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Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 23 JANUARY 1997

Defence Mechanisms of Plants Against Herbivores

Klaas Vrieling, Eddy van der Meijden, Henk Nell and Karin van der Veen van Wijk. Institute of Evolutionary and Ecological Sciences,
Van der Klaauw Laboratory, PO Box 9516,
2300 RA Leiden, The Netherlands

The world is green despite numerous herbivores. Coley & Barone (*Ann. Rev. Ecol. Syst.* 1996, 305–335) estimated that *c.* 10% of the green biomass is consumed by herbivores. This equals the amount of resources that plants on average allocate to generative reproduction.

Plants defend themselves in different ways to the armada of herbivores. Well known are the morphological and chemical defences (mostly secondary metabolites) employed by plants. If these defences are not effective plants can sometimes tolerate herbivory (e.g. regrowth capacity after defoliation) or rely on the association with other species like toxin-producing endophytic symbionts or attracting natural enemies of the attacking herbivores.

Within a plant species a large amount of genetic variation in defences is often found. The content of pyrrolizidine alkaloids, that reduced herbivory by generalist herbivores, in ragwort in the field varied from 0·05% to 1% dry weight. This variation is proposed to be maintained by a balance between the benefits of reduced herbivory by generalist herbivores and the costs associated with alkaloids. Costs of alkaloids can either be direct: energy and nutrients allocated to the production, transport, storage and maintenance of alkaloids, or indirect: herbivory of adapted specialist herbivores which use alkaloids as a feeding stimulant.

Is Soil Fauna Community Affecting Diversity and Structure of Natural Vegetations?

M.P. Berg, Vrije Universiteit, Amsterdam,
Department of Ecology and Ecotoxicology Section Soil Ecology, De Boelelaan 1087,
1081 HV Amsterdam, The Netherlands

Plant/foliar–fauna interactions have been the subject of active ecological research in recent years. Effects of subterranean herbivores, and belowground fauna in general, on single plant species or natural vegetation have been virtually unexplored, although it has received considerable attention in agriculture. The following is based on a literature search.

Consumers of roots appear to reduce root biomass leading to reduced water and nutrient uptake. Plants respond often with a reduction in growth, reproductive output and survival. Severe root herbivory negatively effects anchorage and storage of reserves. Root herbivores may act as vectors for a range of plant pathogens.

Mycorrhizae consumers prefer the small, water and nutrient absorbing, hyphae of mycorrhizal fungi and this accounts for the reduced nutrient uptake by plants in the presence of fauna. Root colonization, by propagulae distribution, is increased by fauna but negative effects on root length infection are also reported.

Detritivorous fauna release nutrients immobilized in dead organic matter and microphytophages from microbes in the rhizosphere, and excrete excess nutrients in the soil solution. Plants respond to increased nutrient availability with growth and reproduction increment. Microphytophages may consume plant pathogens.

Although effects of soil fauna on individual plants species are relatively well known, possible effects on vegetation are largely ignored. The few existing examples indicate that a reduction in belowground fauna positively influences plant diversity, probably due to reduced seed predation, and alters the direction and rate of vegetation succession. It is concluded that soil fauna, especially due to their spatial and temporal heterogeneity in soil, may modify the structure of vegetation.

VLINDEREXPERT, an Expert System for Development of Nature Values

Frans Maaskamp, DLO Institute for Forestry and Nature Research, PO Box 23,
6700 AA Wageningen, The Netherlands

The computer program VLINDEREXPERT is a tool for managers in order to make the optimal decision about mowing time and the choice of machinery for the preservation of butterflies. Also for urban and rural planners involved in designing former arable areas in nature areas, the program is useful. VLINDEREXPERT has a number of databases which are to be completed by the user with information about the site.

When this information is entered, a prognosis is available about the butterflies to be expected. The species concerned are subdivided into inhabitants, guests and migrators. The prognosis also provides a selection of host plants and nectar sources on the basis of an inventory list.

The program gives insight in the consequences of the management regime for butterflies and enables the user to concentrate his management of as many species as possible, or on rare and vulnerable species. The program describes all Dutch butterfly species with their biology and management aspects; each species is also depicted.

Interaction between Pollinators and *Spiranthes spiralis* (I.) Chevall

J.H. Willems[1], A. Stroo[1] and M.-L. Lahtinen[2].
[1]Department of Plant Ecology and Evolutionary Biology, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands and
[2]Laboratory of Plant Systematics and Ecology, University of Turku, FINN-20014 Turku, Finland

Spiranthes spiralis can be considered both a rare and threatened orchid species in NW Europe. At the European Continent the species reaches its most north-western border of its south-eastern distribution area in the southern part of The Netherlands. Only two populations remain in the South of this country of *c.* 35 sites in the first half of this century, namely on the Island of Goeree in the south-western and in South Limburg in the south-eastern part, the latter being the subject of this study.

Experiments carried out on this isolated border population revealed that *Spiranthes spiralis* is a non-autogamous species. Visiting insects, mainly bumblebee species, are needed for seed production either by geitonogamy or cross-pollination. Artificially-pollinated plants had *c.* 75% fruit set compared to 35% for flowers pollinated by insects in 1994. However, this percentage varies considerably from year to year; 30–35% is the long-term average fruit set in this population. The number of seeds varies between 20 to 2000 per fruit.

Removal of flower heads of other species surrounding clumped inflorescences of *Spiranthes spiralis* (>3 near to each other) increased fruit set, indicating interspecific competition for pollinators. Fruit set per inflorescence varied dramatically, up to 80%. In general there was a positive correlation between the numbers of flowers and the numbers of fruits per inflorescence. This is probably a result of flowering strategy of this species which shows that during flowering only 3–5 of the tiny, white flowers are open. Flowering starts at the lowest flower at the flowering stalk.

Insect pollination does not appear to be a significant bottle-neck in the sustainability of this border population in South Limburg. The management of the semi-natural and species-rich grassland in which *Spiranthes spiralis* occurs, mowing in late July and aftermath grazing by sheep in winter, seems to be appropriate. Since the introduction of this management some 15 years ago, the population has increased 10-fold. Therefore this population seems to be sustainable, at least for the near future.

Ground Beetles and their Habitats

H.H. de Vries, Biological Station Wijster,
Centre for Soil Ecology, Wageningen Agricultural University, Kampsweg 27, 9418 PD Wijster,
The Netherlands

Investigations on the ground beetle fauna of heathlands in the north-east part of The Netherlands showed a decrease in the presence of heathland species in fragmented habitats. The research, which was carried out in areas where still an abundance of heath was present, showed increasing extinction rates of populations in habitats smaller than 50 ha. In particular the presence of heathland species with low dispersal ability appeared to be strongly related to area. However, those species that also occur in agricultural areas did not show such a relationship. It can be concluded that the possibilities for (re)colonization determine the presence of species in fragmented habitats.

Fragmented habitats often deteriorate. As many heathland ground beetle species are not phytophagous, estimates of the suitability of small areas as habitats for these species are difficult to make. The habitat conditions necessary for these species are partly formed by the special microclimatic conditions in heathlands and partly by the availability of suitable food, mostly small arthropods. Transplant experiments with two endangered heathland ground beetle species, *Olisthopus rotundatus* and *Pterostichus lepidus*, showed that small heathlands were not, in all cases, suitable. Both species are generally considered xerophilic, but in this region they also can be found in wet heathlands, and *O. rotundatus* even occurs in peatmoors. The results of the experiments indicate for these two species a negative influence of the presence of grasses and of shade of nearby trees. Opportunities for exposure to sunlight seems to be an important factor for the suitability of heathlands for at least part of the heathland ground beetle species.

Seed Removal and Dispersal by Forest Rodents: Effects of Vegetation Structure

Jan den Ouden and Ruben Smit, Department of Forestry, Wageningen Agricultural University, PO Box 342, 6700 AH Wageningen, The Netherlands

The predation and dispersal of seeds are key factors in the regeneration ecology of many plant species. Within Dutch forests, seeds of *Quercus robur* are mainly predated and dispersed by small rodents. The local population densities of these rodents are greatly determined by the vertical structure of the understorey vegetation. We hypothesized that differentiation in vegetation structure, in this study represented by the presence or absence of bracken (*Pteridium aquilinum*) stands, affects seed removal rates and dispersal directions by forest rodents.

The amount of captured rodents was significantly higher under bracken stands than in directly adjacent vegetation without bracken. Bank voles (*Clethrionomys glareolus*) were only caught inside bracken stands, while wood mice (*Apodemus sylvaticus*) were more or less indiscriminate to vegetation structure. Seed removal rates were generally higher inside bracken stands. Overall removal rates were correlated with the population density of rodents on the site.

Dispersal patterns were studied by inserting magnets in the acorns, and relocating dispersed seeds with a magnet detector. Dispersal distances were related to natural seed crop. In a year with low acorn crop in the site, mean dispersal distance was twice (20–25 m) the distance than in a year with an abundant crop (10–15 m). Seeds that were dispersed from plots within bracken stands showed a random dispersal pattern. Acorns from outside the bracken stand were not randomly dispersed, but instead showed a preferred direction towards patches of adjacent bracken vegetation or low oak scrub. This study indicates that vegetation structure has an indirect influence on local seed availability through the directed dispersal of seeds by rodents.

Effects of Grazing by Bewick's Swans and Other Herbivorous Waterfowl on Submerged Macrophytes

Jan H. Beekman and Luis E. Santamaría Galdón,
Netherlands Institute of Ecology, Centre for Limnology (NIOO-CL), Rijksstraatweg 6,
3631 AC Nieuwersluis, The Netherlands

Fennel-leaved pondweed (*Potamogeton pectinatus*) reproduces mainly by means of underground asexual propagules (tubers). These energy-rich tubers form the main food resource for Bewick's swans (*Cygnus columbianus bewickii*) for replenishing their fuel (fat) reserves during their annual migration between northern Russia and western Europe (approx. 3500 km). Bewick's swans have a stepping-stone migration strategy, which is dictated by their high flying costs, low fat loads and slow rates of fat accumulation. They fly stretches of 800–1200 km with intermittent stop-over periods of 2–4 weeks. Here, the swans can deplete *P. pectinatus* tuber banks up to 20% of the initial biomass. However, we found a threshold level of 3–8 g dry weight m^{-2}, below which the swans can no longer efficiently exploit this food resource. Although tuber bank depletion may lead to a reduced pondweed standing crop in the following season, reduced competition for light and nutrients can result in a (interannually) sustained standing crop. Our findings also suggest that the swans feed preferentially on larger-sized tubers. Such tuber size selection could affect the pondweed populations negatively, as growth experiments showed that small tubers suffer higher mortality, sprout later and have a delayed peak plant biomass.

Swan utilization of Lake Lauwersmeer did not decrease during 1980–96, although interannual variation in utilization was almost 10-fold. In a multiple regression analysis, 80% of this interannual variation was explained by two weather variables: the total summer duration of sunshine (which probably determines the primary production of the pondweed population) and wind speeds in early autumn (which influence tuber formation by affecting the detachment of the aboveground material).

Grazing of pondweed shoots, leaves and seeds by Mallard (*Anas platyrhynchos*) and Coot (*Fulica atra*) during summer was studied using exclosures. Grazing increased plant species diversity, but also reduced shoot and seed biomass, and the autumn tuber production of *P. pectinatus*. The latter effect leads us to believe that there is a time-lag interspecific food competition between Bewick's swans and other waterfowl species. In addition, the effect of waterfowl grazing on the relative abundance of different waterplants may have a strong relevance for the succession in submerged plant communities, and diversity and stability of fresh water ecosystems.

The Rabbit—Key Factor for Vegetation Development of Dry Dunes?

Matthijs J. ten Harkel, Landscape and Environmental Research Group, University of Amsterdam, Nieuwe Prinsengracht 130,
1018 VZ Amsterdam, The Netherlands.

A 6-year experimental study has been carried out in the Meijendel dune area north of The Hague (The Netherlands) to examine the combined effects of grazing and atmospheric nitrogen deposition on the vegetation of three dry dune grasslands: one short, species-rich stand, one short, species-poor stand and one stand dominated by tall graminoids. In each stand four experimental plots were created: (1) control; (2) fertilized; (3) excluded from grazing by rabbits and (4) combination of fertilization and exclusion of grazing.

Additional fertilization with nitrogen had no significant effect, both in grazed and non-grazed plots. The exclusion of grazing by rabbits in the three sites resulted in an increase in the frequency of perennial graminoids and a decrease in the frequency of annual graminoids and herbs. During the experiment the aboveground biomass increased and the amount of bare soil and mosses of the three non-grazed sites decreased.

The vegetation changes occurred mainly within 1 year after the exclusion of grazing. An exception is the grass-dominated site where the amount of *Calamagrostis epigejos* increased gradually from about 20% in the first two years to about 50% in the fourth and fifth year.

Grazing by rabbits seems essential to prevent the predominance of graminoids in the dry dunes. If graminoids are dominant, grazing by horses can be an appropriate method to restore the original grassland vegetation. After 6 months of grazing by horses the grass-dominated site showed a decrease of the frequency of perennial graminoids, namely from 95% to 80%, and an increase of the frequency of perennial herbs, namely from 2·5% to 13–20%. After excluding the grazing by horses, the grazed plots gradually became dominated by graminoids again. It seems that with today's rabbit population, large herbivores will always be necessary to prevent grass-encroachment of part of the dunes.

Unexpected Impact of Beavers on a Riparian Willow Wood

B.A. Nolet, Institute for Forestry and Nature
Research, PO Box 23, 6700 AA Wageningen,
The Netherlands

In winter, beavers predominantly eat bark, showing a clear preference for soft-wooded trees (*Populus*, *Salix* and *Betula* species). These early-successional trees are believed to invest in regrowth rather than plant defences, especially when they have outgrown the sapling stage. The hypothesis is that these trees are preferred by beaver because of their low fraction of lignin, making them more easy to fell and to digest (Fryxell, J.M. *et al.* 1994, Oikos **71:** 207–214).

In the Biesbosch, this remarkable ability of willows to regrow has been used for decades to harvest stacks of willow coppices. However, from *c.* 1960 onwards this was no longer profitable. Nature conservationists hypothesized that beavers would be the perfect substitute for man to exploit the willow coppices. This exploitation by beaver was thought to enhance the predicted succession from the present wood (*Salicetum albae* Issler 1926) to hardwood forest of the category *Fraxino–Ulmetum*, subassociation *alnetosum* (Van der Werf, S. 1991, *Bosgemeenschappen*, Pudoc, Wageningen).

Between 1988 and 1991, 42 beavers were released. The territories held by the beavers were 2–4 times as large as in other temperate areas (Nolet, B.A. & Rosell, F. 1994, *Can. J. Zool.* **72:** 1227–1237). Willows formed the staple food of beavers which they mainly ate close to their dens, but non-willow species had higher probability to be cut at the edge of the territories or away from the water. A diet consisted of willows and poplars would not meet the beavers' Na and P requirements during reproduction (Nolet, B.A. *et al.* 1995, *Neth. J. Zool.* **45:** 315–337). *Corylus avellana* and *Fraxinus excelsior* appeared to contain relatively much Na, and *Prunus padus* and *Populus* × *canadensis* were relatively rich in P, suggesting that the beavers ate these trees to avoid dietary deficiencies (Nolet, B.A. *et al.* 1994, *Biol. Conserv.* **70:** 117–128). I conclude that beavers will retard rather than enhance the succession to a more mixed woodland.

THE 'WILLIE COMMELIN SCHOLTEN' MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON 24 JANUARY 1997

Involvement of a New Salicylic-Acid Based Siderophore in Suppression of Fusarium Wilt by *Pseudomonas fluorescens* WCS374

J. Mercado-Blanco[1], P.E. Olsson[1], I. van der Sluis[1], K.M.G.M. van der Drift[2], J.E. Thomas-Oates[2], L.C. van Loon[1] and P.A.H.M. Bakker[1].
[1]Department of Plant Ecology and Evolutionary Biology, Utrecht University, PO Box 800.84, 3508 TB Utrecht and [2]Department of Mass Spectrometry, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands

Siderophore production by fluorescent *Pseudomonas* spp. has been demonstrated to be involved in control of soil-borne plant pathogens by these bacteria. It has been suggested that both competition for iron and induction of systemic resistance (ISR) are the effective mechanisms of siderophore-mediated disease suppression. *P. fluorescens* WCS374 suppresses fusarium wilt in radish by induction of systemic resistance. Strain WCS374 produces the siderophore pseudobactin and salicylic acid (SA) at low iron availability, and it has been suggested that both compounds are involved in ISR. After Tn*5* insertion mutagenesis of strain WCS374, several pseudobactin deficient mutants were obtained. These mutants were demonstrated to produce a second siderophore, the production of which is linked to SA biosynthesis.

We have been able to clone a region of the WCS374 genome that contains the loci necessary for SA and the second siderophore (fluorebactin) production, and part of this clone has been sequenced. Preliminary mass spectrometric data obtained from fluorebactin reveal that an SA moiety is present in the molecule but that the structure differs from SA-based siderophores already described. Using purified fluorebactin and different mutants of strain WCS374, the involvement of this siderophore in disease suppression will be investigated.

ASP-1, a *Pseudomonas chlororaphis* Biocontrol Strain in the Tomato–*Fusarium oxysporum* f.sp. *radicis-lycopersici* System

T.F.C. Chin-A-Woeng[1], A.J. van der Bij[1], J.E. Thomas-Oates[2], K.M.G.M. van der Drift[2], J. Schripsema[3], B. Kroon[4], R.J. Scheffer[4], G.V. Bloemberg[1] and B.J.J. Lugtenberg[1]. [1]Institute of Molecular Plant Sciences, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, [2]Department of Mass Spectrometry, University of Utrecht, Sorbonnelaan 16, 3584 CA Utrecht, [3]Department of Organic Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark and [4]S & G Seeds BV, Westeinde 62, 1600 AA Enkhuizen, The Netherlands

ASP-1 is one of several *Pseudomonas* strains isolated from a Spanish tomato field near Granada that shows excellent biocontrol ability against the plant pathogen *Fusarium oxysporum*. Factors considered to be important for a biological control agent are (i) the production of a factor inhibiting the growth of the (fungal) pathogen, and (ii) efficient root colonization to deliver the anti-fungal factor at the right place and the right time on the root. Research is focused on the identification of anti-fungal factors and their biosynthetic regulation.

ASP-1 has been typed (H.-V. Tichy & R. Simon, TÛV Energie und Umwelt GmbH, Freiburg, Germany) as a *Pseudomonas chlororaphis* species and produces factors such as hydrogen cyanide, chitinase and proteases, molecules that are expected or have been shown to inhibit the growth of plant pathogens. After HPLC purification, mass spectrometric and NMR analyses showed that ASP-1 produces two phenazine antibiotics: phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCX), the latter compound being the major phenazine produced. Based on the proposed biosynthetic pathway for phenazine, PCX is assumed to be a derivative of PCA. It was shown that the *in vitro* anti-fungal activity of PCA was pH-dependent, whereas that of PCX was not.

To isolate genes involved in the biosynthesis and regulation of phenazine production in this strain, Tn*5*-*luxAB* and Tn*5*-*lacZ* transposon libraries of ASP-1 were screened for mutants impaired in phenazine production. Screening of 18 000 transposon mutants resulted in a number of mutants that are defective in phenazine production.

Survival of and Root Exudate-(Proline-) Induced Promoter Activity of *Pseudomonas fluorescens* R2fR Mutant RIWE8 in the Rhizosphere of Wheat

L.S. van Overbeek, J.D. van Elsas and J.A. van Veen. DLO-Research Institute for Plant Protection (IPO-DLO), Wageningen, The Netherlands

A bank of promoter probe (Tn*5*-B20, *lacZ* as reporter gene) mutants of *P. fluorescens* R2fR was differentially screened for specific induction of

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reporter gene expression by wheat root exudates. Four mutants responded to wheat root exudate and these were further screened for their response to 125 different carbon sources. One mutant, denoted RIWE8, responded only to proline. Mutant RIWE8 also showed increased reporter gene expression to maize and grass root exudates.

Reporter gene expression in strain RIWE8 was detected in Flevo silt loam soil amended with proline, but not in malic acid-, glutamic acid-, or arginine-amended soils. In a model rhizosphere microcosm with wheat, maize and grass plant roots, reporter gene expression was highest near the roots and decreased with the distance from the roots.

Expression of *lacZ* in RIWE8 was also detected near roots of wheat plants growing in Flevo silt loam soil under greenhouse conditions. Survival of mutant RIWE8 was slightly reduced in the rhizosphere of wheat as compared with the parent strain R2fR, 3 weeks after introduction.

Reporter gene expression in RIWE8 under field conditions was detected near wheat roots between 1 and 2 weeks after introduction. Survival of R2fR and RIWE8 was determined by CFU, immunofluorescence cell counts and most probable number (MPN) PCR (RIWE8 only). No difference in root colonization and survival was observed between RIWE8 and the parent strain R2fR under field conditions. However, RIWE8 immunofluorescence cell numbers were up to three orders of magnitude higher than corresponding CFU and MPN-PCR numbers after 4 months, indicating the presence of large numbers of non-culturable RIWE8 cells. Mutant RIWE8 CFUs could still be detected 18 months after introduction into the field plot.

Novel Colonization Traits in Tomato Root Colonization by *Pseudomonas fluorescens* Biocontrol Strain WCS365

L.C. Dekkers, A.J. van der Bij, C.C. Phoelich,
I.H.M. Mulders and B.J.J. Lugtenberg. Leiden
University, Institute of Molecular Plant Sciences,
Clusius Laboratory, Wassenaarseweg 64,
2333 Al Leiden, The Netherlands

In order to obtain more knowledge about traits that are important for root colonization, 1300 Tn*5* and Tn*5*-*lacZ* mutants of the efficient root colonizer *P. fluorescens* WCS365 were generated. These mutants were pairwise screened for their ability to colonize the tomato root tip. Putative colonization mutants were subsequently tested for traits previously described to be important for root colonization. Nine colonization mutants were isolated that had no detectable defect with respect to known colonization traits. All mutants colonized the root tip equally well when inoculated alone.

Here we describe the molecular characterization of one of these mutants, strain PCL1210. A 3·1 kb fragment was found to be able to complement the observed mutation. The nucleotide sequence of the region around the Tn*5*-*lacZ* insertion showed three open reading frames. The deduced amino acid sequences of the first genes of the operon, designated as *col*R and *col* S, show strong similarity with known members of two-component regulatory systems. ColR has homology with the response regulators of the OmpR-PhoB subclass whereas ColS, the product of the gene in which the mutation resides, shows similarity to the sensor kinase members of these two-component systems. Only part of the *orf*222 gene is present in the complementing 3·1 kb region, and it therefore does not seem to play a role in colonization. No experimental evidence for a role of the ColR/ColS two-component system in the suspected colonization traits, chemotaxis and transport of exudate compounds, could be obtained. The function of this novel two-component system therefore remains to be elucidated. We conclude that colonization is an active process in which an environmental stimulus, through this two-component system, activates a so far unknown trait which is crucial for colonization.

A Novel Signalling Pathway Controlling Induced Systemic Disease Resistance in Plants

C.M.J. Pieterse, J.A. van Pelt and L.C. van Loon.
Department of Plant Ecology and Evolutionary
Biology, Section Plant Pathology, Utrecht
University, PO Box 800.84, 3508 TB Utrecht,
The Netherlands

Systemic acquired resistance (SAR) is a pathogen-inducible defense mechanism in plants effective against a broad spectrum of plant pathogens. The resistant state is dependent on endogenous accumulation of salicylic acid (SA) and is characterized by the activation of a specific set of genes including genes encoding pathogenesis-related (PR) proteins. Recently, selected non-pathogenic, root-colonizing *Pseudomonas fluorescens* bacteria with disease-suppressive properties have been shown to trigger a phenotypically similar systemic resistance response. This type of systemic resistance is called induced systemic resistance (ISR). To investigate whether pathogen-induced SAR and non-pathogenic rhizobacteria-mediated ISR is controlled by the same signalling pathway, *Arabidopsis* mutants and transgenics that are altered in their response to the signalling molecules SA, ethylene or jasmonic acid were tested for their ability to express SAR and ISR, respectively.

Transgenic NahG plants, that readily convert SA into inactive catechol by the action of the salicylate hydroxylase (*nah*G) gene, expressed non-pathogenic rhizobacteria-mediated ISR to the same level as wild-type plants, whereas pathogen-induced SAR was blocked in these plants. In contrast, the ethylene response mutant *etr*1 and the jasmonic acid response mutant *jar*1 readily expressed SAR, whereas ISR was impaired in these mutants. Furthermore, ISR did not coincide with the accumulation of PR mRNAs, which is a typical characteristic of SAR. These results clearly demonstrate that nonpathogenic rhizobacteria induce a signalling pathway different from that controlling SAR, leading to a form of systemic resistance that, in contrast to SAR, is independent of SA accumulation and PR gene expression but dependent on the ethylene and the jasmonic acid response. Interestingly, mutant *npr*1 which was originally isolated for its inability to express PR genes and as a result was unable to express SAR, was also not responsive to induction of ISR. This suggests that the *npr*1 gene is a regulatory gene controlling both SAR and ISR.

Processing, Localization and Antifungal Activity of *Urtica dioica* Agglutinin in Transgenic Tobacco

M.P. Does, P.M. Houterman, D. Ng and B.J.C. Cornelissen. Institute for Molecular Cell Biology, BioCentrum Amsterdam, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands

Genomic sequences encoding the precursor of *Urtica dioica* agglutinin (UDA) were isolated from genomic stinging nettle DNA by polymerase chain reaction (PCR). Primers used were homologous to parts of the 5′- and 3′-untranslated sequences as published (Lerner, D.R. & Raikhel, N.V. 1992, *J. Biol. Chem.* **267**: 11085–11091). Both partial sequencing of cloned PCR fragments from five independent PCR reactions and Southern blotting of genomic stinging nettle DNA, revealed the presence of a family of at least 16 genes encoding at least 7 isolectins. One fully sequenced clone showed the presence of two introns, located at identical positions as introns in genes encoding plant class I and class II chitinases.

A construct containing the sequence encoding the UDA precursor under control of the CaMV 35S promoter and the 3′ NOS terminator was introduced into *Nicotiana tabacum* cv. Samsun NN by *Agrobacterium* transformation. Western blotting and immunological detection with antiserum raised against a synthetic peptide, showed the production and vacuolar targeting of mature UDA in transgenic tobacco plants. To investigate the presence of a C-terminal vacuolar targeting signal, a construct lacking the sequence for 25 C-terminal amino acids of the UDA precursor was also transformed into tobacco plants. UDA was detected predominantly in the extracellular washing fluid (EWF) of transgenic tobacco plants. Future experiments will have to show the absence of UDA in the vacuoles of these plants.

UDA isolated from the EWF retained its agglutination activity on erythrocytes and its chitin binding activity. In an antifungal assay a concentration of 50 μg UDA/mL did not inhibit growth of *Botrytis cinerea.*

Isolation and Characterization of Sesquiterpene Cyclase Genes from Tomato

A.G.M. Porto le Blanch[1], J. Chappell[2], B.-D. Gao[1] and B.J.C. Cornelissen[1]. [1]Section of Plant Pathology, Institute of Molecular Cell Biology, BioCentrum Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands and [2]Plant Physiology/Biochemistry/Molecular Biology Program. Agronomy Department, University of Kentucky, Lexington, Kentucky 40546-0091, USA

Rishitin is the predominant phytoalexin found in tomato after infection with *Fusarium oxysporum* f.sp. *lycopersici.* Rishitin is a product of the sesquiterpenoid biosynthesis route. Sesquiterpene cyclase, a key enzyme in this pathway, converts farnesyl pyrophosphate (FPP) presumably into vetispiradiene which is then converted by several enzymatic steps into rishitin. To study the role of rishitin in defence reactions against pathogens, we want to inhibit the biosynthesis of this phytoalexin either by antisense expression or co-suppression of the sesquiterpene cyclase genes. Transgenic plants no longer able to synthesize rishitin will be inoculated by *Fusarium oxysporum* f.sp. *lycopersici.*

Southern blot analysis showed the presence of four to six sesquiterpene cyclase genes in the genome of tomato. Four genes have been isolated from a λ EMBL3 tomato genomic library by the use of a tobacco cyclase gene (Facchini, P.J. & Chappell, J. 1992, *Proc. Natl. Acad. Sci. USA* **89**: 11088–11092) as a probe. Sequencing revealed that the primary structure of the product encoded by one of them shows 86% homology (73% identity) with the amino acid sequence of a tobacco sesquiterpene cyclase. The other three genes contain several deletions, some of them resulting in a frame-shift and, therefore, they seem to be pseudo genes. Restriction analysis revealed that three sesquiterpene cyclase genes are located closely on the same chromosome.

Basic Research on Chitinolytic Soil Bacteria: a Prerequisite to Successful Application in Crop Protection

W. de Boer. Netherlands Institute of Ecology,
Centre for Terrestrial Ecology, PO Box 40,
6666 ZG Heteren, The Netherlands

In the early 1960s Mitchell & Alexander (1963, *Can. J. Microbiol.* **9**: 169–177) observed that bacteria which were able to lyse living fungal hyphae excreted chitinases. In addition, they obtained evidence that these chitinases had a functional role in the degradation of fungal cell walls. Later on, research by others showed that the potentially promising biocontrol agents were often chitinolytic. Based upon these results, it has been hypothesized that stimulation of chitinase activity in soils might aid in the suppression of plant pathogenic fungi. Attempts to use chitinous materials for such a purpose have often been unsuccessful, however, and the reasons for this failure are not yet clear.

A possible explanation could be that the composition of the micro-organisms which colonize and degrade the chitin particles determines whether or not the chitin amendments will be helpful in suppressing plant pathogenic fungi. This idea is based upon the observations that many chitinolytic bacteria are not able to lyse fungal hyphae. Hence, it is quite possible that added chitin becomes colonized by micro-organisms that have no effect on pathogenic fungi. Preliminary results of an experiment on the composition of the chitinolytic microbial community in dune soils after addition of chitin indicate that there can be strong differences between soils with respect to the dominant chitin-degrading micro-organisms.

Perhaps a more promising strategy for use of chitin for biocontrol purposes could be to inoculate chitin-containing amendments with chitinolytic bacteria that are able to (i) suppress fungal growth while degrading chitin, and (ii) maintain themselves as the dominant chitin-degraders in the soil.

Influence of Temperature and Wetness Duration on Infection of Lily by *Botrytis elliptica*

J.E. van den Ende and M.G. Pennock-Vos.
Bulb Research Centre (LBO), PO Box 85,
2160 AB Lisse, The Netherlands

Infections of fire on lily, caused by *Botrytis elliptica*, appear as small spots on green leaves, but may progressively invade the tissue under favourable environmental conditions, causing large brown lesions and early die-back of the leaves. Studies on the effects of wetness duration and temperature have been focused on the leaf-spotting phase of the disease. As sporulation takes place on dead host tissue the second phase is of great importance to the epidemiology of fire, and justifies more study of the factors favouring progressive colonization and leaf die-back.

Experiments were carried out under controlled conditions in climate chambers. Maximum spot numbers developed within 24 h on lily leaves under continuous leaf wetness at 20°C following inoculation with conidia of *B. elliptica.* At lower post-inoculation temperatures (5–15°C) at least 48 h of leaf wetness was needed to get similar disease severity. Within 24 h of leaf wetness only a limited number of fire lesions developed at 15°C and 20°C. An increase of lesion numbers was found with increasing leaf wetness duration (1–3 days) at moderate temperatures (10–20°C). No lesions appeared at low temperature (5°C). Interruption of the leaf wetness period during the first 24 h after inoculation reduced the number of spots. Interruptions of the wetness period after an initial 24 h of leaf wetness restricted lesion number and lesion growth with decreasing relative humidity during a dry period. With increasing relative humidity during the dry period an increasing number of spots are able to survive and will cause fire lesions after rewetting the lily leaves.

The data presented are used to optimize a warning system for fire control under field conditions.

Specificity of the Plant Parasitic Nematode *Heterodera* ssp. in the Coastal Foredunes

C.D. van der Stoel. Netherlands Institute of
Ecology, Centre for Terrestrial Ecology,
PO Box 40, 6666 ZG Heteren, The Netherlands

In Dutch coastal foredunes, as well as elsewhere in north-western European foredunes, *Ammophila arenaria* (marram grass) is the most important sand-fixing plant species. Degeneration of *A. arenaria* is a natural feature in stabilised dunes, which may be negative for coast protection. In the degeneration of *A. arenaria*, plant parasitic nematodes and pathogenic soil fungi are supposed to be involved. Thus, these soil organisms may influence vegetation succession. Nematode species of the genus *Heterodera* are frequently found in the root zone and on the roots of *A. arenaria.* In both a field survey and a pot experiment it is investigated whether the cyst-forming *Heterodera* sp. are specific to *A. arenaria*, to determine whether these nematodes contribute to the degeneration of *A. arenaria.*

The dominant foredune plant species, *Elymus farctus*, *Ammophila arenaria*, *Festuca rubra* ssp.

rubra, *Carex arenaria*, *Elymus athericus* and *Calamagrostis epigejos*, in succeeding order, were examined during the field survey. On all plant species cysts were found, either on the roots or in the bulk soil. The total number of cysts found on the roots of *A. arenaria*, expressed per unit of root weight, was higher than on the roots of later successional plant species. Larvae were only found in very low numbers.

In the pot experiment with plants inoculated with *Heterodera* sp. larvae significantly higher numbers of both cysts and larvae were found on *Elymus farctus* and *Ammophila arenaria* than on the succeeding plant species. *Carex arenaria* was the only plant species on which no cysts had been formed.

It is concluded that *Heterodera* sp. are not strictly specific to one plant species, although they occur in larger amounts on the species early in the succession.

Significance of ABC-Transport Proteins in *Aspergillus nidulans*

A.C. Andrade[1], G. Del Sorbo[2] and M.A. De Waard[1]. [1]Department of Phytopathology, Wageningen Agricultural University, PO Box 8025, 6700 EE Wageningen, The Netherlands and [2]Institute of Plant Pathology, Via Università 100, 80055 Portici (Naples), Italy

ABC-transporters are members of the *A*TP-*b*inding *c*assette (ABC) superfamily of membrane transporters which may function as energy-dependent drug efflux pumps in a variety of organisms. From *Aspergillus nidulans* we have isolated two ABC-transporter encoding genes, coded *atr*A and *atr*B. The products of these genes share the same topology as PDR5 and SNQ2 of *Saccharomyces cerevisiae* and CDR1 of *Candida albicans*. The function of both genes from *A. nidulans* has been characterized by comparison of gene expression in fungicide-sensitive and resistant isolates, induction of transcription with drugs, and by complementation studies in yeast.

Particular fungicide-resistant isolates have a relatively high basal transcription level of *atr*A. In the wild-type isolate, transcription of *atr*A and, in particular, *atr*B is strongly enhanced by treatment with drugs such as antibiotics, azole fungicides and plant defense toxins. The enhanced transcription is detectable within a few minutes after drug treatment and coincides with the beginning of energy-dependent drug efflux activity, reported previously in the fungus for azole fungicides. Complementation studies in yeast demonstrated that cDNA of *atr*B could reduce drug sensitivity levels, using *PDR5* knock-out mutants of *S. cerevisiae*. Current research focuses on further analysis of the genes by construction of knock-out mutants of *A. nidulans* followed by phenotypical characterization. In addition, two other ABC-transporter encoding genes have been isolated and are being characterized.

Genetics of Biological and Molecular Markers in *Mycosphaerella graminicola* (Anamorph *Septoria tritici*)

G.H.J. Kema, C. Waalwijk, E.C.P. Verstappen, J.R.A. de Koning, J.A.G. Koeken, P.J.M. Bonants and M. Hagenaar-de Weerdt. DLO-Research Institute for Plant Protection (IPO-DLO), PO Box 9060, 6700 GW Wageningen, The Netherlands

Mycosphaerella graminicola is a bipolar heterothallic ascomycete pathogenic on wheat. A recently described crossing procedure enables genome analysis of this fungus. We study the genetics of (i) avirulence, (ii) mating types and (iii) diverse molecular markers in this fungus.

Avirulence. Isolate IPO323 is avirulent on cvs. Shafir, Veranopolis and Kavkas whereas isolate IPO94269 is virulent on these cultivars. Both isolates are virulent on cv. Taichung 29 and avirulent on cv. Kavkaz/K4500. IPO323 and IPO94269 were crossed and a random F_1 progeny was recovered. In addition, 32 BC_1 progenies were generated by back-crossing. Sixty F_1 and 60 BC_1 isolates, from three progenies, were assayed for avirulence on the aforementioned cultivars. As expected, all progeny isolates were virulent on cv. Taichung. None of these isolates carried virulence for cv. Kavkaz/K4500, indicating that the parental isolates carry the same avirulence factor(s) for this cultivar. Avirulences for each of the differentiating cultivars co-segregated in a 1:1 ratio. We therefore hypothesize the presence of a complex locus of tightly linked avirulence genes.

Mating types. Back-crossing was also performed to identify the mating types (MAT1-1 and MAT1-2). Following a bulked segregant approach we identified one AFLP marker that appears to be tightly linked to the MAT1-1 allele.

Molecular markers. All isolates within a single ascus contained a 800 bp RAPD marker produced with primer OPB-6. However, analysis of a progeny derived from a cross between two of these isolates (IPO94265 and IPO94266) revealed that the 800 bp RAPD markers were inherited independently. We hypothesized that both fragments were inherited independently as a result of a translocation event. Pulsed field gel electrophoresis (PFGE) and RFLP analyses were used to test this hypothesis and to study the inheritance of the supposed translocation. PFGE showed polymorphism among the mono-ascospore isolates, supporting the translocation hypothesis.

Molecular Studies on the Cell Wall Degrading Enzymes from *Botrytis cinerea*

A. ten Have[1], W. Mulder[2], W. Oude Breuil[1],
P. van de Vondervoort[2], J. Visser[2] and J.A.L. van Kan[1]. [1]Department of Phytopathology,
Wageningen Agricultural University, Binnenhaven 9,
6709 PD Wageningen, The Netherlands and
[2]Section of Molecular Genetics of Industrial
Micro-organisms, Wageningen Agricultural
University, Dreijenlaan 2, 6703 HA Wageningen,
The Netherlands

Botrytis cinerea is a plant pathogen with a very broad host range. Although much research has been performed on this devastating fungus, not much is known yet about its pathogenesis. As many other plant pathogenic fungi, *B. cinerea* secretes a number of cell wall-degrading enzymes, such as pectin lyase and polygalacturonase. Previous studies on *B. cinerea* have indicated that endopolygalacturonases play a role in pathogenesis. Up to 13 isozymes have been described. To further investigate the role these enzymes play in pathogenesis, we started a molecular–genetic analysis of the corresponding genes. We cloned a pectin lyase gene (*Bcpel*1), as well as six members of an endopolygalacturonase gene family (*Bcpgal*-6), homologous to endopolygalacturonases from related fungi such as *Aspergillus*, *Sclerotinia sclerotiorum* and *Fusarium moniliforme.*

The expression of the isolated genes was studied in batch cultures as well as during infection of detached tomato leaves. Data obtained indicate differential expression of these genes in a temporal as well as a substrate dependent manner. *Bcpga*1 is the first gene to be expressed during pathogenesis of detached tomato leaves. Therefore, null mutants in *Bcpga*1 were made by homologous recombination. Pathogenicity assays with these null mutants have been performed on detached tomato leaves and detached broad bean leaves.