

Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON 21 JANUARY 1993

Disease Progress of *Rhizoctonia solani* in Flowerbulbs in Artificially Infested Fields and in a Bioassay

J.H.M. Schneider, G. Dijst and M.T. Schilder.
Research Institute for Plant Protection (IPO-DLO),
P.O. Box 9060, NL-6700 GW Wageningen, The Netherlands

The 'Rhizoctonia disease' in tulip and iris, caused by *Rhizoctonia solani*, occurs in patches. In order to develop alternative means of control, the dynamics of these patches in space and time was studied in a field trial and a bioassay.

Tulip fields on three soil types, i.e. a sandy soil (Lisse), a loamy clay (Creil) and a clay soil (Zwaagdijk), were artificially infested with a cold-preferring isolate of *R. solani* (Doornik, A.W. 1981. *Neth. J. Pl. Path.* 87: 139–147), belonging to the AG-2 complex. The increase of patches was measured by estimating sprout affection from April 1992 to June 1992 every 2 weeks. Disease progress of patches was less on the clay soil compared to disease progress on the sandy and loamy clay soil. At harvest (June) the number of bulbs affected by *R. solani* inside and outside patches was counted. Disease incidence (measured as % affected bulbs per row) depended on soil type, but also differed within fields.

In these field trials with the pathogenic AG-2, disease progress measured by sprout affection of tulips was more pronounced within rows, rather than through rows. In glasshouse experiments with a warmth-preferring isolate of *R. solani* AG-4, disease incidence was more severe at higher plant densities.

A bioassay was developed for estimating soil receptivity. Five iris bulbs in a row were planted in double-walled containers. Soil humidity was computer-controlled at 80 mbar and soil temperature was adjusted to 18°C. The first bulb was inoculated with *R. solani* AG-4 thus allowing the fungus to grow through the row. After 5 weeks in two soils the disease progress was compared to the disease progress in a sterilized soil. All bulbs in the sterile soil were heavily affected by *R. solani*, whereas in the non-sterile soils the fourth and the fifth bulb in the row were hardly damaged. This bioassay will be used for further comparisons of disease progress and soil receptivity of arable soil samples.

Germination of Oospores of *Phytophthora porri*

W.D. Smilde. Centre for Plant Breeding and Reproduction Research (CRPO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands

Oospores of *Phytophthora porri* Foister, the cause of white tip disease of leek, are important for survival of the fungus in the soil during summer. The oospores grow in large numbers in leaf lesions, and *in vitro* in a nutrient-rich medium of leek extract or V8 vegetable juice. Experiments with oospores which were grown *in vitro* revealed that these spores have a dormancy period of about 5 months.

The effect of high temperatures on survival of oospores was tested *in vitro*. Oospores in the control treatment germinated for 5–20%. After exposure to 45°C for 5 h, germination of oospores was reduced to less than 50% of the control treatment. When oospores were exposed to 55°C for 5 h, the germination percentage dropped to almost zero.

The effect of high temperatures on survival of oospores was also investigated in a solarization experiment. Solarization is a method to reduce soil inoculum by raising the soil temperature. Soil temperature were increased by transparent plastic on the soil surface in June 1992. In solarized plots the soil temperature exceeded 45°C for 17 h. Initial infection by *Phytophthora porri* on these plots was clearly reduced, compared with the control plots.

Both the *in vitro* experiment and the solarization experiment support the conclusion that solarization cannot prevent an epidemic of *Phytophthora porri* under Dutch climatic conditions.

Induction and Purification of Extracellular Enzymes Produced by the Nematode-egg Parasitic Fungus

Paecilomyces lilacinus

P.J.M. Bonants. Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands

The nematode-egg parasitic fungus *Paecilomyces lilacinus* is capable of degrading the egg-shell of the root-knot nematode *Meloidogyne hapla*. This shell consists of another vitellin membrane and an inner

chitin and lipid layer. I studied the induction of enzymes that could be used by the fungus to degrade this egg-shell, for example proteases and chitinases. Therefore, I added to a minimal medium with conidia of the fungus several substrates (chitin, vitellin, nematode eggs) and looked for the extracellular production of proteases and chitinases over several days of incubation. Chitin induced proteases as well as chitinases. Vitellin, a protein of the outer layer of the egg-shell, induced only proteases. Whole nematode eggs induced proteases and to a small amount chitinases. SDS-PAGE patterns of culture filtrates of the fungus grown with vitellin and with whole eggs as substrate resembled each other closely. Most of the protease activity present in the filtrates was of the serine protease class.

Purification of the predominant induced protease was performed with a bioaffinity column. To that end, bacitracin was coupled to CNBr-activated Sepharose 4B. The purified protease was a basic serine protease of 33.5 kDa. Optimum pH and temperature for proteolytic activity were 10.3 and 60°C, respectively. The protease activity was only inhibited by PMSF, a common serine protease inhibitor. The serine protease was present in large amounts in the culture filtrate of the fungus with all substrates used.

Three chitinases have been purified from the culture filtrate of the fungus grown with chitin as substrate, using an FPLC (Fast Protein Liquid Chromatography) system with a chromatofocusing column and different pH gradients. Fractions were screened for activity with fluorescent substrates (mono-, di- and trimers of 4-methylumbelliferyl-*N*-acetyl-glycosamine). Chitinases were most strongly induced by colloidal chitin and to a small amount by whole root-knot nematode eggs.

Further research is needed to prove the role of the purified enzymes in penetration of *P. lilacinus* into the nematode egg.

Aspects of the Wheat-*Septoria tritici* Pathosystem

G.H.J. Kema and C.H. van Silfhout. Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, NL 6700 GW Wageningen, The Netherlands

Resistance in wheat to *Septoria tritici* has been observed to vary over time and space. In general, cultivars become steadily more vulnerable to the disease and quick turnovers from resistant to susceptible only rarely occur. Genetic variation for virulence in *S. tritici* was suggested to be involved, but continued to be controversial (Eyal *et al.* 1973, *Phytopathology* 63: 1087-1091; Van Ginkel & Scharen 1988, *Phytopathology* 78: 762-766).

In previous experiments highly significant isolate × cultivar interactions occurred among 50

bread wheat isolates and 15 durum wheat isolates from *S. tritici* prone areas like East Africa, the Mediterranean region and Latin America (Kema *et al.*, pers. comm.). Specific virulence differences were even shown within local populations. In order to estimate the genetic variation for virulence in the Dutch *S. tritici* population, a comparative seedling-adult plant experiment was designed involving 22 cultivars and 15 isolates, spanning the period 1972-91. Data analysis, concerning both pycnidial coverage and necrosis, showed a highly significant interaction component. Applying a cluster analysis on the basis of interactions between cultivars and isolates (Corsten & Denis 1990, *Biometrics* 46: 207-215) enabled the selection of significantly different isolates for both parameters. Interestingly, cultivars showing high levels of necrosis do not necessarily have high levels of pycnidial coverage, which results in non-identical dendrograms for both parameters. Three isolates were selected for inoculation in a field experiment, involving the same set of cultivars in a replicated completely randomized block design. The observed differences between cultivars and isolates in the seedling experiment were much more evident in the adult plant stage. The correlations between seedling and adult plant disease levels were generally low, being due to susceptibility in the latter stage despite resistant seedling responses. For some cultivars this response strongly interfered with the plants' habitus, which might be due to an extreme sensitivity to fungal toxins. Recent histological experiments showed a merely intercellular growth of the fungus, which offers possibilities for the study of this hypothesis. Adult plant resistance compared to seedling susceptibility of specific cultivar-isolate combinations was not observed. These observations confirm and emphasize the importance of genetic variation for virulence among Dutch isolates. The role of *Mycosphaerella graminicola* in the dynamics of virulence is not yet understood and needs to be elucidated. Nevertheless, it is recommended that one or more isolates are applied for appropriate selection procedures.

Genetic Variation and Segregation of DNA-polymorphisms in *Botrytis cinerea*

C.J.B. Bergmans, B.F. Brandwagt, J.W. van 't Klooster, C.A.M. Wagemakers, and J.A.L. van Kan. Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands

Genetic variation between eight Dutch field isolates and two Italian monoascospore isolates of *Botrytis cinerea* Pers.:Fr. was studied by RAPD (=random amplified polymorphic DNA)-analysis. RAPD-profiles generated with a set of 50 primers revealed

15–20 DNA-polymorphisms between every two isolates analysed, indicating that all isolates were genetically diverse.

To study the segregation of these DNA-polymorphisms in the progeny, sexual crosses between the Dutch and Italian isolates were performed. From the resulting apothecia, progeny was collected from complete asci of which all eight ascospores were released in their natural sequence. This ordered release of ascospore sets, representing single meiotic events, allowed us to analyse a smaller number of progeny for the segregation ratio of DNA-polymorphisms and, secondly, to show changes in the spore order within the ascus. In addition, these crosses allowed the determination of the mating type of the Dutch isolates, since the mating type of the Italian isolates was known.

A first observation in the monoascospore cultures of the progeny was the occasional segregation of morphological characteristics.

Most of the DNA-polymorphisms, as was shown in the analysis of the Progeny of one cross, segregated into a Mendelian ratio of 1:1. However, two of a total of 17 DNA-polymorphisms segregated into 1:0 ratio. Three DNA-polymorphic markers were absent in the progeny, and three new markers appeared in a 1:1 ratio. These observations are discussed in view of the multinucleate, heterokaryotic nature of *B. cinerea* and its so far unknown ploidy level.

Detection of *Botrytis cinerea* With Specific Monoclonal Antibodies

J. Salinas, G. Schober and A. Schots. Laboratory for Monoclonal Antibodies, P.O. Box 9060, 6700 GW Wageningen, The Netherlands

Infections caused by *Botrytis cinerea* have become an important quality-limiting factor for the production and export of cut flowers in The Netherlands. Conidia produced by the fungus spread easily through the air. After landing on the flowers, conidia remain dormant until water, for instance through condensation, is available for the fungus to germinate; flowers then become infected within a few hours. In autumn and spring in particular, lesions caused by the fungus lead to serious economic losses. Symptoms can be visible in glasshouses during the growth of the flowers, but can also develop later. During storage, transport and shipment of the flowers, changes in temperature often occur which lead to high humidity and subsequent infection when conidia of *B. cinerea* are present. Therefore, a rapid serological test, preferably based on specific monoclonal antibodies (MAbs), would be useful to monitor for the presence of dormant conidia on flowers at all stages of production and transport.

From hybridoma fusion experiments three MAbs directed against conidia of *B. cinerea* were selected by using an immunofluorescence test. The MAbs recognized 42 *B. cinerea* isolates from six different countries and cross-reacted with a few other *Botrytis* species. In the latter case, the immunofluorescence patterns differed from those observed with *B. cinerea*. The MAbs did not cross-react with healthy flowers or with other airborne fungi and bacteria isolated in greenhouses and from the surface of the flower.

A method was developed to collect conidia from the flowers. By using these methods, the three MAbs can be applied in a routine test for the detection of conidia of *B. cinerea* on rose and gerbera flowers.

Binding Characteristics of the Fungal Phytotoxin Fusicoccin and Two Derivatives to the Plasma Membrane-bound Fusicoccin-binding Protein

P.C.J. van der Hoeven, H.A.A.J. Korthout and A.H. de Boer. Department of Plant Physiology and Biochemistry, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087 HV Amsterdam, The Netherlands

The plant pathogen *Fusicoccum amygdali* causes a wilt disease on several fruit species. The wilting is caused by the constant opening of the stomata. Fusicoccin (FC) is the phytotoxin responsible for the disease. The most striking effects of FC are stimulation of the plasma membrane H⁺/ATPase and hyperpolarization of the plasma membrane. The mode of action of FC is probably mediated by specific receptor sites for FC, the plasma membrane-bound fusicoccin-binding protein (FCBP). Although the fungus grows mainly on peach and almond trees, most plant species investigated so far are affected by the toxin. Therefore, the question arises what the function of the FCBP may be, and how FC affects the H⁺/ATPase. Our aim is to study the FCBP by using several FC derivatives. We synthesized a biotinylated FC derivative (FCBio) which will be used for isolating the FCBP by affinity chromatography in a system with immobilized avidin. Another objective is to synthesize a fluorescent FC derivative, which is useful for localization studies of the FCBP. The following binding characteristics of FC and its derivatives were examined in binding experiments using ³H-dihydrofusicoccin and oat (*Avena sativa*) root plasma membranes (at 30°C): K_d ³H-dihydro-FC: 0.7 nM, K_d FCBio: 70 nM, K_d FCBio-avidin: 73 nM. B_{max} 65 picomol mg⁻¹ plasma membrane protein. Hill constant: 1. The dissociation rate is low (t_{1/2}=9.3 h). The results indicate that FCBio can be

used as a probe for further research. The characteristics of the fluorescent FC derivative are now investigated and seem promising.

Purification of the Plasma Membrane Receptor of the Phytotoxin Fusicoccin

H.A.A.J. Korthout, P.C.J. van der Hoeven and A.H. de Boer. Department of Plant Physiology and Biochemistry, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Fusicoccin, a phytotoxin produced by the fungus *Fusicoccum amygdali* Del., binds to a high-affinity binding protein, located in the plasma membrane of higher plants. Our aim is to isolate and purify this fusicoccin-binding protein (FCBP), and to elucidate the signal transduction pathway induced after binding of fusicoccin. Further, the purified FCBP will be used to develop a biosensor for measuring fusicoccin concentrations in fungus and plant extracts.

Several attempts to purify the FCBP in active form have not, so far, been very successful. We have developed a method to purify the FCBP with avidin-biotin affinity chromatography, an application which is fairly unknown in plant biochemistry. This method is based on the strong and specific interactions between avidin and biotin. We used a column with immobilized monomeric avidin together with biotinylated fusicoccin (FCBio). After synthesis, the covalently linked FCBio was purified on reverse phase HPLC and the mass was determined with mass spectrometry. Despite the presence of a biotin group, the specific binding of FCBio to the FCBP still remains. Moreover, FCBio is able to bind the monomeric avidin and can be eluted from the avidin in a mild and specific way with an excess of biotin.

The first attempt to isolate and purify the FCBP from solubilized plasma membrane proteins, using the avidin-biotin affinity chromatography, resulted in a major protein band with an apparent molecular mass of 31 000 daltons on SDS-PAGE, and a minor protein band of 30 000 daltons. Binding experiments with [³H]dihydrofusicoccin demonstrated binding activity in the eluted fractions. These data suggest that the protein eluted from the avidin-column is the FCBP.

The Role of Pseudobactins in Control of Fusarium Wilt and Iron Nutrition of Plants

B.J. Duijff, W.J. de Kogel, P.A.H.M. Bakker and B. Schippers. Department of Plant Ecology

and Evolutionary Biology, Section of Plant Pathology, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands

Under iron-limited conditions micro-organisms and grasses produce iron-chelating metabolites, the so-called siderophores. The siderophores produced by root-colonizing fluorescent *Pseudomonas* spp. (Pseudobactins) may be involved in suppression of soil-borne plant pathogens by competition for iron, but could also influence the iron nutrition of the plant. In the present study we investigated the role of pseudobactin-358 (PSB358), produced by *Pseudomonas putida* WCS358, in control of Fusarium wilt of carnation and in iron nutrition of carnation and barley.

Our results demonstrate that production of PSB358 is involved in reduction of Fusarium wilt in carnation. Treatment of the plants with WCS358 reduced disease incidence. When competition for iron was reduced by increasing the iron availability, disease reduction decreased. Moreover, treatment with a Tn5 mutant, defective in siderophore biosynthesis, did not reduce disease incidence.

The influence of (Fe)PSB358 was studied on iron nutrition of carnation and barley, plants with a ferric reductase-mediated and a phytosiderophore-mediated iron uptake strategy, respectively. A carnation cultivar with a high ferric reducing capacity could use FePSB358 as an iron source. A cultivar with a lower ferric reducing capacity could not. Barley was stimulated in iron nutrition by PSB358 under non-sterile conditions, when, probably due to microbial degradation, phytosiderophore accumulation was low. Under axenic conditions, when phytosiderophore accumulation was higher and possibly adequate for iron nutrition, no differences were observed in iron nutrition of plants grown with FeCl₃ or FePSB358 as iron source.

It is concluded that the mode of action of *Pseudomonas putida* WCS358 in biocontrol of Fusarium wilt, viz. Pseudobactin-358 production, does not interfere negatively with the iron nutrition of the plant.

Biochemical Effects of the Phenylpyrrole Fungicide Fenpiclonil in *Fusarium sulphureum*

A.B.K. Jespers and M.A. de Waard. Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands

Fenpiclonil (CGA 142705) is the first phenylpyrrole fungicide developed for seed treatment by

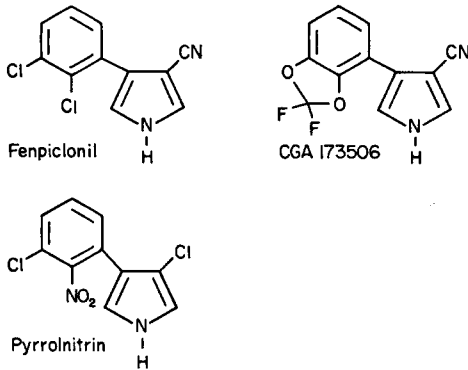


Fig. 1. Structural formulae of fenpiclonil, CGA 173506, and pyrrolnitrin.

CIBA-GEIGY Ltd, Basel. It is available under the trade names 'Beret[®]' and 'Galbas[®]'. Fungi among the Ascomycetes, Basidiomycetes and Deuteromycetes are sensitive to the fungicide. The more potent analogue CGA 173506 has been developed as a foliar fungicide as well and is marketed under the trade name 'Saphire[®]'. Benzimidazole- and dicarboximide-resistant isolates of various fungal species are sensitive to phenylpyrroles.

Fenpiclonil and CGA 173506 are analogues of the antifungal antibiotic pyrrolnitrin, which is produced by several *Pseudomonas* species. Fungicidal activity of these bacteria is caused by the antibiotics produced. Direct application of pyrrolnitrin to crops is hampered by a low chemical stability of the compound. Chemical modifications of pyrrolnitrin resulted in the more stable and highly active compounds fenpiclonil and CGA 173506.

The biochemical effects of fenpiclonil on several physiological processes were studied in the sensitive fungus *Fusarium sulphureum*, one of the causal organisms of damping-off in cereals, of which control by fenpiclonil is recommended. EC_{50} of fenpiclonil for radial growth on PDA and mycelial growth in liquid medium was 0.5 and 10 μ M, respectively. At low concentration, fenpiclonil did not immediately affect oxygen consumption, nuclear division and DNA-, RNA-, protein-, chitin-, ergosterol- and (phospho)lipid biosynthesis. However, the accumulation of amino acids was instantaneously affected at concentrations ranging from 4.2 to 42 μ M. The reduction in amino acid accumulation was accompanied by an increased accumulation of the membrane potential probe tetraphenylphosphonium bromide and a marginal

change of the proton gradient probe propionic acid. The accumulation of sugars was inhibited at even lower concentrations. These results suggest that the biochemical mechanism of action of fenpiclonil may be related to membrane-dependent transport processes.

RFLP Patterns, Vegetative Compatibility Groups and Races in *Fusarium oxysporum* f.sp. *dianthi*

R.P. Baayen, C. Aloï* and B.Q. Manicom†. DLO Research Institute for Plant Protection, P.O. Box 9060, 6700 GW Wageningen, The Netherlands; *University of Turin, Department of Valorization and Protection of Agro-Forestral Resources, Plant Pathology Section, Via Pietro Giuria 15, 10126 Turin, Italy; and †Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, Republic of South Africa

Vegetative compatibility groups (VCGs) were identified among 51 isolates of *F. oxysporum* f.sp. *dianthi*, 18 isolates of *F. oxysporum* from *Dianthus* spp. not belonging to f.sp. *dianthi* and, for comparison, 11 isolates of *F. proliferatum* from *Dianthus* spp. Vegetative compatibility was found between isolates of *F. oxysporum* f.sp. *dianthi* race 4 (VCG 0020), races 2, 5 and 6 (VCG 0021), races 1 and 8 (VCG 0022), and wilt-causing isolates previously classified as *F. redolens* from *D. caryophyllus* (VCG 0023) and *D. barbatus* (VCG 0024) which both were identified as new races. Three self-compatible wilt-causing isolates were the sole members of a VCG and were also identified as new races.

DNA restriction fragment length polymorphisms (RFLPs) of the isolates were determined using total DNA digested with restriction enzyme Hind III and a previously described probe (Manicom, B.Q., M. Bar-Joseph, A. Rosner, H. Vigodsky-Haas and J.M. Kotze 1987, *Phytopathology* 77: 669–672). Isolate sets in *F. oxysporum* f.sp. *dianthi* derived from RFLPs were consistent with those defined by VCGs.

Three VCGs were found among isolates of *F. oxysporum* from *Dianthus* spp. not belonging to f.sp. *dianthi*; five non-pathogenic isolates were the sole members of a VCG. All isolates of *F. proliferatum* belonged to a single VCG. These isolates had simpler patterns, with one exception, which were not associated with VCGs.

VCG and RFLP analyses offer the prospect for rapid identification of races, although inoculation tests continue to be necessary to differentiate races that belong to a single VCG.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 14 MAY 1993

Brefeldin A Effects on Pollen Tubes

Twan L.M. Rutten, Jan Derksen and Bart Knuiman. Department of Experimental Botany, University of Nijmegen, Toernooiveld, NL-6525 ED Nijmegen, The Netherlands

Brefeldin A (BFA) rapidly and reversibly blocks anterograde membrane transport (i.e. ER towards Golgi towards cell surface) in animal cells by inhibiting the formation of nonclathrin-coated vesicles (Orci *et al.* 1991, *Cell* **64**, 1183–1195). In the field of plant cell research pollen tubes represent an attractive model as they are tip growing cells. Tip growth requires the continuous production of secretory vesicles by the golgi apparatus. BFA effects were thus expected at both the morphological and ultrastructural level.

Pollen germination and pollen tube growth were clearly affected by the drug: both processes could be stopped completely at concentrations as low as $3 \mu\text{g ml}^{-1}$. Notable thickening of the cell wall was not observed in non-growing pollen tubes, and plasma streaming remained vigorous in the presence of brefeldin A. Over time the tip region became filled with a dense cytoplasm that lacked large organelles.

Ultrastructural observations showed the rapid dissociation and disappearance of Golgi bodies. The concomitant appearance of vesicle-like structures attached to and continuous with the ER suggests a fusion of the Golgi with the ER similar to that which happens in animal cells (Lippencott-Schwartz *et al.* 1990, *Cell* **60**, 821–836). Additional proof was the presence of coated vesicles attached to the dilated parts of the ER. In untreated pollen tubes coated vesicles were only found in the vicinity of dictyosomes.

Secretory vesicles in growing pollen tubes accumulate in a cone-shaped area in the extreme tip that is enveloped by a thick layer of tubular ER. As BFA inhibits the formation of new secretory vesicles, the tip becomes depleted of secretory vesicles, and the tubular ER shrivels up to fill the pollen tube tip. Interestingly, the cone-shaped accumulation site of secretory vesicles could still be discerned at a time when few vesicles were left. This raises questions about the mechanisms by which this zone is defined.

After removal of the drug, the dictyosomes reappeared. Although a *cis-to-trans* polarity was again evident, the restored dictyosomes contained only two to four cisternae. Coated vesicles were present near the restored dictyosomes. Though these vesicles lacked the spiky coating typical for clathrin their diameter of 80–100 nm was almost twice that of

nonclathrin-coated vesicles present before drug treatment.

The fact that pollen tube growth is stopped without the tip bursting or a thick wall layer in the tip being deposited is remarkable since secretion and tube growth are considered to be two separate processes (Picton & Steer 1983, *J. Cell Sci.* **63**, 303–310). Further studies on the concentration-dependent effects of BFA on pollen tube growth may provide new information on the mechanisms regulating wall deposition and tube growth.

Phenotypic Plasticity of Capitulum Morphogenesis in *Microseris pygmaea* (Asteraceae: Lactuceae)

Johannes Battjes and Konrad Bachmann. Hugo de Vries Laboratory, Kruislaan 318, 1098 SM Amsterdam, The Netherlands

Inbred populations of the annual Chilean species *Microseris pygmaea* genetically diverge in many quantitative characters, including numbers of florets per capitulum. We have previously determined how genetic differences in floret numbers are expressed in meristem form and size during early head morphogenesis. In order to find out how these meristem parameters vary in time and under different environmental conditions we performed an experiment on two highly divergent inbred lines, A92 and C96. After growth under short-day conditions we transferred three groups of plants with 2-week intervals to long-day conditions. In this way we introduced artificial variation for plant size at the onset of flowering, defined as the number of leaves per plant. The number of florets per head increased linearly with plant size. Meristems were harvested on 14 consecutive days after transfer to long days. Vegetative meristems are flat. In C96, elevation of the capitulum apex started on the fourth day. Increase was exponential thereafter. The first capitulum bract was observed on the sixth day, the first floret primordium appeared after 9 days. Three days later the apex was completely filled with floret primordia. Initiation of floret primordia was about five times faster than initiation of bract primordia. In A92 the developmental sequence was similar, but all events occurred a few days later than in C96. In order to detect the effect of plant size on morphogenesis, we performed a multiple regression analysis of the developmental parameters on time and number of leaves. Apex diameter and height of the receptacle are significantly correlated with plant size. Differences in meristem size were already detected in the vegetative state. In

contrast, there was no influence of plant size on floret primordium size. We combined the multiple regression models in one simple model for prediction of floret numbers from numbers of leaves per plant at onset of flowering. Predictions of this model agree reasonably well with observed relationships in both inbred lines.

Organization of the Microtubular Cytoskeleton in Dividing Root tip Cells of *Vicia faba* L., Visualized with Confocal Laser Scanning Microscopy

N.C.A. de Ruijter, J. Pietrusiewicz, J.L. Oud*, J.H.N. Schel and A.A.M. van Lammeren.
Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands; and *Department of Molecular Cell Biology, Section of Molecular Cytology, University of Amsterdam, Plantage Muidergracht 14, 1018 TV Amsterdam, The Netherlands

Elongated cells and short cells present in root tips of *Vicia faba* L. exhibit different chromosome orientations during cell division (Oud & Nanninga 1992, *J. Cell Sci.* 103, 847–855). We analysed the three dimensional organization of the microtubular (MT) cytoskeleton by light and confocal laser scanning microscopy to reveal if and how the different chromosome orientations are related with MT configurations during karyokinesis and cytokinesis.

To increase the mitotic frequency, seedlings were incubated with 2.5 mM hydroxyurea. After that root tips were fixed in paraformaldehyde. Cryosections (10–15 µm) and PEG-1500 sections were treated with 1% hemicellulase, 1% Triton X-100 and labelled with anti- α -tubulin and GaM-Bodipy. DNA was stained with propidiumiodide.

In the meristematic cells, nuclei were always in central position. At preprophase, MTs formed a cortical band around the nucleus, both in long and in short cells. MTs also arose at the nuclear membrane, forming a basket of pole-to-pole spindle MTs around the nucleus. At prometaphase, dense MT bundles developed from kinetochores to poles. Spindle axes in short cells tilted and occupied the longest, diagonal cell axis. Since MTs were always oriented perpendicularly to the equatorial plane this plane tilted, too. Anaphase spindles in elongated cells were parallel to the axis of the cell files. In short cells, however, oblique spindles were often seen. Sometimes MT arrays curved, permitting the spindle to fit in the cell. Elongated telophase cells exhibited numerous MTs running from pole to pole, parallel to the length axis of the cell and perpendicular to the phragmoplast. Short cells showed MTs in oblique position to the cell axis at telophase and cytokinesis. At the end of cytokinesis cell plates became sigmoid, because MTs near the parental walls oriented parallel to the length axis, despite an oblique spindle orientation. Thus, while chromosome separation and MT configurations were different in narrow-spaced cells, straight files of cells were still formed.

COMBINED MEETING OF THE SECTION FOR PLANT SYSTEMATICS AND GEOGRAPHY AND THE BION WORKING GROUP SYSTEMATICS OF CULTIVATED PLANTS AND THEIR WILD RELATIVES ON 14 MAY 1993

Cultivated Plants and Systematics: Living Apart Together?

W.A. Brandenburg, DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands

Cultivated plants are the result of interference of biological processes and human action. This interference is called the domestication process. Systematics deal with the classification of living organisms as seen by human beings: systematic results can be considered the human projection of biodiversity. Biodiversity today is the result of evolutionary processes thus far.

Evolution and domestication have much in common. A striking difference is the speed with which both processes proceed. Furthermore, the development of typical features of cultivated plants imply a decrease of natural fitness. The exception to this are those cultivated plants which have been selected for an improvement of reproductive structures.

As to the interference between evolution and domestication, crop-weed complexes have to be studied. It appears that the variation in these complexes is often misunderstood. Depending on the crop-weed complex, the weedy population may have been introduced in cultivated fields whether or not by human action, may have originated from the cultivated plants or may have arisen simultaneously with

the cultivated plant from the ancestral population. The classification of cultivated plants can be improved considerably revealing interrelationships of crop-weed complexes. By accepting separate starting-points for botanical classification and the classification of cultivated plants, the concept of taxon and culton respectively, it is possible to define unequivocal criteria for the connection of both botanical classification and classification of cultivated plants. As a consequence, stabilization of the nomenclature of cultivated plants will be greatly improved.

The Culton Concept: Recent Developments in the Systematics of Cultivated Plants

Wilbert L.A. Hetterscheid. VKC, Linnaeuslaan 2a, 1431 JV Aalsmeer, The Netherlands

The last few decades have seen a substantial development of new philosophies in the systematics of wild plants (e.g. phenetics, evolutionary systematics and phylogenetic systematics). In marked contrast to this, the systematics of cultivated plants has not seen major progressive developments for many years. The main reason for this near stand-still is the persistent idea that systematic groups of cultivated plants behave like taxa. However, when one screens the literature on the systematics of taxa, one never encounters any reference to cultivated plants. On the other hand, taxonomic literature on cultivated plants suffers from a lack of proper concept of what actually constitutes groups of cultivated plants. This results in, e.g. destabilizing nomenclatural discussions, classification proposals with impractical hierarchies, a fair number of impractical and ill-defined classification categories (e.g. grex, specioid, notho-cultivar, subcultivar, etc.). Classifying and naming groups of cultivated plants is an effort that only serves special needs of human society. Classifying wild plants is servant to research in an evolutionary content. It is therefore highly confusing to use the same classification categories (and the resultant nomenclature) for objects that need to be studied in very different contexts (teleologies). The taxon concept never has and never will fulfil this demand and a second general classification concept is proposed to embody classification categories of cultivated plants, viz. 'culton'. A culton is a group of cultivated plants, based on one or more user criteria. The nomenclature of culta is governed by the International Code of Nomenclature for Cultivated Plants. With this concept in mind, all counter-productive ties between

the taxonomy of wild plants and that of cultivated plants can be eliminated.

Systematics of Crop-Weed Complexes

L.J.G. van der Maesen. Department of Plant Taxonomy, Wageningen Agricultural University, P.O. Box 8010, 6700 ED Wageningen, The Netherlands

Crop-weed complexes are defined as complexes of cultivated and related wild or weedy plants growing together and influencing each other through introgression. The level of introgression is usually low and does not necessarily occur each year. Weeds or companions of crop species originate through man's activities. disturbed areas accommodate non-wanted plants with pioneer habits, a pre-adaptation that is also useful for potential domesticates. Many weedy plants have no domesticated relatives (classification hence unambiguous, as they are not members of a crop-weed complex), others are conspecific or closely related to domesticates. Weeds can be agrestals, occurring in cultivated fields, or ruderals. In both conditions introgression between domesticate and conspecific weeds is possible. The degree of weediness varies considerably, and weeds related to the crop species are often mimetic.

Weeds that are conspecific with the crop create no taxonomic problems at the species level, but at the infraspecific level usage of categories is not consistent. Weeds that are not conspecific, either mimetic to the crop or not, are covered by the usual categories in the ICBN: species, subspecies and (botanical) varieties. The level subspecies is commonly used for weeds (and cultivated plants) in the botanic sense, either inconsistently with or without a geographic content (du Rietz 1930, *Svensk bot. Tidskr.* 24), or in the informal sense (Harlan & de Wet 1970, *Taxon* 20; Pickersgill, in Styles, *Infraspecific Classification of Wild and Cultivated Plants* 1986). Opinions differ whether to use non-hierarchical, open, non-complementary classification for cultivated plants, but the ICNCP and its newest proposed version do not preclude the use of hierarchy. Open classification is the practical solution for cultivar classification, but weeds do not have domesticated genes only. The use of notho-taxon epithets is conceivable, but these are not met with frequently. The use of a category such as subspecioid is advocated by Jeffrey and Pickersgill to attain a more uniform treatment of wild/weed/domesticated complexes. Acceptance is needed at the level of the Code. Meanwhile, the use of the subspecies is most common for those weeds that are closely related to our cultivated crop species. In the context of any biological paper the nature of the subspecies, etc. has to be made clear, since use is

not unambiguous and perhaps never has been between scientists.

The systematic Relationship of *Lactuca sativa* and *Lactuca serriola*, in Relation to the Distribution of Prickly Lettuce

F.T. Frietema. Rijksherbarium/Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands; and Center for Plant Breeding and Reproduction Research CPRO-DLO, P.O. Box 16, 6700 AA Wageningen, The Netherlands

The realms of taxonomy of cultivated plants and of the wild flora are different in dealing with plant species, even to the extent of having separate languages; the same plant may be known under different names in either of them.

In the literature of cultivated plants the name 'lettuce' refers mainly to *Lactuca sativa*, with often light-coloured, edible, very variable rosette leaves, often forming large heads. There are also wild species of lettuce, which are of potential breeding interest only.

In the botanical literature, 'lettuce' is the vernacular name of a genus, *Lactuca*, of which prickly lettuce, *L. serriola*, is known to be part of the flora of The Netherlands, on waysides, dykes and ruderal areas. They are recognized by having bluish-green, prickly, often lobed leaves, and a plume-like inflorescence. Consequently, only a wild growing lettuce with yellowish green leaves and no prickles is considered to be an escape, whereas a vegetative specimen of a more or less prickly, bluish-green cultivar will be recorded as wild. This implies that the recorded number of escaped cultivated lettuce in The Netherlands is low, probably lower than it actually is. In contrast, prickly lettuce is spreading rapidly over Europe, and acts like an invasive plant.

When information from the literature of the two taxonomies is combined (de Vries, F. T. *et al.* 1992. Botanical Files: a study of the real chances for spontaneous gene flow from cultivated plants to the wild flora of the Netherlands. *Gorteria* Suppl. 1) the question arises, whether the recent spread of prickly lettuce can be linked to the cultivation of lettuce. This question is under study at the moment.

Systematics of Wild Tuberiferous *Solanum* Species in South America in Relation to the Domestication Process

R.G. van den Berg. Department of Plant Taxonomy, Wageningen Agricultural University, P.O. Box 8010, 6700 ED Wageningen, The Netherlands

The taxonomy of cultivated plants involves two processes: evolution and domestication. Evolution

has produced a large group of tuber-bearing *Solanum* species, a few of which have been domesticated by man. The domestication process, the adaptation of plants to the influence of man, causes variation patterns that should be interpreted differently from those caused by natural evolution. The boundaries between wild and cultivated plants constitute an area where different classification principles may be applied.

The wild species in section *Petota* are classified in series, i.e. groups of related species, supposedly reflecting evolutionary lineages. The cultivated potatoes are to be found in series *Tuberosa*, together with approximately 100 wild relatives. They are classified as seven 'cultivated species' in the generally accepted system of Hawkes. One of these species, *Solanum tuberosum*, is divided in two subspecies: subsp. *tuberosum*, accommodating the modern cultivated varieties, and subsp. *andigena* with the local Andean clones. Clearly, in this way the taxon concept is applied both to the wild species and to the many cultivated forms. For the latter, the culton concept (see abstract by Hetterscheid) would be preferable, leading to a classification of cultivars in cultivar groups which would replace 'species' like *Solanum stenotomum*, *S. phureja*, *S. × chaucha*, and *S. tuberosum* with its subspecies.

Many of the wild species occurring in south America are weedy: they grow preferably in disturbed habitats, often close to cultivated fields. Gene exchange between wild and cultivated plants is therefore possible and even likely, leading to crop-weed complexes (see abstract by van der Maesen). Recent field work in Bolivia emphasized the problematic classification of a group of species, some of which are very similar to the cultivated forms. Species boundaries within this group are extremely difficult to establish due to the extensive variability in many characters. Members of this group, which has been dubbed the '*brevicaule*-complex' by Ugent and the '*leptophyes*-group' by Grun, have been considered as parental species of the cultivated potato. An alternative view would be to interpret some of these 'species' as escapes from cultivation, often with on-going gene exchange with the crop. Species like *Solanum hoopesii* and *S. ugentii* have been described rather recently and much has been made of their tetraploid chromosome number which was noted to be remarkable for wild species. Further research might establish conspecificity of these taxa with an '*andigena*-group' and, similarly, of certain wild diploid species with diploid forms of the crop. This would lead to a reclassification of a number of taxa. One way or another, the consequences of the domestication process must be taken into account in the classification of these close relatives of the cultivated potato.

Systematics of the Genus *Lolium* L.

B.P. Loos. Centre for Plant breeding and Reproduction research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands

Linnaeus recognized two *Lolium* species in his *Species plantarum* (1753): *L. perenne* and *L. temulentum*. In 1968 Terrell (Terrell, E.E. *USDA Technical Bulletin* 1392, pp. 65) reports over 480 intra-generic names for the classification of the genus *Lolium*. In general, seven species are recognized nowadays, three cross-breeding species (*L. perenne*, *L. multiflorum* and *L. rigidum*), and four inbreeding species (*L. loliaceum*, *L. remotum*, *L. temulentum* and *L. persicum*). A review was given on the experimental results available from literature on the intra-generic taxonomic classification. The main techniques used were: intra- and intergeneric crosses, chromosome counts, chromosome morphology determinations, DNA content measurements, Giemsa banding of chromosomes and seed-protein determinations. With all these techniques the cross-breeding species could be distinguished from the inbreeding species. Species distinction within both groups was more difficult, especially in the cross-breeding group. A drawback of all techniques mentioned was the limited amount of material used in the experiments. Morphological analysis of plant material mostly resulted in fairly good species separation, although often not all species were represented in the trials. There is a lack of information on molecular markers for the species relationships within *Lolium*.

Results of own experiments were reported. Fifty-one *Lolium* populations, divided over seven species, were compared using 19 morphological characters. Results showed that the several *Lolium* species could be distinguished, but *L. temulentum* and *L. persicum* showed strong intergradation. Also, 25 populations were compared using five isozymes. These results showed that separation of the cross-breeding *Lolium* species was possible, but that separation of the inbreeding species was impossible due to fixation for the same allelic variants of four of the five enzymes in all inbreeding populations.

Cultivar groups in *Tulipa* L. (Liliaceae)

J. van Scheepen. Royal General Bulbgrowers' Association (KAVB), P.O. Box 175, 2180 AD Hillegom, The Netherlands

In 1601 Clusius recognized in his *Rariorum Plantarum Historia* three major groups within *Tulipa* (cultivars and species together): early, mid season and late flowering. These main groups are still used (Stuurman, J.R. (ed.) 1987, *Classified List and International Register of Tulip Names*). The colour and colour pattern, even when caused by virus, were used

for grouping (and in some cases they still are), e.g. Lacken: violet with broad white margin, Bransons, Agathen, Rosen, Violettes, Bizarres, etc. Morphological characters were of course also used for grouping: double-flowered tulips, parrot tulips for lacinated flowers (known since about 1645). Tulips were only cultivated in England, France, Belgium and The Netherlands. The cultivar classification was, with a few exceptions, used internationally. In 1917 the British-Dutch Report of the Tulip Nomenclature Committee 1914-15 listed all cultivars and 13 major cultivar groups in existence, together with their synonymy. Since this time lists of tulip cultivars and cultivar groups have been published frequently, first by the Royal Horticultural Society, since the invitation to act as international registration authority for tulip names by the Royal General Bulbgrowers' Association. The appearance of new and disappearance of old cultivars has made it necessary to amend the classification. New cultivars may not fit completely in a group and the more this happens, the greater the need for changes. Groups like Mendel, Darwin and Cottage tulips were introduced, redefined and eventually abandoned. The 1987 classification scheme is currently under revision.

The Systematics of the Genus *Tulipa* L. (Liliaceae)

L.W.D. van Raamsdonk. Centre for Plant Breeding and Reproduction Research CPRO-DLO, P.O. Box 16, 6700 AA Wageningen, The Netherlands

The genus *Tulipa* consists of two subgenera with approximately 55 species. The subgenus *Eriostemon* includes three presumably monophyletic sections, viz. *Australes*, *Biflores* and *Saxatiles* with seven, seven and five species, respectively (van Raamsdonk & de Vries 1992, *Pl. Syst. Evol.* 179: 27-41). The other subgenus *Tulipa* (*Leiostemones* Boiss.) includes five sections, viz. *Chusianae* (3 species and 3 intraspecific taxa), *Kolpakowskianae* (3 plus 2, respectively), *Tulipanum* (*Oculus-solis*; 6 species), *Eichleres* (13 and 1, respectively) and *Tulipa* (*Gesnerianae*; 5 plus 2, respectively). The cultivated tulip, *T. gesneriana*, is type species of the genus (van Raamsdonk & de Vries, *Pl. Syst. Evol.* submitted). This classification is based on a phenetic analysis (principal component analysis) of living material. The CPRO-DLO collection contains about 300 accessions, representing the almost complete variation of the genus. Phylogenetic analysis of section *Biflores* revealed a phylogeny which was also deduced from the phenetic analysis, using polyploidy to detect presumed ancestry.

The two subgenera are completely isolated reproductively. Species belonging to different sections are generally intersterile. Between the sections *Eichleres*

and *Tulipa* hybrids can be obtained in a number of combinations. The possibility of combining *T. gesneriana* with a range of related species has been used in breeding research (van Eijk, van Raamsdonk, Eikelboom & Bino 1991, *Sex. Pl. Repr.* 4: 1-5).

A total of 80 *T. gesneriana* cultivars belonging to 12 cultivar groups have been used in a study of phenetic variation over 2 years. The main axis after principal component analysis in both years is determined by flowering time and correlated characters. Cultivar groups originally raised as hybrid groups

between early and late flowering cultivars took an intermediate position. Earliness appeared to be the main characteristic in every cultivar classification since the first one from the early seventeenth century. Since this predominant use of earliness is supported by the principal component analysis, the historical, hierarchical classification is best used with earliness as the main division of tulip cultivars, with a further subdivision of the main groups according to other (floral) characters like lily-flowered, single versus double, fringed and parrot.