium oedipodium*, and disfavouring *Empetrum nigrum, Vaccinium vitis-idea* and *Cladonia* spp. Acid-treated plots without nitrogen addition form another distinct group, with a low cover of phanerogamic species and a number of crustaceous lichen species on bare soil, e.g. *Trapeliopsis granulosa* and *Placynthiella oligotropha.*

Further details were studied by means of redundancy analysis (RDA). The first axis (eigenvalue: 0·31) is almost completely determined by nitrogen addition, while the second (eigenvalue: 0·04) is largely determined by acidification/liming. The effect of urea is equivalent to a combination of nitrogen and acid; the effect of Mg is similar to that of lime though not as distinct. No clear effects were found for sulphur, micronutrients and irrigation. The effect of P was only determined in N-fertilized plots; *Chamerion angustifolium* is favoured by P, *Vaccinium myrtillus* disfavoured, and *Deschampsia flexuosa* is indifferent.

(Thanks are due to the Swedish Agricultural University (Uppsala) for permission to visit the experiment and support in the field.)

MEETING OF THE SECTION FOR THE PRESERVATION OF THE WILD FLORA, 3 MARCH 1988

AN ECOLOGICAL APPROACH TO STUDYING POPULATIONS OF ENDANGERED PLANT SPECIES

J. VAN ANDEL

Laboratory for Plant Ecology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Theories on population dynamics of plant species mainly concern processes of colonization and persistence. Little attention has been paid to processes of extinction. Populations are local and temporal representatives of a species. Whether or not a population represents an 'endangered species' depends on: (i) the area and pattern of distribution of the species, and (ii) the evolutionary history of the species. As far as local populations are concerned, symptoms of extinction are: (i) a decrease in vitality of the individuals, and (ii) a decrease in population size. A chain of causes and consequences of decreasing vitality and declining population size may result in extinction. If habitat factors become more and more unfavourable (due to natural or man-induced changes), decreasing vitality and fecundity of individuals may result in a lower rate of gene exchange within the population. If the population is isolated from neighbouring populations, the genetic variability decreases further. The effect of a decreasing genetic variation on the fitness of a population may well differ between common and rare species.

Demographic life-history studies are a prerequisite for evaluating the status of a local population, compared with its relatives. Experimental analyses are required to detect causes and consequences of a decline in vitality or the size of a population. Response-analysis of plants from different seed sources (populations of different size and isolation rate, individual parents, experimental crossings between field plants) reveals information on the regenerative potential of populations or plants. Results from response analyses under controlled conditions should be tested in the field, both within and outside the range of actual distribution. For this purpose sowing and transplanting into synecologically characterized gradients is necessary. Causes of differences in responses between

progeny groups may have a genetic basis or result from environmentally induced changes in plant performance.

FRITILLARIA MELEAGRIS IN THE NETHERLANDS

A. CORPORAAL

Ministry of Agriculture, Directory of Nature Milieu and Faunabeheer

Fritillaria meleagris L. is widespread throughout the temporate zone of Europe. The main distribution area, however, can be found in the atlantic part of Europe. The species is restricted mainly to Alno-Padion vegetation and their derivates, e.g. plant communities that belong to the class Molinio-Arrhenatheretea. Haylands with wet to moist soil conditions, and on which low-level manuring has been applied, can be considered to be their optimal habitat. These haylands are often situated in either temporarily inundated areas or at sites where water seepage is prevalent. Sites with favourable conditions for this species are often encountered in delta areas of rivers and in hilly areas.

The number of *Fritillaria* sites in The Netherlands has decreased enormously during the last decade. This is also true for the number of individuals per population occurring in the remaining sites. The declining number of sites and individual plants per site is caused by water regulation measurements, changes in agricultural practice and by the widespread use of flower picking and bulb excavation. The main distribution area in The Netherlands is the delta of the river Ijssel, near the town of Zwolle.

Fritillaria flowers 6–12 years after their first appearance above ground. Observations in a population near Zwolle showed an average seed production of 225 seeds/m^2, and a recruitment rate of one seedling per 1000 seeds. During the flowering period the above-ground phytomass can amount to 60–70% of the total weight. This means that plants can be threatened severely when the hay is cut during the flowering time of *Fritillaria*. As early mowing of grasslands is now the norm this poses a serious threat to the future preservation of *Fritillaria*. An early mowing regime can destroy a population of this species within a few years.