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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON OCTOBER 21, 1982

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Evaluation of siderophores as a factor in soil mycostasis

Recent work in Baarn has shown that certain fluorescent Pseudomonads inhabiting the rhizospheres of potato and wheat can increase plant growth. The ability to increase growth is associated with the production of iron-chelating compounds (siderophores), which appear to starve as yet unknown deleterious rhizosphere micro-organisms of iron, thus preventing their growth and activity. The possible role of such bacteria and their siderophores in soil mycostasis was investigated.

Two siderophore-producing fluorescent Pseudomonads produced zones of inhibited growth of the following six fungi on King's medium B: *Cochliobolus sativus, C. victoriae, Trichoderma hamatum, T. koningii, T. harzianum,* and *Botrytis cinerea. Fusarium solani* f. sp. *phaseoli* was not inhibited. Spore germination within the zones showed little or no inhibition by isolate WCS 29 J, but isolate WCS 74 strongly inhibited germination of the *Trichoderma* spp. and *B. cinerea.* When Na-Fe-EDDHA (100 μM) was present in the medium, the zones of growth and germination inhibition either were not present (isolate WCS 29 J), or were reduced in size (isolate WCS 74), indicating the mycostatic potential of siderophores in vitro.

Addition of Na-Fe-EDDHA or $FeCl_3$ (80 μM) to a sandy clay (Lelystad) or a sandy loam (Lienden) soil did not reduce the mycostatic effect of the soils, either when conidia were incubated on membrane filters on the soils, or directly on the soil surface. When glucose (60–1000 μg/g soil) was added to soil to promote partial germination of conidia of *C. victoriae*, the further addition of Na-Fe-EDDHA did not stimulate greater germination than occurred in its absence. Addition of EDTA (80 μM) to soil containing 1000 μg glucose/g, did not reduce germination of conidia of *C. victoriae* or *B. cinerea.*

These results provide no evidence for a role for siderophores in soil mycostasis.

G. J. BOLLEN, D. VOLKER and G. A. VAN DEN BERG (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 6709 PD Wageningen*)

The effect of selective heat treatment on microbial activity in soils

The microbial activity of unamended soils and of those amended with various carbon sources, supplied with ammonium nitrate to a C/N ratio of 14, was estimated by measuring the CO_2 evolution. The soils used were a greenhouse soil (sand, org. matter 6%, pH 6.6) and a potting soil mixture (peat with marl and clay, org. matter 62%, pH 5.5). The temperatures at which treatments for 30 min. were given ranged from 50 to 100 °C.

In the laboratory, respiration was measured of samples kept under sterile conditions. Total amounts of CO_2 produced by unamended soils was highest for samples treated at 50 °C. It decreased with increasing temperatures of treatment in spite of the availability of more freshly-killed biomass after treatments at higher temperatures. Soils amended with glucose and alfalfa meal showed the same pattern in their response. Decomposition of cellulose was appreciably more sensitive to heat treatments than that of alfalfa meal.

A greenhouse soil was treated in situ. In plots treated at 70 and 85 °C respiration was stimulated for two weeks, but in 100 °C-treated plots it took ten weeks before it had declined to the level of that in untreated plots. Cellulolysis measured at a depth of 25 to 35 cm was unaffected in 70 °C-

treated plots, but completely eliminated in those steamed at 85 and 100°C. Four weeks after treatment the first cellulolytic micro-organisms had returned into the soil of these plots. Soon after their appearance the rate of cellulolysis attained a level three to four times that recorded for untreated plots.

H. VELVIS (*Instituut voor Bodemvruchtbaarheid, Oosterweg 92, 9751 PD Haren (Gn)*)

Verticillium biguttatum, a valuable antagonist for the biological control of Rhizoctonia solani

Verticillium biguttatum is a mycoparasite, very frequently occurring on sclerotia of *Rhizoctonia solani* on potato tubers from different soils. It was found to be able to reduce the viability of sclerotia more strongly than other mycoparasites isolated from sclerotia. The minimum temperature for this activity lies between 10 and 15°C.

Disease of potato sprouts, originating from sclerotia on the seed tuber, could be decreased considerably in laboratory experiments by treating the seed tubers with a suspension of *V. biguttatum* conidia. Presence of *V. biguttatum* could be demonstrated on the subterranean sprout surface of the treated plants and, after a few weeks, also on sprouts of the heavily infected untreated plants. An interaction between *R. solani* hyphae and *V. biguttatum* is suggested. This is supported by the observation that sprout infestation by *Rhizoctonia*, originating from surrounding soil, is also decreased by seed treatment with *V. biguttatum* under laboratory conditions.

There is a clear difference in effectivity among different strains of *V. biguttatum*.

SUDHIR U. MESHRAM (*Instituut voor Bodemvruchtbaarheid, Oosterweg 92, 9751 PD Haren, Gn.*)

Azotobacter chroococcum as an antagonist of Rhizoctonia solani and its use as a biological control agent

To determine the antagonistic effect of *Azotobacter chroococcum* on *Rhizoctonia solani*, 40 isolates of *A. chroococcum* were tested against *R. solani* on potato dextrose agar by a diffusion technique. Among these, five isolates strongly inhibited growth of *R. solani*. They were selected for micro-pot experiments (*in vitro* with an inoculation) with different soils infected with a pathogenic culture of *R. solani*, at various temperatures, viz., 25, 20, 15 and 10°C. Interaction between *A. chroococcum* and *Verticillium biguttatum* M73 was also studied.

In micro-pot experiments with sterilized and natural soil, isolates J4 and J6 of *A. chroococcum* proved highly effective against *R. solani* (no disease infection of potato sprouts). Similar results were obtained in pot experiments under glasshouse conditions. No formation of sclerotia was found on the harvest from seed potatoes inoculated with isolate J4 plus *Verticillium biguttatum*. Consequently, this appears to be a very promising treatment for the control of *R. solani*. A significant increase in yield of tubers was also recorded due to inoculation with *A. chroococcum*.

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

Efforts to biological control of Rhizoctonia solani in potato fields

From results of laboratory and pot experiments by Velvis and by Meshram it seems that *Verticillium biguttatum* and *Azotobacter chroococcum* might be valuable tools for biological control of *R. solani* in field experiments.

Field experiments have the drawback of strongly variable temperatures and soil moisture conditions and the fields are usually rather heterogeneous with regard to the presence of *R. solani*.

The seed potatoes (variety Bintje) were treated in the following ways: disinfected, non-disinfected and non-disinfected with one of two *V. biguttatum* isolates or one isolate of *A. chroococcum* or a mixture of these. Each treatment was present in twelve-fold. Stems and leaves were killed in mid-July with a herbicide; the tubers were harvested three weeks later. The tubers were washed and

the sclerotium indices were determined. Only four out of ten fields could be examined up to now.

The variation in the values of the sclerotium index (S.I.) of each treatment is considerable. The harvest from seed potatoes treated with the mixture of antagonists had the lowest average S.I., sometimes significantly lower than that of the harvest from untreated or disinfected seed potatoes. The harvest from seed potatoes treated with *V. biguttatum*, isolate M 73, had the next lowest S.I. Statistical significance was often absent, especially in soils that became very dry. The activity and the population density of *V. biguttatum*, which is sensitive to dry conditions, was strongly limited in the dry soils. This strong reduction in population density may result in a more severe *R. solani* infestation in next year's crop.

G. DIJST (*Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, 6709 PD Wageningen*)

The production of sclerotia on potato tubers by Rhizoctonia solani

During the cropping season the amount of sclerotia produced by *Rhizoctonia solani* on the surface of potato tubers increases gradually. For sanitary reasons haulm destruction is an obliged measure when cropping seed potatoes in The Netherlands. Haulm destruction, however, enhances the production of sclerotia on the tubers enormously. This increase can be detected within ten days after treatment.

Greenhouse and field experiments showed that mechanical removal of the shoot (cut off just above soil surface) equals the stimulatory effect of chemical destruction.

In another experiment, plants (cv. Bintje grown from meristems) were raised in steamed perlite in a double-compartiment system which allows tubers to develop separately from the roots. Fourteen days before harvest the tubers were inoculated with fungal hyphae on water agar. Seven days later shoots of half of the plants were cut off. At harvest date on all tubers of these treated plants sclerotia had developed, whereas all control tubers showed only skin damage caused by the fungus. The experiment was repeated with equal results. These results indicate, that the stimulation of sclerotia production is caused by some direct interaction between the fungus and the tubers, and is not primarily due to effects caused by biotic or abiotic components of the soil, root exudates, decaying roots and shoots, the destructive chemical or a sudden redistribution of components from dying shoots towards the tubers.

Chemical destruction of the shoots halts the transpiration of water, whereas the water uptake by the roots continues for a while. This results in an increased water content of the tuber (results from a field experiment). If the roots are cut through the water content of the tuber is much decreased. In a greenhouse study this practice reduced the amount of sclerotia on tubers of five out of seven varieties tested.

M. A. WILLIAMSON and N. J. FOKKEMA (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Javalaan 20, 3742 CP Baarn*)

Interactions between saprophytic yeasts and Colletotrichum graminicola on maize leaves

Conidia of *Colletotrichum graminicola* sprayed onto maize leaves caused considerably less necrosis when the leaves had an established population of saprophytic yeasts (*Sporobolomyces roseus* and *Cryptococcus laurentii*).

Microscopic observations of *C. graminicola* conidia on cleared leaves and polystyrene leaf replicas prepared in the first few days after pathogen inoculation, indicated that percentage germination, germ tube length and appressorium formation were not affected by the presence of yeasts. This means that the reduction of infection cannot be explained by a reduction in the pre-penetration development of *C. graminicola*. This is in contrast to previous observations of the pre-penetration development of other necrotrophic pathogens e.g. *Cochliobolus sativus* (BLAKEMAN & FOKKEMA 1982).

However, examination of the penetration process of *C. graminicola* in cleared tissue or in fresh material using epi-illumination autofluorescence, demonstrated that yeasts reduced the number of

penetrations from appressoria by 50%. This may indicate an effect by yeasts on the production of enzymes necessary for penetration by the pathogen. The reduction in penetrations corresponds to a 50% reduction of lesion numbers and necrotic area on similarly treated leaves. In addition, preliminary observations suggest that yeasts may affect the colonization of necrotic tissues by *C. graminicola* and the formation of sporulation structures.

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Leaf-Blight of winter wheat caused by Gerlachia nivalis

A duplicate field plot experiment was designed to investigate several factors which may influence the development and spread of leaf-blight, caused by *Gerlachia nivalis* (perf. stat. *Monographella nivalis*).

Factors such as fungicides, the aphid-honeydew-complex, antagonists, insects, other pathogens and nitrogen were examined. Regular observations were made on samples of 15 single plants from each field plot (min. size 60 m^2).

G. nivalis causes infections of wheat throughout the year; the disease proved to be weather-dependent. Although all organs can be infected independently, the various forms of the disease may stand in direct relation to each other.

The attack of stem bases, primary roots and leaves of young plants beneath snow cover is well known; hence the name "snow mould". It appears that these primary infections develop from contaminated seed or infected soil.

However, infections by conidiospores can be common in the spring and summer, but ascospores produced in these primary and secondary infections from June until harvest, add to the inoculum and thus are important in the further spread of the disease.

The first symptoms of the leaf-blight appear on the lower leaves in early June, while leaf spots on the upper leaves develop during the middle of the flowering period, simultaneously with ear infections. No evidence of systemic spread could be found.

Plants sampled from the plots which were fertilized with extra nitrogen at GS 30 and 50 had more leaf and ear infections than plants grown in the plots which received only one nitrogen fertilization in spring.

The leaf-blight proved to be sensitive to antagonistic saprophytes ("pink and white yeasts"). When the natural mycoflora was reduced leaf-blight was more severe than in plots where saprophytes were stimulated.

The sudden appearance of this leaf-blight in 1979 remains unexplained. It is probably due to a combination of differences in fungicide resistence and pathogenicity of *G. nivalis*.

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R. VANTOMME, C. RIJCKAERT, J. SWINGS and J. DE LEY (*Onderzoekscentrum voor Fytobacteriosen, Sectie II-I.W.O.N.L., Laboratorium voor Microbiologie en microbiële Genetica, RUG, Gent, Belgium*)

Phenotypical, serological and phytopathological characterization of 144 E. amylovora strains from nine countries

The authors examined 144 *E. amylovora* strains; 90 strains were isolated in Belgium from *Crataegus* (55 strains), *Cotoneaster* (24), *Pirus* (10) and *Sorbus* (1). The foreign strains, including 15 from the Netherlands, came from a broader host spectrum comprising also strains from: *Cydonia, Dichotomanthes, Malus* (11 strains), *Mespilus, Prunus, Pyracantha, Rubus* and *Stranvaesia*. Our main objective was a serological, phytopathological and biochemical characterization of the isolates.

The serological characterization was performed by slide agglutination. All strains showed a posi-

tive reaction.

The morphological, biochemical and physiological features described in BERGEY's Manual (8th ed., 1974) were checked. All corresponded well for all isolates except for the utilization of Na-citrate and Na-D,l-lactate. Strain differentiation was possible by growth factor requirements, gelatin and Tween 60 hydrolysis, cell morphology, litmus reaction, sensitivity towards antibiotics and acidification of certain carbohydrates. Strain 100-4, isolated from *Cydonia oblonga* in Denmark, showed a very strong acid production, whereas the *Rubus* isolates NCPPB 2291, 2292 and 2293) failed to do so.

Pathogenicity was checked by Billing's green pear test and by a hypersensitive reaction on tobacco leaves. For fourteen isolates inoculations were made into 15 different host plants. The French strain CNBP 1987, isolated from *Malus* in the Landes, was shown to be highly aggressive, producing fire blight symptoms on all hosts inoculated.

Phenotypic features are not correlated with geographic origin, host plant or aggressiveness.

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Bacterial rot of *Dieffenbachia maculata* (Lodd.) G. Don caused by *Erwinia chrysanthemi*

Several bacterial strains were isolated from diseased *Dieffenbachia maculata* (Lodd.) G. Don cv. Compacta, cv. Camillo and cv. Veerle plants, which were cultivated in a nursery near Gent (Belgium). They were identified with API 20 E and API 50 CHE systems as *Erwinia chrysanthemi* strains. These strains and some other strains from culture collections were pathogenic to all three *D. maculata* cultivars tested: "Camillo", "Compacta" and "Tropic snow". Inoculation of the plants in the stem or petiole was the only effective method for obtaining systemic infection. The petiole seemed to be most susceptible to the disease. Wounding was indispensable for induction of the disease. Temperatures between 25° and 30°C, high relative air humidity (75% RH and more) and low light intensity (0,5 ft-c) favoured disease whereas higher inoculum concentrations (10^7 and more cells pro ml) accelerated it.

Histological studies showed tissues degradation in infected areas and supported the finding that petioles are the most susceptible tissues to the disease. Rapid transport of bacteria was possible after vessel infection in stem or petiole tissues.

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Transport and activity of soluble antigens of Erwinia salicis in willows

Erwinia salicis populations present in the xylem vessels of watermarked *Salix alba* trees release soluble antigens into the transpiration stream which are then transported to the leaves.

The SAL detection method (S = soluble, A = antigen, L = leaves) has been developed to detect these antigens, using enzyme-linked immunosorbent assay. These soluble antigens contribute to the expression of symptoms of the Watermark disease: this was shown in experiments on excised twigs and 3-year-old trees in the field. The antigens are spread evenly through the whole leaf blade, but occur in higher concentrations in the lower leaves of the shoot.

Using this technique it is possible to ascertain the occurrence of *E. salicis* inside the host without disturbing host or parasite.

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Endogenous elicitors from bean cell walls (Phaseolus vulgaris L.)

Purified cell walls of etiolated bean hypocotyls (*Phaseolus vulgaris* L., var. Prélude) were incubated in a pectinase solution (Macerozyme R-10, partially purified by gel filtration over Sephadex G-25). The incubation was terminated by filtering the suspension over glass wool and, subsequently, over a 10 kD-filter. The filtrate mainly contained uronic acid-rich material. The amount of uronic acid-rich material in the filtrate increased for at least 24 h (when it represented about 9% of the wall dry weight). The filtrate was able to induce lesions on bean hypocotyls. In the browned tissue phytoalexins (predominantly, phaseollin and kieviton) accumulated and the incorporation of wall-bound hydroxyproline (presumably "extensin") was accelerated. The biological activity of the filtrate reached a plateau after 4 h of incubating, when the uronic acids in the filtrate represented about 4% of wall dry weight.

The filtrate was chromatographed on Bio-Gel P-4 and P-2. The columns (P-4: 29 × 0.8 cm; P-2: 48 × 1 cm) were equilibrated in H_2O. The void (Vo) and included (Vi) volumes were determined with Dextran Blue and $NiCl_2$, respectively. The eluates were analyzed for biological activity, for uronic acids and for total sugar content (P-2 only). On the P-4 column (fractionation range: 800–4,000) the biological activity coeluted with the uronic acid-containing components in a single peak at Kav = 0.9. On the P-2 column (fractionation range: 100–1,800) total sugar content peaked at Kav = 0.59 followed by a broad peak at Kav = 0.70–0.76 and by a minor peak at Kav = 0.84. Uronic acid content showed a major peak at Kav = 0.70 and a minor peak at Kav = 0.84. The bulk of the biological activity eluted at Kav = 0.76 and a minor peak of activity eluted at Kav = 0.84. The results indicate that the biological activity freed from bean cell walls by Macerozyme R-10 resides in oligosaccharides containing both uronic acids and neutral sugars.

P. C. MOL, W. SLOBBE and A. FUCHS (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 6709 PD Wageningen*)

The possible role of sesquiterpenes in the response of tobacco to various stress factors

In a study on the possible role of sesquiterpenes in the response of tobacco, *Nicotiana tabacum*, to injury or inoculation, leaves of the cultivar Samsun NN were subjected to various stress factors. To this end, leaves 3–5 of about 6 to 8-week-old plants were either treated with a 10^{-3} M solution of $HgCl_2$ or inoculated, by injection, with *Pseudomonas tabaci* or, after being dusted with carborundum, with TMV. Six days after treatment or inoculation the leaves were extracted with 40% ethanol; the ethanolic extracts were taken to dryness, and the residues dissolved in 60% methanol. The sesquiterpenes present in the methanolic solutions were partitioned into chloroform, the chloroform extracts dried down, and the residues taken up in a small amount of acetone. The presence of fungitoxic sesquiterpenes was established using a TLC-bioassay (HOMANS & FUCHS 1970) with chloroform/methanol 95:5 as the solvent system and *Cladosporium cucumerinum* as the test fungus. Purification and identification of sesquiterpenes was achieved by TLC and GC/MS analysis (FUCHS et al. 1983). In addition, in a time-course study, leaves were inoculated with TMV and harvested after 1, 2, 3, 4, 6, 8, 10 and 13 days, respectively. Extracts were made and analysed as before.

Upon treatment with $HgCl_2$, irregular necrotic areas were observed after 6 h, whereas upon inoculation with *P. tabaci* or TMV discrete necrotic spots became visible after 18–24 and 40–48 h, respectively. Upon $HgCl_2$-treatment no sesquiterpenes were found, while after inoculation with *P. tabaci* only capsidiol was formed. Inoculation with TMV, on the other hand, resulted in a large number of sesquiterpenes, capsidiol being the one produced first, followed – more or less stepwise – by at least seven sesquiterpenes of the oxyglutinosone pathway (MASAMUNE et al. 1978), four of the latter, viz. 3-hydroxylubimin, epirishitin, glutinosone and oxyglutinosone, being unknown so far for *N. tabacum*. Remarkably and contrary to other reports (UEGAKI et al. 1980, 1981), phytuberin and phytuberol, two sesquiterpenes belonging to the phytuberol pathway, were never observed. The results obtained suggest a positive correlation between the time elapsed before symptom expres-

sion and the number of sesquiterpenes formed: it seems as if a rapid response prevents the plant from inducing sesquiterpene synthesis at all ($HgCl_2$) or only activates the capsidiol pathway (*P. tabaci*), while a more gradual reaction leads to activation of the oxyglutinosone pathway as well (TMV). However, the results described could also be accounted for by assuming a specific pattern of induction for each of the three stress factors.

Another objective of our study was the phenomenon of induced systemic resistance. Therefore, in parallel experiments, leaves 6–8 of tobacco plants, of which leaves 3–5 either remained untreated (controls) or were treated or inoculated with $HgCl_2$, or *P. syringae*, *P. tabaci* or TMV, respectively, were challenged by applying the same stress factors, in all possible combinations of treatments of lower (3–5) and upper (6–8) leaves. Systemic resistance was only induced by TMV against TMV and expressed by the formation of distinctly smaller necrotic spots. In all other cases no signs of induced systemic resistance whatsoever were observed. Whereas in healthy upper leaves no sesquiterpenes were detected, whether or not the lower leaves were inoculated with TMV, inoculation with TMV of upper leaves resulted in the same sesquiterpenes as did inoculation of the lower ones. The relation between the rate of induction of sesquiterpene synthesis and the onset of symptom expression in challenge-inoculated leaves is currently being investigated.

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H. J. VAN TELGEN and L. C. VAN LOON (*Vakgroep Plantenfysiologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)

Isolation and Electrophoretic Analysis of Chromatin Proteins from Virus-Infected Tobacco Leaves

Nuclei from young tobacco leaves were isolated by tissue homogenization in an Omnimixer and repeated grinding in a Potter homogenizer. With this method up to 45% of the total leaf DNA was released. Examination of the nuclei by interference-contrast microscopy showed them to be intact. Chromatin prepared from these nuclei contained DNA, RNA, and protein in a ratio of 1:0.05:2.8. A slightly lower yield of nuclei was obtained from young, mosaic-diseased leaves from plants infected with tobacco mosaic virus (TMV), but infection did not affect the overall compositional characteristics of the chromatin.

Analysis of the chromatin proteins in polyacrylamide gels containing SDS showed that upon TMV infection an apparently new protein of *c.* 100,000 D appeared. Furthermore, a protein band of 32,000 D increased in intensity together with two adjacent bands at 29,000–35,000 D. These changes were not observed upon electrophoresis in an acidic urea system. Thus, whereas TMV multiplies in the cytoplasm, upon infection discrete alterations in the nuclear chromatin proteins are induced that may be related to the expression of the characteristic mosaic symptoms.

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Resistance in cucumber powdery mildew to fungicides with different mechanisms of action

Chemical control of cucumber powdery mildew (*Sphaerotheca fuliginea*) in Dutch glasshouses is necessary. Frequent application of fungicides like benomyl, dimethirimol and pyrazophos has, how-

ever, led to development of resistance to these chemicals. For this reason they have hardly been used during the last years. Recent use of fungicides with a mode of action based on inhibition of ergosterol biosynthesis (imazalil, fenarimol and triforine) has not yet led to failure of disease control. In view of these data, cucumber powdery mildew was chosen to study the epidemiology of fungicide resistant strains.

A monitoring survey performed in 1981/82 showed that fungal isolates from glasshouses, located in different parts of The Netherlands, were less sensitive to six fungicides (benomyl, dimethirimol, fenarimol, imazalil, pyrazophos and triforine), as compared with the five reference isolates. Only dinocap controlled all isolates at approximately the same concentration.

Resistance to benomyl, dimethirimol and pyrazophos indicated that a certain level of resistance remained present for years in the absence of the fungicide. Factors which might explain such a stability are a relatively high fitness of the resistant isolates and the absence of wild-type isolates, excluding the possibility of competition with resistant isolates.

In glasshouse experiments the decreased sensitivity of isolates to the sterol biosynthesis inhibiting fungicides was not apparent at the dosage recommended for practical use. However, half of these doses resulted in only partial control of the disease incited by the resistant isolates, while the original wild-type isolates could still be eradicated at $\frac{1}{50}$th of the recommended rate.

At present fenarimol, imazalil and triforine are the most important fungicides for control of cucumber powdery mildew. Because of the decreased sensitivity of the fungus, these compounds have to be sprayed more frequently than shortly after their introduction. It is important to know whether isolates, with a decreased sensitivity to fungicides, have a fitness comparable with that of the original wild-type isolates.

To that end epidemiological parameters of the various isolates will be studied.

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Factors influencing the formation of prepenetration structures of Botrytis cinerea on French bean leaves

Several lines of evidence indicate that infection of intact tissues of many host plant species by *Botrytis cinerea* will, in general, evoke a susceptible reaction only if this fungus can dispose of infection-stimulating substances. In nature, infection stimulants become available from e.g. dead plant material and pollen grains. The identity of some of these substances is being investigated. Combinations of a simple sugar, e.g. glucose, with inorganic phosphate or with some purine-related or Fe-chelating compounds were also found to stimulate infections by *B. cinerea*.

The mode of action of infection stimulants is largely unknown. They may either act as nutrients, thereby enhancing conidial germination and further development of the fungus directly, or act indirectly by predisposing host tissue to infection. The stimulations are dependent on concentrations of infection stimulants. Stimulant deficiency may give rise to a resistant reaction, being expressed as restricted lesions, a superficial browning or no visible necrosis at all.

Light microscopy of inoculation sites revealed many penetrations per site in susceptible reactions, but only a few in resistant ones. Therefore, the effect of some stimulants on the infection process of *B. cinerea* in primary leaves of French bean was examined further. Glucose stimulated the formation of prepenetration structures, such as conidial germ tubes, elongated superficial hyphae, hyphal appressoria and infection cushions, but penetration itself was enhanced by additional stimulants mainly. The conidial concentration in the inoculum appeared to be a major factor determining the type of prepenetration structures from which penetration occurred.

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON THE 26TH OF OCTOBER 1982 ON THE OCCASION OF THE 25TH JUBILEE OF THE ITAL RESEARCH INSTITUTE

R. B. FLAVELL (*Plant Breeding Institute, Trumpington, Cambridge, U.K.*)

The structure and evolution of repeated sequences in plant chromosomes

A large fraction of the DNA of most plant genomes, especially those with larger DNA contents, is composed of families of repeated DNA sequences. Repeats of the same family are rarely confined to a single site or a single chromosome showing that DNA transposition between chromosomes is a common event in evolution. Studies into the structure of many cloned segments of chromosomal DNA have shown that molecular mechanisms which create, delete and transpose repeated sequences are major contributors to the architecture of plant chromosomes. Furthermore, comparative studies between related cereal species have shown that a large fraction of the DNA "turnsover" during evolution, old sequences being replaced by new ones. Some of the mechanisms which create DNA changes during evolution are particularly interesting because they may facilitate the fixation of new variants in populations in the absence of selection and thus help explain how so much non-coding repeated sequence DNA has accumulated in plant chromosomes.

P. J. J. HOOYKAAS (*Biochemisch Laboratorium Rijks Universiteit, Wassenaarseweg 64, 2333 AL Leiden*)

The use of Agrobacterium tumefaciens for the genetic manipulation of plant cells

The Gram-negative soil bacteria *Agrobacterium tumefaciens* and *A. rhizogenes* are the causative agents of the neoplastic plant diseases crown gall and hairy root. Large extrachromosomal DNA elements – the Ti and Ri plasmids – contain the DNA sequences responsible for the transformation of plant cells into tumor cells. It has been found that a piece of plasmid DNA is integrated into nuclear plant DNA during transformation. This DNA – the T-DNA – is expressed in plant cells and contains loci which cause an auxin- or a cytokinin-like effect in plant cells. However, the T-DNA can be freed from its tumorigenic properties, and then be used for the introduction of desirable genes into plant cells.

K. J. PUITE (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

First steps to genome transformation of plants using uptake of isolated chromosomes

In this preliminary stage of our chromosome transplantation study the following steps have been realized in joint effort using the model plant *Haplopappus gracilis* (2n = 4):

1. isolation and culture of the protoplasts. In some instances cell colonies have been observed.
2. analysis of DNA-distribution patterns of fixed protoplasts stained with the fluorescent dye Hoechst 33342 and measured by flow cytometry.
3. partly synchronization of the cell culture with hydroxy urea or thimidine followed by a colchicine treatment. A mitotic index of at least 25% has been reached for cells and protoplasts.
4. isolation of cell lines which differ in their C- and N-metabolism. Also a zinc tolerant line has been obtained. The characters will be tested for inheritance and may be used for chromosome transfer.

W. A. BOVENBERG and A. J. KOOL (*Biologisch Laboratorium Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Analysis of chloroplast DNA in interspecies somatic hybrids of Petunia

Heterozygosity of extra-chromosomal genes can be obtained by somatic hybridization by induced protoplast fusion, followed by selection of viable heterokaryons capable of plant regeneration. Somatic hybrids can be characterized with respect to their chloroplast and mitochondria content by comparing the restriction endonuclease cleavage patterns of their chloroplast (cp)DNA and mitochondrial (mt)DNA with those of the parental plant species.

We describe here the fate of chloroplasts in three different somatic hybrid combinations within the genus *Petunia*. These somatic hybrids were produced by fusion of wild-type (wt) mesophyll protoplasts of *Petunia parodii* with protoplasts from cell suspension cultures of either wt *P. hybrida*, the nuclear albino mutant *P. parviflora* or the cytoplasmic albino mutant *P. inflata*. Characterization of these hybrids with respect to floral morphology and chromosome numbers indicated that both parental nuclear genomes were present in each of the hybrids. Analysis of the cpDNA from the hybrid and parental plants with various restriction endonucleases revealed that in all three hybrid combinations exclusively the *P. parodii* type cpDNA was present (Kumar et al. 1982). Recombinations between the different parental type cpDNAs were not observed. The presence of only *P. parodii* type cpDNA in the *P. parodii-P. inflata* hybrids and in the *P. parodii-P. hybrida* hybrids can be explained to be the result of the physical selection conditions that were used to isolate and regenerate these hybrids. The presence of only *P. parodii* cpDNA in the *P. parodii-P. parviflora* hybrid is less clear. The observed sorting-out of chloroplasts in favour of *P. parodii* chloroplasts may be explained by mechanisms such as nuclear-cytoplasmic incompatibility.

A. KUMAR, E. C. COCKING, W. A. BOVENBERG and A. J. KOOL (1982); Restriction endonuclease analysis of chloroplast DNA in interspecies somatic hybrids of Petunia. *Theor. Appl. Genet.* **62**: 377–383.

A. J. KOOL and C. M. COLIJN (*Biologisch Laboratorium Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Induction, selection and regeneration of fluorotryptophan resistant mutant cell lines of Petunia hybrida

The aim of our study was to develop conditions for effective mutagenesis of plant cell cultures of *Petunia hybrida* and to determine if mutations selected at the cellular level would be stably maintained and expressed in plants regenerated from such mutant cells.

By using 6-fluorotryptophan (6FT) as positive selection condition we were able to show that N-methyl-N'-Nitro-N-Nitrosoguanidine in a concentration of 5–40 μg/ml significantly increases (up to 100 times) the number of 6FT-resistant calli (COLIJN et al. 1979). The drug-resistant character of these cell lines was stable for at least 35 generations, even when grown in the absence of 6FT.

A number of these resistant cell lines were also auxin-autotrophic, suggesting that, in analogy to the 5-methyltryptophan-resistant carrot cell lines (Wildholm, 1977), an increased free tryptophan level in these cells is responsible for the resistant phenotype. Plants were regenerated from a number of the 6FT-resistant cell lines. Calli initiated from the regenerated plants still showed the resistant phenotype. After crossing of the 6FT-resistant plants with one of the wild-type parental plants, about 50% of the progeny showed the drug-resistant phenotype, indicating that the mutation behaves as a dominant, nuclear trait.

Aberrant morphology of the resistant plants such as the formation of numerous little shoots on the flower stem, which could be caused by an increased auxin synthesis, suggests expression of the mutation at the plant level. Present research is aimed at mapping the mutation that causes 6FT-resistance in these petunia plants.

WIDHOLM, J. M. (1977). Relation between auxin autotrophy and tryptophan accumulation in cultured plant cells. *Planta* **134**: 103–108.

COLIJN, C. M., A. J. KOOL & M. J. J. NIJKAMP (1979). An effective chemical mutagenesis procedure for Petunia hybrida cell suspension cultures. *Theor. Appl. Genet.* **55**: 101–106.

F. A. KRENS (*Biochemisch Laboratorium Rijks Universiteit, Wassenaarseweg 64, 2333 AL Leiden*)
In vitro DNA transformation of tobacco protoplasts; the introduction of foreign DNA in plant material

Posters

J. B. M. CUSTERS (*Instituut voor de Veredeling van Tuinbouwgewassen, Postbus 16, 6700 AA Wageningen*)
Embryo culture as an aid in interspecific hybridization in Cucumis

The objective of this study is to determine in vitro culture medium requirements for obtaining plants from inviable hybrid embryos resulting from crosses between agronomically interesting *Cucumis* species. The embryos in situ abort at various stages of development depending on the parental combination.

In preparatory experiments with non-abortive embryos we succeeded in regulating the continuation of embryonic development as well as the precocious germination of embryos following excision at different stages. Sucrose, kinetin, age of the embryo, and duration of culture were decisive factors for development in vitro.

Using the culture procedures as established for the non-abortive embryos, we could induce embryos with arrest of growth during their maturation to grow into plants, but those with breakdown in the proembryo and globular stage did not grow. As an alternative for the latter embryos we tried to obtain callus from these and to regenerate plantlets afterwards. All media which were developed so far for the initiation of callus from various tissues of cucumber plants appeared unsuitable for such initiation from immature embryos.

P. MIEDEMA (*Stichting Voor Plantenveredeling, Postbus 117, 6700 AC Wageningen*)
The effects of various cytokinin treatments on shoot initiation and shoot morphogenesis in Beta vulgaris

A tissue culture technique for clonal propagation of *Beta vulgaris* was investigated. The technique consisted of two or three steps: (a) adventitious shoot formation on flower buds, (b) shoot multiplication by stimulating axillary bud development, and (c) rooting of shoots from (a) or (b). Media consisted of half strength Murashige & Skoog with 3% sucrose and 0.8% agar, supplemented with 10 µmol/l BAP for (a), 1 µmol/l BAP for (b) and 10 µmol/l IBA for (c).

Various genotypes have been tested. Genotypic variation was found in shoot formation, shoot multiplication and rooting. Secondly, repeated subculture on BAP containing media resulted in stunted shoots with thick leaves in some genotypes.

The shoots derived from flower buds were vegetative. Some genotypes, characterized by a low vernalization requirement, formed generative shoots (bolters) when the flower buds were transferred from 10 µmol/l BAP to media without growth regulators, 1–2 weeks after incubation. A similar effect was obtained when BAP was replaced by IPA or zeatin; the latter cytokinins are presumably sooner inactivated than BAP. Shortening the BAP treatment or replacing BAP by IPA or zeatin also prevented shoot malformations.

It is concluded that cytokinin is required for shoot initiation.

A. M. HEMRIKA-WAGNER, E. J. VERSCHOOR and L. H. W. VAN DER PLAS (*Biologisch Laboratorium Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)
Influence of temperature on respiratory characteristics of potato tuber callus

A positive correlation was demonstrated between the growth temperature of potato tuber callus and the capacity of the CN-resistant alternative oxidase pathway. In mitochondria isolated from

callus grown at 28 °C this capacity amounted to approximately 40% of uninhibited respiration, whereas in callus grown at 8 °C only about 10% of the mitochondrial respiration was CN-resistant. When respiration of intact callus tissue was measured, these capacities were 70% and 30% respectively.

Participation of alternative oxidase pathway in uninhibited in vito respiration was not related to the capacity of this respiration. Irrespective of culture temperature of the callus a constant part of uninhibited respiration (about 20%) was caused by alternative pathway respiration. As a consequence, a greater part of alternative pathway capacity was operating in uninhibited respiration during growth at low temperatures.

It is suggested that the differences in alternative oxidase capacities are a direct effect of temperature (perhaps via alterations in mitochondrial membrane composition), but that these changes do not reflect a changed need to divert electrons to the alternative oxidase pathway.

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

Respiratory physiology of potato tuber callus cultures

Cultured potato tuber callus has a high capacity for the transfer of electrons to oxygen via the CN-resistant, alternative pathway. In studying the physiological significance of this pathway, the *in vivo* activity of this pathway (in uninhibited respiration) has been determined using callus grown with or without sucrose (3 or 6%) as carbon source. The *capacity* of the alternative pathway, induced during the first week of incubation on the nutrient medium, was nearly independent of the sugar concentration. The *in vivo* activity of the alternative pathway appeared to be dependent on the age of the callus and on the sucrose concentration in the medium. The participation of the alternative pathway in uninhibited respiration was maximal 2–3 weeks after start of the incubation. With increasing sugar concentration this participation increases, but under the experimental conditions tested the alternative pathway capacity never is fully used.

Addition of chloramphenicol to the nutrient medium leads to slower callus growth and inhibition of the synthesis of various cytochrome route components. A normal induction of alternative oxidase during the first week occurs. Under these stress conditions the alternative pathway became for more than 80% operative *in vivo* after 2 weeks of incubation.

K. SREE RAMULU and P. DIJKHUIS (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Analysis on genetic stability of in vitro cultures of protoplasts and cells and regenerated plants in potato (Solanum tuberosum cv. Bintje, 2n = 4x = 48)

When protoplasts or cells are explanted *in vitro*, genetic instabilities of various kinds (polyploidy, aneuploidy, mutations, etc.) occur during growth and differentiation. These greatly limit the regeneration potential, as is the case with cell cultures, or lead to the generation of large variability, as found amongst the protoplast-derived plants. With increasing experience with the phenomenon, ways to avoid instability can be found, and this is of practical importance for genetic manipulation. One among the important factors seems to be the specific combination of cytokinin and auxin, their amount and ratio, which influence regeneration and the frequency of variation. By optimalizing the balance of these growth hormones that varies according to the species or genotype, it may be possible to minimize the severity of genetic instability.

E. JACOBSEN, M. J. TEMPELAAR and E. W. BIJMOLT (*Biologisch Centrum Rijks Universiteit, Kerklaan 30, 9751 NN Haren*)

Cytophotometric determination of ploidy levels in *Solanum tuberosum* callus and plants

Ploidy level determination in cells from callus- and cell suspension cultures of monohaploids is

required to verify the presence of sufficient haploid cells for induction and recognition of recessive mutations. This is achieved by cytophotometric measurement of Feulgen-DNA content (TEMPELAAR 1980) in separate protoplasts, obtained by enzymatic tissue-digestion. In addition, protoplasts and cells were obtained in the same fashion from leaves and roottips of control plants. These provided reference data for C-values and frequency distributions. DNA content was determined by comparison with Chicken erythrocytes to amount to 3–4 picograms in the presynthetic interphase nuclei of tetraploids.

As opposed to the situation in control plants, callus cells of a monohaploid and a dihaploid turned out to have several ploidy-levels. This is also the case for a doubled plant, regenerated from callus (JACOBSEN 1981) of a dihaploid.

JACOBSEN, E. (1981): Polyploidization in leaf callus tissue and in regenerated plants of dihaploid potato. *Plant Cell Tissue Organ Culture* **1**: 77–84.

TEMPELAAR, M. J. (1980): DNA-content in isolated nuclei. of postembryonic stages of progeny from normal and irradiated males of Tetranychus urticae Koch (Acari, Tetranychidae). *Chromosoma* **77**: 359–371.

K. J. PUITE and W. R. R. TEN BROEKE (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Flow cytometry of plant protoplasts using Hoechst 33342

The use of flow cytometry with fixed and vital plant protoplasts will be of great importance in cell kinetic and somatic hybridization studies. Also fusion experiments of selected plant chromosomes and protoplasts leading to transformed cells will necessitate the use of flow cytometric techniques.

Haplopappus gracilis protoplasts were isolated from a cell suspension culture with 5% Driselase and 0.5% Pectolyase in 0.4 M sorbitol during 3–4 h at 28°C. After 1 h fixation in cold 75% 3:1 ethanol/acetic acid with addition of 3.6 g sorbitol/100 ml the protoplasts were washed in 0.2 M sorbitol and resuspended during 10 min in 0.002% pepsin added to 0.2 M sorbitol at pH 2. Washing in 0.2 M sorbitol and staining with 1 µg/ml Hoechst 33342 in 0.1 M citric acid-phosphate buffer (pH 6) and 0.2 M sorbitol resulted in staining of the nuclei without non-specific staining of other cell constituents. Flow cytometric data from log phase cells, S-phase blocked cells using hydroxy urea and colchicine treated cells resulting in G_2/M arrest and tetraploidy, were collected with the FACS IV flow cytometer.

Vital protoplasts show no loss of vitality after passage through the flow cytometer, as measured by fluorescein diacetate staining. Up till now staining of vital protoplasts with Hoechst 33342 could only be realized under extreme pH conditions. Also a treatment of the protoplasts at elevated temperatures (20 min at 37°C) results in staining of the nuclei. FCM data indicate that also here we have no quantitative DNA staining. Protoplasts in 0.4 M saccharose and 1–2 mM $CaCl_2.2H_2O$ at pH 10 exhibit staining of the nuclei after adding Hoechst 33342 to a final concentration of 5 µg/ml. This procedure, however, does not result to date in a sufficiently homogenous staining required for flow cytometric analysis probably due to differences in cell membrane permeabilities.

A. M. M. DE LAAT and J. BLAAS (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Synchronization of cell cultures and chromosome isolation in Haplopappus gracilis

Metaphase chromosomes are potential vectors for intra- and interspecific transfer of genetic information. Chromosome-mediated gene transfer in plants requires further progress of synchronization, the isolation and purification of chromosomes and an efficient uptake of chromosomes by protoplasts. Synchronization of *H. gracilis* suspension cells was performed by incubating log-phase cells in the presence of inhibitors of DNA synthesis like hydroxyurea (5 mM) or excess (4 mM) thymidine for 20 h. Subsequently cells were arrested in metaphase by 0.05% colchicine. Synchronization was followed a) microscopically (determination of mitotic index) or b) by flow cytometer (measurement of DNA content in ruptured protoplasts using fluorescent DNA stains). Mitotic index often excee-

ded 25%.

Protoplasts were prepared from such synchronized cultures. During this incubation rapid decondensation of DNA occurred. This could be circumvented only partially by lowering the temperature (12 °C) and decreasing the incubation time, followed by mechanical disruption of the "semi-protoplasts", according to GRIESBACH et al. (1982).

Present research is focussed on the purification and sorting of metaphase chromosomes.

GRIESBACH, R. J., R. L. MALMBERG & P. S. CARLSON (1982): An improved technique for the isolation of higher plant chromosomes. *Plant Sci. Lett.* **24**: 55–60

L. J. W. GILISSEN and M. J. VAN STAVEREN (*Stichting ITAL*, Postbus 48, 6700 AA Wageningen)

Variant cell lines of Haplopappus gracilis

From the wild type cell suspension culture of *Haplopappus gracilis* (Nutt) Gray stable variant cell lines were selected:

1. The A0-line was obtained after selection on solid medium containing asparagine as the sole nitrogen source. This cell line could also metabolize valine or histidine or lysine as the sole nitrogen source. From this A0-line the cell lines A6, A14, A15, A53, A54, A55, A56 and A61 were selected on medium containing alanine as the sole nitrogen source. Prior to the selection, the cell lines A15–A61 were irradiated with 30 Gy.

In a growth test on several sugars as alternative carbon and energy source, all the A-lines grew well on galactose, in contrast to the wild type cell line. Except the wild type, A54 and A55, all the other A-lines were also able to grow well on lactose. Besides, A15 was the only line which grew well on mannose. However, neither the wild type cell line nor the A-lines were able to grow on maltose as the sole carbon source in the medium. Selection from the A0-cell line and the wild type cell line on mannose or maltose has recently resulted in twelve and eight well growing cell lines, respectively.

The A54-line ceased to grow within a few days after culture in nitrate medium, probably due to an inhibition of the nitrate uptake.

2. The zinc-tolerant CL7-line was selected from the wild type cell line at the toxic concentration of 7 mmol Zn^{2+}. Four sublines have been produced after reselection at 8 or 10 mmol Zn^{2+}. However, these lines did not show an increased zinc-tolerance as compared to the CL7-line. These CL7-(sub)lines appear to be tetraploid. The wild type cell line tetraploidized following treatments with hydroxyurea and colchicine showed no increase in the zinc-tolerance. Therefore, it is suggested that the character of zinc-tolerance in the CL7-(sub)lines is not based on their doubled gene dose.

H. J. SCHOLTEN, W. J. FEENSTRA, H. NIJDAM and G. DATEMA (*Biologisch Centrum Rijks Universiteit, Kerklaan 30, 9751 NN Haren*)

Characterization of a new nitrate-reductase deficient mutant of Arabidopsis thaliana

Mutant plants disturbed in the reduction of nitrate by the enzyme nitrate-reductase (NaR) can be isolated by selection for resistance to chlorate. NaR of *A. thaliana* consists of subunits with cytochrome-c-reductase (CcR) activity and a cofactor which is shared by NaR and xanthine dehydrogenase (XDH). Measurement of activity of NaR, CcR and XDH in mutants can give an indication about the nature of the mutation.

A mutant (G1) was selected which could grow only on ammonium as N-source in a humid environment. G1 died on nitrate as N-source or when transferred to soil. Chlorate-resistant callus from F2-seeds grew well on a mixture of amino acids as N-source. In combination with amino acids nitrate was no longer poisonous and even a positive effect of nitrate addition could be demonstrated. No NaR-activity could be found in G1 which indicates that G1 may be a structural mutant. Measurement of CcR-activity after sucrose gradient fractionation showed a G1-GcR banding in the same fractions as wt-CcR. Apparently in G1 subunits can be coupled to a complete apoenzyme. In G1

callus no XDH-activity could be detected showing that no effective cofactor is available in G1. From the results it was concluded G1 is a NaR-deficient, possibly structural, cofactor mutant.

H. J. WICHERS and H. J. HUIZING (*Laboratorium voor Farmacognosie Rijks Universiteit, Antonius Deusinglaan 2, 9713 AW Groningen*)

Transformation of L-tyrosine into L-dopa by alginate entrapped cells of Mucuna pruriens

Cells of *Mucuna pruriens*, grown as suspension cultures, are able to synthesize L-DOPA endogenously; however 90% of the L-DOPA is accumulated intracellularly.

Calcium alginate entrapped cells of *Mucuna pruriens* are able to transform L-tyrosine into L-DOPA; in this case 90% of the L-DOPA is released into the medium. Furthermore, immobilized cells transform L-tyrosine almost exclusively into L-DOPA, whereas in suspension cultures at least one more product is formed.

These observations make immobilized cells of *Mucuna pruriens* very suitable for use in a bioreactor for the production of L-DOPA.

C. M. COLIJN and A. J. KOOL (*Biologisch Laboratorium Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Chloroplast DNA expression in white cell cultures and a light-sensitive mutant of Petunia hybrida

E.M.-analysis of white suspension culture cells of *Petunia hybrida*, grown on MS medium supplemented with 2, 4-D (0.5 mg/l), shows that these cultured cells contain only plastids with very few membranes and incomplete stacking.

The proplastids isolated from these cell cultures are capable of in vitro polypeptide synthesis when supplied with an ATP generating system (COLIJN et al. 1982). The polypeptide pattern, syntesized in these proplastids, differ from those synthesized in isolated green leaf chloroplasts. In the cell culture plastids only a very small amount of the large subunit polypeptide of RuBPCase is synthesized. Furthermore, in the proplastids at least 5 polypeptides with high moleculair weights (more than 70 Kdalton) are synthesized. Such HMW-polypeptides are also synthesized in the proplastids present in yellow leaves of the nuclear mutant plant *P. hybrida* E5059 (This plant has yellow leaves when grown at normal light intensities (>10,000 lux) and green leaves when grown at low light (>3,000 lux). These HMW-polypeptides were not present in the green plastids isolated from the green leaves of this mutant plant grown at low light, nor in normal leaf chloroplasts. Therefore these results suggest that the presence of these high molecular weight polypeptides is specific for the proplastid stage of chloroplast development.

COLIJN, C. M., A. J. KOOL & H. J. J. NIJKAMP (1982): Protein synthesis in Petunia hybrida chloroplasts isolated from leaves and cell cultures. *Planta* **155**: 37–44.

L. J. W. GILISSEN, Ch. H. HÄNISCH TEN CATE and A. KEEN (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)

Characteristics of the growth of cells in suspension culture

The development of a new method described below resulted in a non-destructive and very simple way to measure the amount of cells in one Erlenmeyer flask at any time during the incubation period. Besides, this method in combination with a computer program enables to calculate the relative growth rate, being the most important character, of the growing suspension culture.

Erlenmeyer flasks containing the cell suspension are placed in a standardized oblique position. After a few minutes, when the cells are settled out, the width of the surface of the packed cells is measured with a vernier calliper. Each width corresponds to a specific cell mass volume according to a calibration relation. Due to the shape of the Erlenmeyer flasks the relationship between the

width and the logarithm of the amount of cells is linear. Therefore, the maximal relative growth rate (during the exponential growth phase) is determined directly from the widths measured, by means of linear regression.

J. H. L. SEGERS, P. C. W. M. VOSSEN, A. A. M. VAN LAMMEREN and J. H. N. SCHEL (*Vakgroep Plantencytologie en -morfologie Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)
Ultrastructural changes during in vitro culture of embryo and endosperm tissue from Zea mays L.

J. H. N. SCHEL, A. A. M. VAN LAMMEREN and W. M. J. POELMA (*Vakgroep Plantencytologie en -morfologie Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)
Isolation of nuclei from endosperm protoplasts of Zea mays L.

A. A. M. VAN LAMMEREN (*Vakgroep Plantencytologie en -morfologie Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen*)
Direction of cytoplasmic and spindle microtubules in plant cells by indirect immunofluorescence

C. J. VENVERLOO and N. PRONK (*Botanisch Laboratorium Rijks Universiteit, Nonnensteeg 3, 2311 VJ Leiden*)
Regulation of the plane of division in epidermis cells of Nautilocalyx leaf explants

P. W. EVERS (*Rijksinstituut voor Onderzoek in de Bos- en Landschapsbouw "De Dorschkamp", Postbus 23, 6700 AA Wageningen*)
Growth and morphogenesis of shoot initials of Douglas fir in vitro. 3. Photosynthesis in vitro

S. ROEST and G. S. BOKELMANN (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)
The isolation, culture and regeneration of protoplasts of potato (Solanum tuberosum L. cv. Bintje)

Ch. H. HÄNISCH TEN CATE and E. ENNIK (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)
Suspension culture of Solanum tuberosum L. cv. Bintje

R. J. BINO and H. J. W. WIJSMAN (*Genetisch Instituut Universiteit Amsterdam, Kruislaan 318, 1098 SM Amsterdam*)
Regeneration from epidermis tissue of Petunia

G. M. M. BREDEMEYER and H. C. J. BURG (*Stichting ITAL, Postbus 48, 6700 AA Wageningen*)
Secretion products of plant cells in vitro

A. J. KOOL[1] and G. A. M. VAN MARREWIJK[2] ([1] *Biologisch Laboratorium Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam;* [2] *Instituut voor Plantenveredeling Landbouwhogeschool, Lawickse Allee 166, 6709 DB Wageningen*)
Analysis of mitochondrial DNA from cytoplasmic male sterile and restored fertile Petunia hybrida plants

W. A. BOVENBERG and A. J. KOOL (*Biologisch Laboratorium Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)
Analysis of chloroplast segregation in interspecies somatic hybrids of Petunia

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON SEPTEMBER 30, 1982
Theme: SEEDBANK AND VEGETATION

J. H. WILLEMS (*Vakgroep Vegetatiekunde & Botanische Oecologie, Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht*)

The seed bank as a part of the vegetation

According to the generally accepted definition of vegetation: "... a system of largely spontaneously growing plant populations, growing in coherence with their sites and forming an ecosystem with these sites and all other forms of life occurring in these sites" (WESTHOFF & VAN DER MAAREL 1973), the seed bank is a part of the vegetation. As a consequence the study of the seed bank is an essential task for phytocoenology. Yet, the seed bank is not studied by plant ecologists as comprehensively as the above ground plant specimens. Nevertheless, the number of papers concerning the role of the seed bank as part of the ecosystem is increasing.

Though outstanding scientists like LINNAEUS and CHARLES DARWIN already paid attention to the seed bank, the detailed investigations of PUTENSEN (1882) in Germany can be considered the beginning of seed bank research. PUTENSEN paid attention to, i.a., the vertical distribution of the seeds of arable weeds. During the following decennia the investigations were mainly carried out for agricultural purposes, e.g., weed control.

The most commonly employed method of seed bank research is to keep soil samples in the greenhouse. Identification and counting of the seedlings will give an impression of the amount of viable seeds in the soil.

The enormous number of viable seeds in the soil is striking. A research program concerning Dutch production grasslands (VAN ALTENA & MINDERHOUD 1972) showed an average presence of about 10000 seeds per m^2 in the upper 5 cm of the soil. The highest number of seeds even exceeds 100000 seeds per m^2. The weight of the buried viable seeds of the eight most common grass species is about 13 kg per hectare. This is about one-third of the amount used when new production grassland is created by sowing.

A research program concerning the seed bank of a chalk grassland, carried out in the Gerendal Nature Reserve in The Netherlands, clearly showed the presence of seeds of plants of former successional stages. No seeds were found of plants of expected future stages in vegetation development, although seed sources of these species were present on only a short distance (some hundreds of metres). The number of viable seeds varied from 5000–16000 per m^2 (depth 20 cm).

The composition of the seed bank varied considerably both in space and time. Variety in space was especially studied in a small, isolated area of old aged chalk grassland near Maastricht. The composition of the seed bank showed but little resemblance to the above ground phytomass, e.g. no seeds were found of the dominant *Brachypodium pinnatum*. This species produced at least 100000 seeds per year, which amounts to more than one seed per dm^2. In the greenhouse the seeds germinated well, however. A more detailed paper concerning the seed bank of chalk grassland is in preparation.

The importance of seed bank investigations in the study of population dynamics and biology is evident as shown by the great number of papers on this theme. The seed bank is of great interest, too, in order to get more insight in structure and functioning of a plant community as a whole. This insight is necessary for management for Nature Reserves aiming at maintaining or enlarging biological richness.

ALTENA, S. C. VAN & J. W. MINDERHOUD (1972): Keimfähige Samen von Gräsern und Kräutern in der Narbenschicht der Niederländischen Weiden. *Z. Acker- und Pflanzenbau* **136**: 95–109.

PUTENSEN, H. (1882): Untersuchungen über die im Ackerboden enthaltenen Unkrautsämereien. *Hannov. land-u forstwirtsch. Vereinsdl.* **21**: 514–524.

WESTHOFF, V. & E. VAN DER MAAREL (1973): The Braun-Blanquet Approach. In: *Handbook of Vegetation Science*. V. p. 617–726. Ed. R. H. WHITTAKER. Junk, The Hague.

M. J. M. OOMES and M. HAM (*Centrum voor Agrobiologisch Onderzoek CABO, Postbus 14, 6700 AA Wageningen*)

Some methods of determining the seed bank

The term seed bank implies all the living seed present in the soil at a certain time. The seed bank changes because some seeds die and new seeds are produced by the vegetation. Usually, only those seeds that are viable under the prevailing conditions during a given period are considered to determine the seed bank. This is because seeds of different plant species may differ widely with regard to the conditions under which they germinate. Apart from qualitative differences between the species caused by dormancy, quantitative differences within the species may occur, caused by seed-polymorphism. Quantitative interpretation of the results of germination experiments is limited, because these results are highly dependent on the method applied. Soil samples were subjected to different pretreatments to determine which pretreatment would give the best qualitative approach of the seed bank.

The soil samples were taken from an Arrhenatherion elatioris grassland on a heavy river-clay soil, at a depth of 0–5 cm. Before the samples were laid out at 20 °C under 12 hrs light and 12 hrs dark, they had been kept in moist storage under various conditions:

A: 3 week; 12 hrs light, 25 °C, 12 hrs dark, 10 °C;
B: 4 weeks; dark, 3 °C; then 2 weeks as A;
C: as A, but wetted with 2000 ppm KNO_3;
D: as A, but wetted with 2000 ppm KNO_3 and 500 ppm GA_3;
E: 3 weeks; 16 hrs light, 30 °C, 8 hrs dark, 20 °C;
F: 4 weeks outdoors, protected against rain and drying out, from 22 February to 21 March;
G: no pretreatment.

Results and conclusions:

1) Pretreatments F and G yielded the largest number of monocot and dicot species.

The other pretreatments gave fewer monocots and all these species also germinated under treatments F and G. Striking for the dicots was that under each pretreatment at least one species occurred that did not occur under the other treatments. Most species will germinate under a combination of treatments F and G, one of these two treatments is insufficient.

2) Pretreatment with 2000 ppm KNO_3 did not yield a positive effect, neither did the addition of 500 ppm GA_3. The use of GA_3 is discouraged, because due to elongation of the hypocotyl, seedlings may die before they can be identified.

3) To obtain good results at least 800 cc soil is needed per sample.

4) Superficial drying of the samples should be prevented, because this might induce dormancy.

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The distribution in space and time of viable seeds of some short lived forbs on two chalk grasslands in S. Limburg

Since 1979 the authors are investigating the ecology of short lived species in two chalk grasslands, i.e. the Gerendal and the Vrakelberg. The occurrence of these species appeared to be correlated with the vegetation structure: most species have the greatest abundance in less productive stands, preferring open microhabitats, while a few others are found mostly in denser vegetation.

Factors influencing this distribution are in study. One of these factors is the availability of viable seeds. This paper deals with the influence of the distribution of viable seeds in space and time on the occurrence of the species, especially in the seedling-phase. In the way the viable seeds are distributed the short lived forbs of the Gerendal and the Vrakelberg can be divided in two groups.

Gentianella germanica and *Euphrasia officinalis* are representatives of the first group. Viable seeds are only present during winter and early spring. Fresh seeds of this group have a chilling requirement of two to three months to become viable. The maximum number of viable seeds is reached in February. In March there is a steep decline probably due to germination. Seeds occur only in the upper-most layer of the soil profile. These species have a transient seed bank (sensu THOMPSON & GRIME 1979). The horizontal distribution of the seeds is heterogeneous especially under dense vegetation, where

seed density is also low. Probably this distribution has something to do with the micro-habitat of the species. These are gaps in the vegetation turf. Gaps occur mainly in early spring after severe winter conditions and in open vegetations. They have a patchy distribution.

Species of the second group, represented by *Daucus carota* and *Scabiosa columbaria* among others, have a persistent seed bank (sensu THOMPSON & GRIME 1979). The number of viable seeds is small during summer. After summer there is a two step increase: one in autumn after seedfall and one in winter, since a part of the fresh seeds needs a chilling period of at least two months. The number of viable seeds is highest in February-March and decreases slowly in spring because of germination and secundary dormancy. Seeds are also found in lower regions of the soil profile. The horizontal distribution is less heterogeneous than the one of the first group, particularly in dense vegetation. Life strategy of species of the second group seems to be more competitive: life span is less short (at least more than two years in the study area), vegetative growth is more vigorous etc.. In addition, dispersal from the mother plant is more extended, for the fructiferous stems exceed the vegetation turf, which is an advantage, in particular in dense vegetations.

One can conclude that emerging of seedlings on a certain place for species of the first group almost totally depends on the availability of viable seeds. Viable seeds are only present during winter and early spring. All these seeds germinate in early spring during a short period. Seedling mortality is high. Germination is only temperature sensitive.

Seedling emergence of species of the second group is more gradual and takes place over a longer period. Viable seeds are present during the whole year. Germination is a process regulated by mechanisms which are also sensitive to other abiotic factors like R/FR ratio. The total set of abiotic factors on a certain place determines whether there is germination or not. This set may give an indication on the suitability of that place for seedling-establishment. Seedling mortality is low.

THOMPSON, K. & J. P. GRIME 1979: *Seasonal variation in the seed bank of herbaceous species in ten contrasting habitats. J. Ecol.* **67**: 893–921.

A. M. M. VAN BREEMEN and B. H. VAN LEEUWEN (*Vakgroep Oecologie, Zoölogisch Laboratorium, Kaiserstraat 63, 2311 GP Leiden*)

The seed bank of three short-lived monocarpic species, Cirsium vulgare (Compositae), Echium vulgare and Cynoglossum officinale (Boraginaceae).

In Meijendel, a dune area north of The Hague (The Netherlands), local populations of most short-lived monocarps have only very limited survival periods. Out of three investigated species, *Cirsium vulgare* (Savi) Ten., *Echium vulgare* L. and *Cynoglossum officinale* L., populations of *Cirsium* appeared to have the shortest survival period, whereas all marked populations of *Cynoglossum* were still present after 4 years.

Disappearance percentage of populations in Meijendel (1977–1981)

Cirsium vulgare	c. 45% (n = 9)
Echium vulgare	c. 5% (n = 14)
Cynoglossum officinale	0% (n = 13)

n = the number of marked populations in 1977.

For maintenance in the dune area, *Cirsium* has to colonize new spots most frequently. This species is therefore supposed to be most benefited by spreading its seeds in time (a persistent seed bank) and/or in space (dissemination).

The size of the viable seed bank (seeds in innate, induced or enforced dormancy), measured by sieving soil samples in the period between germination and new seed rain, appeared to be relatively small (60 seeds/m^2 maximally). Most seeds were present in the top 1-cm layer.

Two months after sowing in a field experiment (in the period of dissemination) c. 10% of the *Cirsium* and *Echium* seeds and c. 60% of the *Cynoglossum* seeds were still present in a viable state. Most of the other seeds vanished completely. Those seeds which could be recovered apart from the viable ones, showed predation marks (*Cirsium* and *Echium*). Loss of seeds out of the experimental plots for *Cynoglossum* was due to horizontal secondary dispersal.

In an experiment in which seeds were buried, protected against predation, at c. 2 cm depth, the seeds appeared to be short-lived. After 3 years less than 1% *Cirsium*, 5% *Echium* and 0% *Cynoglossum*

seeds were still viable. About 50% of the *Cirsium* seeds had germinated and the other 50% had died. *Echium* and *Cynoglossum* seeds had practically all germinated. After 3 years at c. 15 cm depth 55% *Cirsium*, 70% *Echium* and 0% *Cynoglossum* seeds were still viable.

Cynoglossum does not have a persistent buried seed bank. The persistent seed bank appeared to be formed partially by seeds at the soil surface and partially by seeds at the infructescences (the dissemination in sheltered habitats is spread over about 2 years).

The survival times of buried viable seeds of the investigated species did not answer the supposition that the species with the highest disappearance percentage of its populations, is most benefited by a persistent seed bank. The importance of dissemination in this respect is beyond the scope of this note.

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Seed bank and species diversity in moist grassland communities

During hay-making without fertilization on formerly fertilized lots the vegetation changed from pasture communities poor in species into species richer hayfield communities. The newly appeared species can either originate from the persistent seed bank or from the transient seed bank. The seed bank was measured as germinable seeds after six months in a controlled environment providing a day and night interval at 20 °C.

Persistent seed bank species were classified as such if (i) more seeds are found in 2–4 cm and/or 4–6 cm layers than in 0–2 cm, (ii) they are present in the seed bank and absent in the vegetation, (iii) the number of seeds is not related to the above ground abundancy, (iv) no decline is found in the number of seeds under prevention of seed rain. Transient seed bank species have the opposite characters. It is striking that species of pioneer communities (treading and/or periodically dry and wet soils) viz. *Montia fontana, Stellaria alsine, Gnaphalium uliginosum, Juncus bufonius, Peplis portula, Alopecurus geniculatus, Glyceria fluitans, Juncus effusus, Sagina procumbens, Polygonum mite* and *Callitriche spp.* are persistent seed bank species according to these criteria. Hayfield like species viz. *Holcus lanatus, Cerastium fontanum, Ranunculus repens, R. flammula, Anthoxanthum odoratum, Rhinanthus serotinus, Plantago lanceolata, Caltha palustris, Lychnis flos-cuculi* are transient seed bank species.

Newly appearing hayfield species depend on dispersal due to their transient seed bank. Sowing experiments with hayfield species absent both in the vegetation and in the seed bank indeed demonstrate that germination is possible and hence dispersal is the bottle-neck.

Finally the establishment of newly appearing species both of the transient seed bank type and of the persistent seed bank type depend on the structure of the canopy: the shorter and more open the canopy, the more seedlings of sown hayfield species become juveniles and the more species of pioneer communities establish.

QUIRIN VYVEY (*Laboratorium voor Morfologie, Systematiek en Oecologie van de planten, Rijksuniversiteit Gent, Ledeganckstraat 35, Gent)*

Study of the seed bank in Schoeno-juncetum subnodulosi All 1922 and in Molinietum caeruleae All. 1922

The seed bank beneath managed and unmanaged *Schoeno-Juncetum subnodulosi* All. 1922 in Berg (Prov. of Brabant, Belgium: VYVEY & STIEPERAERE 1981) and in Marest-Dampcourt (Dep. de l'Aisne, France) and in managed *Molinietum caeruleae* All. 1922 in Berg and Vance (Prov. of Luxemburg, Belgium) was analysed. At the same time the seed bank beneath *Caricion davallianae*-vegetations and under carr, on former *Schoeno-Juncetum*, was analysed.

In each of 51 plots 15 soil samples of 100 cm^3 were collected. Subsequently the 15 samples were allowed to air-dry and when dry, the soil was sieved through a 0,5 cm-mesh sieve. The sieved samples were mixed thoroughly and placed in a 2-cm-deep layer covering 3 cm of sterilised humus. These trays were regularly watered from below with tap water, and the number of seedlings were recorded

at frequent intervals during 3 months. The results were analysed using cluster analysis.

In order to interpret this cluster analysis ordination was applied. From this we may conclude that there is a complete absence of any close association between the surface vegetation and the seed flora of the soil beneath. The most remarkable feature of the results is the negative correlation between the number of species in the seed bank and soil moisture. The number of viable seeds is extremely small (5 to 15 seedlings) in water-logged, basic soils beneath *Caricion davallianae*-vegetations.

In the soil under the 20 years' old carr, on former *Schoeno-Juncetum*, some viable seeds of *Juncus subnodulosus, Anagallis tenella, Carex panicea* and *Potentilla erecta* are still present. Under managed *Juncus subnodulosus*-vegetations more species (± 13 species) have viable seeds in the soil. Nevertheless none of the botanical interesting species (e.g. *Schoenus nigricans, Carex pulicaris, Carex hostiana, Pedicularis palustris, Oxycoccus palustris*) occurring in the surface vegetation, do germinate.

The results of this study illustrate the importance of the last surviving plants as to conservation of rare species in wetland vegetation.

VYVEY, Q. & H. STIEPERAERE (1981). The rich-fen vegetation of the Nature Reserve "Het Torfbroek" at Berg-Kampenhout (Prov. of Brabant, Belgium). *Bull. Soc. Roy. Bot. Belg.* **114**: 106–124.

H. J. DURING (*Vakgroep Vegetatiekunde en Botanische Oecologie, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Diaspore bank of bryophytes and ferns in chalk grasslands

The soil of the Dutch chalk grasslands of Wrakelberg and Gerendal (prov. S-Limburg) contains not only a seed bank, but also a large reservoir of diaspores of bryophytes and ferns. From samples of the layers 0–1, 1–3 and 3–6 cm. taken at 9 sample dates in the period February 1980 – January 1981, 37 species of bryophytes and 2 or 3 species of ferns emerged. All bryophyte species occur above-ground on the slopes at present or have been found there some years ago, indicating a low input of weedy species from outside. The fern spores must originate from forests at some km distance. Seasonal variation in the diaspore bank is low for the bryophytes; the ferns show a peak in winter.

The bryophyte species composition and relative abundance of the species in the soil differs markedly from the above-ground vegetation, perennial pleurocarps and hepatics being absent or much rarer in the soil and small, often tuber-bearing acrocarps (Colonists sensu DURING 1979) being strongly over-represented. A large proportion of the diaspores in the soil appear to be vegetative ones: tubers, gemmae, probably also stem fragments. It is suggested, that such vegetative diaspores are very long-lived in the soil.

Species diversity of the samples is high, considering their small size (5–15 cm^3). E.g., in the uppermost cm of the soil of the Gerendal site, from the samples 6–12 (mean 8.7) species were recorded, while in the above-ground vegetation relevés, sized 0.5 × 0.5 m^2, 4–11 (mean 8.1) species were found. This indicates a very fine-grained distribution of the diaspores in the soil. Still, the distribution is very contagious: samples taken less than 10 cm apart sometimes have less than 50% of the species in common, and even the layers of one sample may differ rather much from each other.

The bank of long-lived diaspores in the soil will play an important role in the rapid revegetation of naturally occurring gaps in the vegetation. Whether it also takes part in the autumnal regrowth of the bryophyte layer in chalk grasslands (cf. AL-MUFTI et al. 1977) will be investigated in the future.

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AL-MUFTI, M. M. et al. (1977): A quantitative analysis of shoot phenology and dominance in herbaceous vegetation. *J. Ecol.* **65**: 759–791.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON NOVEMBER 10TH, 1982 SYMPOSIUM ON HEATHLAND MANAGEMENT IN THE NETHERLANDS, HELD AT THE 50TH ANNIVERSARY OF THE STICHTING GOOISCH NATUURRESERVAAT

J. T. DE SMIDT (*Vakgroep Botanische Oecologie, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Heath-land management and ecological research

Management requires knowledge of the structure and function of the ecosystem, and well defined management aims.

The aims in heath-land management have evolved from maintaining open heath-land by cutting trees, to preventing grass invasion by removal of nutrients. Todays aims are maintainance of a diversity of heath-land species and communities, in particular those that are characteristic of or even restricted to heath-land and its particular oligotrophic environment. Diversity linked to differences in vegetation structure depends principally on the methods applied for management: grazing, burning, mowing, cutting sods. Outbreaks of the heather beetle (*Lochmaea suturalis*) result in increase of structural diversity, unless the effect is large scale grass dominance, which may cause the loss of characteristic species and reduction in species and community diversity.

Apart from aims concerning maintaince of attributes of the heath-land ecosystem, management requires also aims on the development of potential attributes. However, the optimal level of diversity and the degree of specificity of species and communities for heath-land is insufficiently known. To a certain degree the specificity is known of vertebrates, insects, cormophytes and plant communities. Knowledge on this aspect of other groups in the plant and animal kingdom is far more fragmentary. Ecological knowledge too is, with the exception of a small number of dominant plant species and some mammals, quite insufficient to design sure-fire management methods.

Permanent plot studies during 30 years indicate that small scale management is in favour of most heath-land species and communities. This means treatment per hectare rather than per 10 hectares or even larger units. For the time being management has to be effected with this general resp. rudimentary type of knowledge. To the conservation of all potential systems, well stocked with characteristic species, it is essential to collect more information by fundamental ecological research on large numbers of plant and animal species, their mutual interactions and their interactions with the environment.

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Removal of organic matter and nutrients as management practices for conservation of heath lands

in 1835 about 800,000 ha of heath lands existed in The Netherlands, providing the fertilizing material for about 300,000 ha of arable land (DIEMONT et al. 1982). To produce enough manure sheep were kept at night in a deep stable with a layer of surface litter from heath lands in which the animal dung was collected. Removal of coherent pieces of vegetation and top soil material from heath land and the product itself are called in Dutch (and German) "plaggen". "Plaggen" and mowing of heather, for manuring and as fuel, were probably most effective in regenerating the vegetation. Controlled burning must have been of minor importance.

Nowadays some 40,000 ha of heath lands are left in The Netherlands, mostly situated in nature reserves, only 5,000 ha grazed by sheep. In 1950–1960 heath land management by "plaggen" or mowing was discouraged because it was believed that the removal of nutrients counteracted the maintenance of the heath vegetation (MÖRZER BRUYNS 1953, WESTHOFF 1960). The advice therefore was controlled burning. Field trials, however, showed losses by burning of up to 420 kg N ha^{-1}, 71 kg K ha^{-1}, 16 kg P ha^{-1}, twice the amounts measured under laboratory conditions (CHAPMAN 1967). All heath land management therefore, including burning, means output of nutrients; but this is essential for the maintenance of heath lands: After half a century of neglected management about 20% of the heath lands in The Netherlands are covered by grasses. The primary aerial produc-

tion in grass heath is higher than in *Calluna* dominated vegetation (3 vs. 1.5–2.2 tons $ha^{-1}y^{-1}$, DIEMONT et al. 1982), indicating an increased nutrient pool in heath lands.

By the re-introduction of "plaggen" grass heath is changed into a vegetation dominatd by *Calluna vulgaris* or *Erica tetralix*, and rare species as *Gentiana pneumonanthe* re-appear. "Plaggen" once in 30–50 years is also a suitable alternative for burning or mowing of still existing heath vegetations. Compost made from the 200–1000 $m^3 ha^{-1}$ of organic matter removed from heath lands by "plaggen" produces a potting earth useful in horticulture (DIEMONT et al. 1982), the returns from which may balance the costs of "plaggen".

DIEMONT, W. H., F. G. BLANCKENBORG & H. KAMPF (Ed.) (1982): Blij op de hei? Innovaties in het heidebeheer. *Rapport werkgr. verwerking/afzet heideplaggen.* Staatsbosbeheer Utrecht.

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Relationship between soil- and vegetation types in Dutch heath-lands

Soil science in the multidisciplinary Heather beetle-project tried to answer the question: can differences in soil be correlated with differences in vegetation. Soil differences may contribute to the formation of a mosaic pattern and the invasion of grasses in Dutch heath-lands.

On the research areas Oud-Reemsterveld and Hoorneboegse heide, four heath-land soil types were described, based on the morphometric classification of the Dutch Soil Survey Institute. These soil types are: a type with a compact black humus illuviation layer and an iron pan, one with a black humus illuviation layer only, one with a brown less compact illuviation layer and one with a weakly developed loose humus illuviation layer. These soil types showed no clear correlation with the vegetation types.

Spatial differences in vegetation should rather be expected to correlate with differences in nutrient content than morphometric differences. However, concentrations of nutrients (N and P) in heath-land soils are kept very low by plants. For this reason soil types could not be characterized by differences in nutrients. Consequently the availability of nutrients for heath-land plants had to be approached by an indirect way. Herb rich heath vegetation types correlate with relatively cations-rich soils. The process of podsolisation is slower in such soils than in cations-poor ones, as mono- and bivalent cations neutralize fulvic and himic acids and will inhibit the leaching of Fe and Al ions from the top soil. This means that transported Fe and Al indicate the richness of the soil. A new soil typology was made on the quantities of transported Fe and Al. These types showed also consistent differences in other chemical parameters like exchange capacity, pH, humus content.

This explains the invasion of grasses on less developed podsol soil types. Formation of mosaic patterns cannot be explained in direct relation to soil properties.

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The heather beetle, Lochmaea suturalis Thomson, (col., Chrysomelidae) as the cause of the origin of vegetational patterns in heath-lands

Vegetations of *Calluna vulgaris* (L.) Hull in Western Europe are attacked by plagues of the heather beetle, *Lochmaea suturalis* Thomson with irregular intervals of 10 to 20 years (BLANKWAART 1977). Often the *Calluna* plants die off and grasses begin to dominate.

During the years 1978 to 1980 the course of a local outbreak of *Lochmaea* in a *Calluna* field in The Netherlands was followed (BRUNSTING 1982). Within two years a heather vegetation changed into a vegetational pattern of heather and grasses. It was shown that this change was entirely due

to the *Lochmaea* outbreak and that the shape of the pattern was determined by the dynamics of the outbreak.

The onset of an outbreak is marked by the appearance in summer of brown areas in *Calluna* stands. In these areas ("foci") larvae of *Lochmaea* have caused extensive damage to the heather plants. During late summer and autumn the young adult beetles walk to the edges of these foci in search of fresh food. This results in a high density margin ("front") of beetles arount the focus, in which densities of up to 2000 per m^2 can be found. This front remains in winter and early spring. In our study area the affected plants in the focus died off and grasses (*Deschampsia flexuosa* (L.) Trin. became dominant from 1979 onwards. Beetles dispersed by flight in spring and spread over the *Calluna* field. The population of *Lochmaea* built up in the area, with exception of the former front. Here many dead *Lochmaea* (hundreds per m^2) were found covered by a virulent and sporulating entomopathogenous fungus *Beauveria bassiana* (Bals) Vuill. This fungus had spread out over this part of the Lochmaea population, favoured by the high beetle density that had prevailed. By a lower natality and higher mortality of *Lochmaea* by the fungus in the former front, the plague subdued and *Calluna* could recover. In the rest of the area *Calluna* died off and from 1980 onwards *Deschampsia flexuosa* became dominant. The pest subdued because the beetles ran out of food, and most beetles flew away. The vegetational pattern that had arisen from the plague still persists now (1982).

It is hypothesised (G. W. HEIL, this symposium), that the *Calluna* did not regenerate and grasses became dominant because of an eutrophication of the soil. This hypothesis seems to be affirmed by experiments. To asses the role of the heather beetle in the proces of eutrophication, heather consumption and faeces production during a plague was estimated. Heather consumption by *Lochmaea* is high when compared to other grazing animals, e.g. sheep. It is supposed that the breakdown of *Calluna* plants affected by *Lochmaea* is a source of high mineral enrichment of the soil, while the production of faeces is an additional important factor.

It is concluded that the heather beetle is an important factor in the heath-land ecosystem and causes the formation of new vegetational patterns.

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J. J. M. BERDOWSKI and R. ZEILINGA (*Vakgroep vegetatiekunde en botanische oecologie, Lange Nieuwstraat 106, 3513 PN Utrecht*)

The effect of the heather beetle (Lochmaea suturalis Thomson) on heather (Calluna vulgaris (L.) Hull) as cause of mosaic patterns in heath-lands

At the Hoorneboegse Heide (near Hilversum) mosaic patterns can be distinguished which vary in shape, size and floristic and structural composition. A clear relation has been found between the present vegetation types and variation in the soil. The heath-lands with a high grass cover were found on the relative nutrient-rich soils, while the pure heath-lands were found on the relative nutrient-poor soils (resp. moderpodzol and haarpodzol).

During the heather beetle outbreaks of 1980 and 1981 a number of patterns of infestation developed, which differed in shape, size and infestation intensity. These patterns were not related to a specific vegetation type. During this period of time a part of the heath-lands with a low grass cover changed into heath-lands with a high grass cover. Some parts of the heath-lands with a high grass cover changed into a *Deschampsia flexuosa* (L.) Trin. grassland. About 80 percent of the changes took place in the severely affected heath-lands on the moderpodzol. The change from heath-land to grassland on the Hoorneboegse Heide takes place in several stages: establishment of grasses during the first heather beetle outbreak and expansion of grasses during subsequent outbreaks.

Usually the *Calluna* plants survive after a light infestation. After a severe infestation the *Calluna* plants usually die off, not directly after the outbreak but progressively after a six-month lag. The ability of *Calluna* to regenerate from the stem base is age-dependent. Six-to nine-year old plants

regenerate the best in terms of numbers of new shoots and shoot weight.

Death is not caused by a direct effect on the waterbalance of the *Calluna* plants. Measurements of transpiration and water potential indicated a more favourable water balance after the outbreak than before. The penetration of air into the xylem vessels through the damaged leaf tissue as a cause of death is under investigation.

Measurements of the carbohydrate reserves in *Calluna* plants during the year after an outbreak indicate disturbance of the carbohydrate balance, due to the removal of the green leaves, to be the cause of death.

G. W. HEIL (*Vakgroep vegetatiekunde en botanische oecologie, Lange Nieuwstraat 106, 3512 PN Utrecht*)

Change of Dutch heathland into grassland as a result of a heather beetle, Lochmaea suturalis Thomson, infestation

Management of the Dutch heath-lands has become a problem because of the increasing supplanting of *Calluna vulgaris* (L.) Hull by grasses. The most important grass species are: *Molinia caerulea* (L.) Moench, *Deschampsia flexuosa* (L.) Trin. and *Festuca ovina* (L.).

From data of J. J. M. BERDOWSKI, A. M. H. BRUNSTING & R. ZEILINGA (this symposium) it has become clear that the interaction between *Calluna* and the heather beetle plays an important role in this process.

Fertilizer treatments were carried out on the Westerheide near Hilversum in The Netherlands to investigate the growth of *Calluna*. Repeated nitrogen treatment of 28 $kg.ha^{-1}.yr^{-1}$ resulted in dramatic replacement of *Calluna* by *Festuca* as dominant. Phosphorus treatments did not result in such a change. The levels of nutrients applied are comparable with those released by the dying-off of *Calluna* after a heather beetle infestation.

It is hypothesized that a heather beetle infestation may result in a similar replacement of *Calluna* by *Molinia, Deschampsia* and *Festuca* as a result of competition for nutrients. *Calluna* dominates when the nutrient level is low. When the nutrient level is relatively high, *Calluna* is less competitive in comparison with the grass species, which have a potentially higher relative growth-rate (R.G.R.).

K. H. VOOUS (*Van der Duyn van Maasdamlaan 28, 1272 EM Huizen*)

The fauna of Calluna heath lands

A general survey of the fauna of the *Calluna* heath lands in The Netherlands reveals a paucity of species and individuals of terrestrial vertebrates, none of which is characteristic of this habitat. This fact was already stressed by authors from the mid-nineteenth century, although at that time wet open bogs with bell-heather *Erica tetralix*, richer floristically and faunistically than drier *Calluna* heath lands, were more abundant.

Grazing herbivores, including the flocks of sheep traditionally attended by a shepherd and dog, are now virtually a thing of the past. Lizards, blindworms and snakes have greatly diminished in numbers but survive in other habitats. The same applies to the Moor Frog *Rana arvalis*, in Dutch known as "heath frog". Even the abundance of insects and spiders has diminished, particularly those whose life cycle is related to plants that have become rare (e.g. the blue Lycaenidae, to gentian species). However, no heather species of butterflies have disappeared from our country or have suffered serious losses. The caterpillars of these butterflies do not live on *Calluna*, as do the larvae of the Heather Beetle *Lochmaea suturalis* exclusively. As a consequence monotonous *Calluna* heath lands can provide the circumstances for the development of this monophagous species as a destructive pest.

At present 30–50 species of birds regurlarly nest in the *Calluna* heath lands, but none is restricted to these, although the names of at least seven species in Dutch (14 in German) bear names connected with heather, e.g. "heath lark" for Wood Lark *Lullula arborea*.

The black Grouse *Tetrao tetrix*, often considered in western Europe as ecologically restricted to *Calluna* heath lands, does not seem to be a heather species at all; it is an inhabitant of the transition

of northern forest edges, glades and bogs and similar montane and subalpine habitats. With the ageing of *Calluna* heather as result of the abandoning of extensive sheep tending, the disappearance of small-scale farming on the surrounding grounds and the increase of the pine plantations, the Black Grouse has lost its range expansions. Throughout the Central European lowlands it has reached a stage of virtual extinction.

The Atlantic *Calluna* heath lands do not possess any kind of characteristic animal life that would ask for immediate conservation measures; they probably never had. But because of their great scenic beauty and as a remembrance of a kind of land use at present no longer practised or feasible, it is more than worthwhile to preserve characteristic and extensive examples which after all are mere remnants of what they formerly have been.

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Heath-land management in the Netherlands, scientific and social aims

The aims of the conservation and management of heath-land ecosystems concern cultural history, the conservation of nature, environmental quality, recreation and science.

Since neolithic times heath-land has played an important role in cultural development in regions with poor soils, and at one time covered extensive areas of Western Europe. Agricultural production in these areas greatly depended on the flow of nutrients from the heath to the crop fields through the practice of cutting sods, which mixed with sheep dung served as a fertilizer. Economic constraints of this farming system gave no opportunities for the accretion of wealth, but its stability meant protection against the famines that ravaged adjacent parts of Europe in the 14th and the 17th centuries. It is valuable, for the understanding of todays culture, that we should preserve the physical world of our ancestors.

In the 19th century 600000 ha of pleistocene sandy deposits in The Netherlands were covered with heath vegetation. This type of landscape extended from Flanders to Jutland. Conservation of the remaining 5–10% of this landscape is essential for the survival of heath-land ecosystems. The larger heath-land reserves are important refugia for oligotrophic systems, both terrestrial and aquatic. The quality of these environments rapidly deteriorates if eutrophication is allowed to occur.

It serves both scientific and social aims to study causal relationships in oligotrophic systems and the effects of eutrophication upon these systems. Heath-land has favourable properties for fundamental research in ecology. It is relatively simple structurally, but is far more stable than most simple ecosystems.

Ideas on management have changed a good deal since the establishment of the first heath land reserves some fifty years ago. The conviction held in the early days that nature should be left untouched in nature reserves was gradually abandoned because of the increasingly evident need to cut invading trees. A better understanding of the semi-natural character of heath land led to the introduction of regular burning in the nineteen-fifties.

The loss of nutrients by fires was minimized by burning at low frequency (every 15 years). This low frequency burning regime resulted in the decrease of *Calluna* as the dominant species.

During the last decade grasses have replaced heather as dominant species in many places. This physiognomic change, and also the frequent outbreaks of the heather beetle (*Lochmaea suturalis*), have led recently to renewed intensive discussions on methods of heath-land management. The input of nutrients with precipitation, and the accumulation in the system since heath-land lost its agricultural function as a source of nutrients, is generally accepted as the most probable factor causing the take-over by grasses. This realization may also throw new light on the hypothesis of endogenous cyclical processes which was at one time postulated to explain mosaic pattern in heath vegetation.