*Acta Bot. Neerl.* 26(3), June 1977, p. 265–272.

# MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

## MEETING OF THE SECTION FOR VEGETATION RESEARCH ON SEPTEMBER 30, 1976

M. J. M. OOMES *(Centrum voor Agrobiologisch Onderzoek (CABO), Wageningen*

### Cutting regime experiments on extensively used grasslands

Extensively used grasslands in the Netherlands are low productive grasslands unsuitable for intensive agricultural use and which are not improved for reasons of economy or landscape planning.

Because of the increasing requirement of grasslands for recreation, nature and landscape conservancy, these grasslands are used for extensive agricultural purposes in existing nature reserves or in national parks to be created.

In this study a number of cutting regimes is compared with respect to their influence on the vegetation and the cost of management. The objectives are:

1) Combining extensive agricultural use with management for nature conservancy;

2) Obtaining grasslands with a greater number of species and increasing the diversity between the grasslands. An important question is, whether low productivity is conditional in this, i.e. whether fertilizers should be completely excluded or not;

3) Promoting the development of the ecological potentialities of such grasslands by stimulating the natural distribution of the native plant species.

The experiments are carried out on various types of grassland, a Poo-Lolietum on sandy soil and an Arrhenatheretum on clay soil.

Some rough conclusions are:

– On the sandy soil the after-effect of the fertilizer application has disappeared after two years and the yield has decreased from 11 to 6 tons of dry matter (DM) per ha per year. A dressing of 50-20-20 kg pure NPK per ha increases the yield by two tons, N fertilizer alone has no effect. Apparently, in these sandy soils N and K soon are the limiting factors and the effect of the remaining phosphate is negligible.

– On clay soils a N dressing of 50 kg has the same effect as a complete dressing on sandy soil, so only N is limiting. An excess of phosphate will not increase the production of the vegetation.

– The number of species in the vegetation did not yet show a decrease due to fertilizing after 5 years, only a few high productive species begin to dominate.

– Of the low productive grasslands with yields below 5–6 tons of DM per ha annually, the dry matter yield later in the season does not increase, when the herbage is cut later than the end of June.

– When the yield of the second cut is below 1–2 tons of DM, the vegetation will not be damaged by omitting this cut, especially not when the grassland is inundated during winter. However, the vegetation is very favourably affected, when this second cut is mown or grazed. This will retard the growth of some dominant grasses to such an extent that the horizontal and vertical build-up of the sward in spring will allow better establishment and growing of other plant species. It may be expected that this will increase the species diversity.

– A similar effect is obtained by cutting the vegetation in May. Moreover, this will retard flowering and seed production of a number of species to later in the season. Generally, variety in cutting dates leads to different effects on the life cycles of plant species, in this way increasing the species diversity of the vegetation and causing different flowering aspects in a complex of grasslands.

– Dominant species dependent on their generative reproduction, like e.g. Holcus lanatus, can be suppressed by cutting before the production of ripe seed, or long afterwards, when the seedlings have been smothered by the dense herbage. So more detailed understanding of the

distribution strategy and of the life cycle of plant species may contribute management and predicting its influence.

## MEETING OF THE SECTION FOR PLANT PATHOLOGY ON NOVEMBER 16, 1976

F. H. RIJSDIJK *(Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen)*
Calculation of yield losses due to cereal diseases in Europe

As part of a series of Agro-ecological Atlasses on Cereal Growing in Europe, published under the auspices of the European Cereal Atlas Foundation, two atlasses have been produced, one on agrometeorology (THRAN & BROEKHUIZEN 1965), in which Europe is divided into some 80 agro-climatic sub-areas, and one on cereal growing in general (BROEKHUIZEN 1969). A third atlas, dealing with pests and diseases, is in preparation at the Laboratory of Phytopathology of the Agricultural University at Wageningen. The third atlas will contain a.o. data on mean annual crop losses of the various cereals as well as information on weather-dependent risks of pests and diseases.

Crop loss data have been collected in three ways:

- *Enquiry*: A questionnaire asking for data on cereal growing and crop losses per harmful agent, was sent out and completed by some 200 colleagues all over Europe.
- *Literature review*: The Publication and Documentation Centre PUDOC in Wageningen started a literature survey of information on crop losses, with special emphasis on eastern Europe.
- *Personal information*: Data were collected by a few informants during extensive travelling.

A procedure was devised to relate losses to disease severities and/or prevalences. For most harmful agents enough data have become available to calculate an equation of crop loss to disease severity. The positions of the lines connecting points of equal losses, the *isoloss* curves, were also calculated. Climate sub-area data calculated from Vol. I have been used to simulate epidemics of yellow stripe rust (*Puccinia striiformis*) on wheat. As only monthly averages were available from the atlas. years with different weather types were simulated; by variation around these values with the help of a random generator. The simulated yearly weather sequences were then used as inputs to a simulator of fungal epidemics, set for yellow rust (RIJSDIJK 1975). The result was a frequency distribution of terminal severities over 50 simulated years for every climate sub-area. The frequency distributions give indications of the risks incurred in the various climate sub-areas when no crop protection is practised. The risks are expressed in five classes: High, medium, low, negligeable, and unknown, and mapped accordingly.

BROEKHUIZEN, S. (1960): *Agro-ecological Atlas of Cereal Growing in Europe.* Vol. II. Atlas of the Cereal-Growing Areas in Europe. Elsevier, Amsterdam. 157 pp.

RIJSDIJK, F. H. (1975): A simulator for yellow rust on wheat. *C. R. Fac. Sci. Agron., Gembloux* **1975:** 411–418.

RIJSDIJK, F. H. & J. C. ZADOKS (1976): Assessment of risks and losses due to cereal rusts in Europe. *Proc. 4th Eur. Mediterr. Cereal Rusts Conf., Interlaken* **1976:** 60–62.

THRAN, P. & S. BROEKHUIZEN (1965): *Agro-ecological Atlas of Cereal Growing in Europe.* Vol. I. Agro-climatic Atlas of Europe. Elsevier, Amsterdam. c 150 pp.

B. H. H. BERGMAN and MARIANNE A. M. BAKKER-VAN DER VOORT *(Laboratorium voor Bloembollenonderzoek, Lisse)*
Latent infections of Fusarium oxysporum f. sp. tulipae in tulip bulbs

Latent infections in plants caused by fungi have been frequently recorded, especially in ap-

ples, strawberries, tomatoes and tropical fruits. Also latent infections of *Fusarium oxysporum* in gladiolus corms and in the basal plate of narcissi have been demonstrated. Evidence was collected about a similar behaviour of *Fusarium oxysporum* f. sp. *tulipae* Apt occurring naturally in the basal plate and in the outer tissue of tulip bulbs. Latent infections could be induced experimentally in bulb scales by inoculations of the undamaged scales followed by a short (e.g. 2 days') incubation at 25°C. After removal of the inoculum, upon subsequent dry storage a number of bulbs developed disease symptoms. These progressed from the inoculation sites throughout the whole bulb. However, some of the bulbs did not rot, but either remained apparently undamaged or developed yellowish-brown and slightly sunken dots or small specks. These did not enlarge during prolonged dry storage for up to 4 months. When after this period the inoculation sites were desinfected by rubbing with cottonwool moistened with 10% formalin, from 60–100% of the bulbs the pathogen could be recovered upon plating on potato dextrose agar. Even dipping of the bulbs in 10% formalin for 5 minutes only partially reduced the outgrowth from the inoculation site tissue. This indicates that the pathogen had penetrated deeply enough to escape killing by the desinfectant. Histological investigations on the atypically damaged tissue have not yet been completed.

C. J. LANGERAK and J. HAANSTRA-VERBEEK *(Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen)*

## The influence of physiological and abiotic factors on the pathogenesis of Fusarium oxysporum Schl. f. sp. narcissi Snyder & Hansen

*Fusarium oxysporum* f. sp. *narcissi* only attacks unprotected parenchymatous tissues in bulbs and roots of narcissus. Initially the fungus grows intercellularly, later on also intracellularly. Tissue decay starts only above 13°C, and if the infected host tissue has a low metabolic activity. Such conditions prevail at the time of bulb maturation in early summer and during storage. Besides mechanical injury between lifting of the bulbs and replanting, wounding is caused when the protective periderm of the basal plate is being ruptured by the outgrowing roots. These conditions facilitate fungal pathogenesis.

Some months after planting a new internal periderm develops parallel to the outer periderm layer and transversally through the parenchymatous tissue, which connects the bulb scales with the central bulb stool. New infections of the basal plate may be fully excluded or insufficiently enclosed by this suberized barrier. In the former case further penetration into the bulb only occurs when this periderm is ruptured again. In the latter case, basal rot always appears either just before lifting or during storage.

Young roots can be infected, as long as the exodermal cell walls do not contain suberin. Suberisation normally starts within two weeks after rupturing of the basal plate. Reddish-brown stripe-shaped lesions arise within two months if the soil temperature exceeds 10°C. Penetration of the bulb by mycelium from old dying roots can be almost precluded, because roots of a maturing plant are internally cut off from the bulb through a suberisation straight across the root base before deterioration by *Fusarium* and saprophytes can begin.

These observations indicate that optimal control of *F. oxysporum* in narcissus can be obtained if bulbs are kept free from growing mycelium during the first month after planting at low soil temperatures.

W. H. M. MOSCH and J. C. MOOI *(Instituut voor Plantenziektenkundig Onderzoek, Wageningen)*

## Identification of Phoma exigua var. foveata rot in potato tubers by thin layer chromatography

Since gangrene, caused by *Phoma exigua* Desm. var. *foveata* (Foister) Boerema, was de-

tected in 1967 in the Netherlands for the first time, strict measures have been taken by the Plant Protection Service as well as by the General Netherlands Inspection Service for Field Seeds and Seed Potatoes (N.A.K.) to restrict its occurrence and eventually eliminate this disease.

In order to test lots of seed potatoes for contamination, samples of 50 to 100 tubers are taken from each lot. The tubers are bruised one by one, then stored at 5°C for eight to ten weeks. After storage from every rotting tuber isolations are made on an agar medium. Five to nine days later *P. e.* var. *foveata* can be identified by a yellow pigment which frequently crystallizes as needles and which rapidly turns red when a drop of alkali is added. The production of this pigment is the only characteristic by which *P. e.* var. *foveata* can be distinguished from *P. e.* Desm. var. *exigua* which also causes gangrene on potato but of a much less noxious type.

BICK & RHEE (1966) stated that the yellow pigment consists of several anthraquinone derivatives, the most important being chrysophanol and pachybasine. In testing all lots of seed potatoes the N.A.K. has to make thousands of isolations. Therefore we investigated whether identification of gangrene, based on detection of anthraquinone pigments, produced by *P. e.* var. *foveata* would have advantages over identification by isolation.

Homogenized cultures of fungi on malt agar, bacteria on nutrient agar or rotted tuber tissue were cut into small pieces and extracted overnight with chloroform. The chloroform was evaporated at 55°C. The extracts were spotted on Silicagel sheets (Merck, layer thickness 0.25 mm) and chromatographed with a mixture of toluene and acetone (95:5 (v/v)). Various components were then visible under U.V. light (366 nm). From cultures and rot of *P. e.* var. *foveata* the most conspicuous component had a Rf value of 0.79. This spot turned red after spraying with 10% KOH in methanol, which according to SHAH et al. (1972) demonstrates the presence of anthraquinone derivatives on thin layer chromatograms.

This component was never found in chromatograms of extracts from cultures or rot produced by isolates of *P. e.* var. *exigua*, The method is sensitive for amounts as small as 60 mg of rotted tissue and may even be used with smaller quantities. No indication of the Rf. 0.79 component was found in cultures of a number of other potato pathogens nor in the rots, caused by these organisms.

To compare the sensitivity of the isolation and chemical method, the rot of 473 diseased tubers, mostly derived from lots of potatoes already tested with the bruise method, was diagnosed. 236 lesions were identified as gangrene caused by *P. e.* var. *foveata*, by both methods, 57 lesions were identified as "foveata" gangrene by the chromatographic method only and 14 lesions were identified as "foveata" gangrene by the isolation method only. In another experiment, including more than 1000 rotting tubers, the chromatographic method performed even better.

C. KLIFFEN *(Instituut voor Plantenziektenkundig Onderzoek, Wageningen)*

The development of a biochemical test to determine the degree of field resistance of potato leaves to Phytophthora infestans, without the use of the fungus.

It is frequently seen that potato varieties have a higher value for field resistance shortly after their introduction for practical use than is the case a few years later. The cause of this is probably that initially vertical resistance is involved and that later, after the appearance of compatible races of the fungus, the real level of resistance becomes apparent. For this reason, there is a need for a test by which the degree of field resistance of a potato variety can be determined, without the use of a compatible race of the fungus.

The biochemical test is based on the hypothesis that the degree of field resistance shows a correlation with the degree of the reaction of the leaves to certain stimuli. In order to test this supposition, a number of experiments are made, in each case with two races which are known to have differing degrees of field resistance. The stimulus used is gassing with $NO_2$; a fairly accidental choice.

The leaves are removed from the plant, weighed, placed with their stalks in water, and then exposed for 4 hours to an environment containing 30 ppm $NO_2$ gas. A physiologically similar group of leaves is subjected to the same treatment, without the $NO_2$, as reference. After the mentioned treatments, the leaves are ground-up in a solution of polyethylene glycol (PEG), magnesium acetate ($Mg^{2+}$), cysteine, polyvinyl pyrrolidone, sucrose and a buffer. The homogenate is added to a mixture of sand and cellulose powder, thoroughly stirred and the complete mixture then added to a column also consisting of a mixture of sand and cellulose powder suspended in the previously mentioned solvent. Elution occurs successively with three solvents, firstly the complete solvent, then the same without PEG and finally, the same without PEG and $Mg^{2+}$. In this way, three fractions are obtained which show a strong absorption of UV light. The extinction value at 260 nm is increased as a result of the $NO_2$ gassing in the first fraction, but decreased in the other two. For the more resistant variety, this increase is larger and the decreases are smaller.

If such experiments are carried out with a variety for which the degree of field resistance is not known and one (or more) varieties for which it is, then it is possible to obtain an impression of the degree of field resistance which the former would show when a compatible race of the fungus appears.

G. C. A. BRUIN, S. A. GIESKES and A. FUCHS *(Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen)*

## Induction of the synthesis of pisatin, and its breakdown by bacteria

According to CRUICKSHANK & PERRIN (1963) bacteria, among which *Pseudomonas pisi* and *Xanthomonas phaseoli*, were not able to induce the formation of significant amounts of pisatin upon inoculation ($10^6$ cells per ml) of endocarps of detached pea pods. Similar results were obtained by STHOLASUTA et al. (1971) who after inoculation of pea leaves with *Ps. phaseolicola* did not observe any accumulation of pisatin.

Preliminary observations by Platero Sanz & Fuchs (unpublished) suggested formation of fair amounts of pisatin upon challenging endocarp tissue with suspensions of the following bacterial species: *Erwinia atroseptica*, *E. carotovora*, *Ps. pisi*, *Ps. syringae* and *X. phaseoli* var. *fuscans*. These experiments have been repeated and extended with similar results: upon inoculation of endocarp tissue of pea pods (cv. Gloire de Quimper) with bacterial suspensions in sterile water (c. $10^8$ cells per ml), invariably pisatin was produced, whether or not the pea pods were slightly injured by needle puncturing. Amounts present after 4 days incubation at 24°C varied from 237 µg/g fresh weight for *Ps. pisi* to 463 µg/g fresh weight for *E. carotovora*, the concentration in non-inoculated control pea pods being less than 47 µg/g fresh weight (this being the detection level of pisatin in the assay system).

In time-course studies with *Ps. pisi* the pisatin concentration was found to increase until 6 days after inoculation (c. 400 µg/g fresh weight), and to slightly drop afterwards. Addition of $^{14}$C-phenylalanine at the time of inoculation resulted in incorporation of radioactivity into one unidentified product, beside pisatin, in the petroleum ether extract, and two unknown phenylalanine metabolites in the ethyl acetate extracts. Autoradiography of thin-layer chromatograms (solvent chloroform/methanol 97:3) showed all unknowns to gradually disappear with time, whereas increasing amounts of radioactivity were incorporated into pisatin until 6 days after incubation, after which the radioactivity incorporated into pisatin dropped slowly.

Since the latter results suggested at least a minor capacity of *Ps. pisi* to break down pisatin, experiments were carried out to study the effect of pisatin on bacterial growth, and their ability to degrade it. The first experiments (PLATERO SANZ & FUCHS, 1975) showed that pisatin inhibited bacterial growth; however, growth of pea pathogens (*E. atroseptica*, *E. carotovora*, *Ps. pisi*) was not inhibited completely, even not at concentrations of 100µg/ml; at these concentrations, growth of non-pea pathogens (*Ps. syringae*, *X. phaseoli* var. *fuscans*) was nil. At the end of the experiments, pisatin was almost quantitatively recovered from the nutrient medium. Because, however, the latter contained appreciable amounts (1–2%) of

glucose, and since with fungal pathogens the synthesis of the pisatin-degrading enzyme system seems to be subject to catabolite repression (DE WIT-ELSHOVE & FUCHS, 1971) these experiments were repeated with bacteria, which were pregrown in a pisatin-containing nutrient medium (with either 1% peptone or 1.5% glucose) and then subcultured, after being concentrated by centrifugation, in a medium with only 0.05% glucose, and 30 μg non-labelled or $^{14}$C-labelled pisatin per ml. Neither with *E. carotovora* nor with *Ps. pisi* any other product but pisatin was detected, the recovery ranging from c. 80 to 100% of the amount added.

Together the results of our experiments revealed that bacteria, pea pathogens as well as non-pea pathogens, can induce the formation of significant amounts of pisatin, and that especially non-pea pathogens are quite sensitive to pisatin; further, as distinct from many fungi (cf. VAN ETTEN et al., 1975; VAN 'T LAND et al., 1975; FUCHS et al., 1976) bacteria seem unable to degrade it.

CRUICKSHANK, I. A. M. & D. R. PERRIN (1963): Studies on phytoalexins. VI. Pisatin: The effect of some factors on its formation in Pisum sativum L., and the significance of pisatin in disease resistance. *Austr. J. Biol. Sci.* **16**: 111–128.

ETTEN, H. D. VAN, S. G. PUEPPKE & T. C. KELSEY (1975): 3,6a-Dihydroxy-8,9-methylenedioxypterocarpan as a metabolic of pisatin produced by Fusarium f. sp. pisi. *Phytochemistry* **14**: 1103–1105.

FUCHS, A., M. PLATERO SANZ & F. W. DE VRIES (1976): In vitro conversion of pisatin by Fusarium oxysporum f. pisi. *Acta Bot. Neerl.* **25**: 253–254.

LAND, B. G. VAN 'T, E. D. WIERSMA-VAN DUIN & A. FUCHS (1975): In vitro and in vivo conversion of pisatin by Ascochyta pisi. *Acta Bot. Neerl.* **24**: 251.

PLATERO SANZ, M. & A. FUCHS (1975): Degradación de pisatina, un compuesto antimicrobiano producido por Pisum sativum L. *Proc. Congreso Nacional de Microbiologia, Salamanca (Spain).*

STHOLASUTA, P., J. A. BAILEY, V. SEVERIN & B. J. DEVERALL (1971): Effect of bacterial inoculation of bean and pea leaves on the accumulation of phaseollin and pisatin. *Physiol. Pl. Path.* **1**: 177–184.

WIT-ELSHOVE, A. DE & A. FUCHS (1971): The influence of the carbohydrate source on pisatin breakdown by fungi pathogenic to pea (Pisum sativum). *Physiol. Pl. Path.* **1**: 17–24.

J. W. L. VAN VUURDE (*Phytopathologisch laboratorium "Willie Commelin Scholten", Baarn*)

## Distribution of micro-organisms on seminal roots of wheat

Microbial colonization of the root surface from tip to base is studied in order to obtain information about population density and antipathogenic potential of micro-organisms at different root zones of wheat.

Plants were cultivated in an observation box placed in a slantwise position, equipped with a removable perspex plate on which root growth was recorded daily. Seminal roots were sampled at various times after initiation. To relate microbial colonization to morphological changes of the root, subsamples from the middle part of the zones grown on successive days were taken from each root and their structure and microflora examined.

On one-week-old roots we observed, by the dilution plate method, an exponential increase of bacteria from tip to root base, whereas numbers of actinomycetes only slightly increased. Ten to twelve days after root initiation the total number of bacteria generally decreased at the root base. This was often accompanied by a strong increase in the number of actinomycetes.

Direct observation of the root zones by means of fluorescence microscopy demonstrated that this high number of actinomycetes was not due to sporulation only. Hyphae of actinomycetes could cover large parts of the surface at the base of 12 day old roots. Whereas numbers of bacteria generally increased from tip to base, they showed two peaks in the covering of the root surface. A first peak on 4-days-old segments probably results from the avail-

ability of root cap material and tip exudates as a nutrient source for rapidly growing strains. The second peak with maximum surface cover was found at 7- to 9-days old root segments, about 4 days after the rupture of the cortex due to the formation of lateral roots.

Microscopic observation of Feulgen and acridine orange stained root cells indicated that dying of epidermal and cortical cells may also be important in causing the second colonization peak.

W. H. VAN ECK *(Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn)*

Lysis of chlamydospores of Fusarium solani in soil

During lysis of chlamydospores of *Fusarium solani* in soil perforation of the cell walls by soil microorganisms was not observed in the electron microscope. Chlamydospore walls contain mainly chitin and $\beta(1-3)$ glucan. Addition of chitin and laminarin (a $\beta(1-3)$ glucan) to soil enhanced lysis of chlamydospores, but this lysis was not accompanied by perforation of the cell walls. Lysis of chlamydospores in soil, therefore, is not of a heterolytic nature. Persistence of chlamydospores in soil may be related to the amount of stored nutrients in the cells. Chlamydospores high or low in lipid could be prepared from macroconidia grown on media with a high or low carbon level, respectively. A high lipid content did not favour persistence of chlamydospores in soil.

G. JAGER and H. VELVIS *(Instituut voor Bodemvruchtbaarheid Haren, Groningen)*

An effort to measure antagonism in soil against Rhizoctonia solani

Antagonism between microorganisms in soil is a general phenomenon, which may result from competition, antibiosis or parasitism. In many cases competitors and parasites are active producers of antibiotics. *Rhizoctonia solani* was chosen as the test organism and antagonism (antibiosis) measured by determining the inhibition of the hyphal growth on filter paper disks, impregnated with the moisture of underlying soil in petri dishes.

It was looked for whether addition of chitin would increase antagonism (antibiosis) in different soils. Increased inhibition of growth of *Rhizoctonia* was compared with increases in numbers of antibiotic active microorganisms. For a reclaimed peat soil, in which streptomycetes were strongly activated by chitin additions, a good agreement between numbers of antibiotic active streptomycetes and growth inhibition of *Rhizoctonia* was found. In a slightly acid sandy soil growth inhibition was due to streptomycetes and fungi. With clay soils an addition of chitin (0.5 % w/w) did not lead to growth inhibition nor to increased numbers of antagonists.

Decreased damage to young potato sprouts was found in soil samples where increased inhibition of *Rhizoctonia* growth occurred.

Further observations are needed to evaluate whether also in other soils increased inhibition of *Rhizoctonia* growth is accompanied by enlarged numbers of antagonists and decreased damage to the host plant.

C. P. DE JAGER *(Laboratorium voor Virologie, Landbouwhogeschool, Wageningen)*

Genetic analysis of cowpea mosaic virus mutants

The genome of cowpea mosaic virus (CPMV) comprises two RNA's which are encapsidated into separate particles. These particles are designated middle- (M) and bottom component (B) according to their sedimentation behaviour in sucrose gradients. Since the components each carry only a portion of the total genome they are separately non-infectious.

Mutants of CPMV could be induced by nitrous acid treatment. In the reaction with the RNA point mutations may arise by conversion of the RNA-bases. The majority of the induced mutants was defective in multiplication and/or gave faulty symptom expression in four differential hosts.

In eleven induced mutants the mutations for changed phenotypic properties have been located in either the M- or B-component by three test methods:

1. *In vitro* recombination. M- and B-components of wild and mutant strains were purified.

From the symptoms observed on the differential hosts after inoculation with either of the two heterologous combinations of components, the component carrying the mutation could be identified.
2. Supplementation. Adding a purified wild-type component to an unfractionated mutant preparation restored wild-type symptom production only if the mutation was in the equivalent component.
3. Reassortment of components. This test was based on the assumption that a mixture of two mutants, defective in the same phenotypic property, may only induce wild-type symptom expression if the mutants carry mutations in different components.

Mutations for decreased multiplication and symptom alterations on each differential host were located in M- as well as in B-components.

D. PETERS *(Laboratorium voor Virologie, Landbouwhogeschool, Wageningen)*

On the subgrouping of plant and animal rhabdoviruses

The *Rhabdoviridae* constitute a family of viruses which have a bullet-shaped or bacilliform morphology. Members of this family replicate in vertebrate, invertebrate and plant tissue. This family is often subgrouped in plant- and animal rhabdoviruses: a division that is based on their host range. However, a division based on a number of genetically inherited stable characters which can not be influenced by the host has to be preferred. A number of characters such as the presence of the proteins L and NS, the presence of one of two matrix proteins (M or M1 and M2), the involvement of the nucleus in the replication of virus, and may be also the occurrence of a detectable transcriptase activity, may be used to divide this family in two subgroups. Those viruses which contain two M proteins (M1 and M2), and no detectable defined minor proteins (L and NS) and transcriptase activity, and depend upon the nuclear involvement in the replication, constituted one subfamily. Rabies virus, as type member, and the plant viruses sowthistle yellow vein virus, *Sonchus* yellow net and eggplant mottle dwarf may be the members of this group. The other group is formed by vesicular stomatitis virus, as type member with lettuce necrotic yellows virus and *Sonchus* virus being viruses infecting plants. This group has one M protein, a L and a NS protein, contains transcriptase activity, and the nucleus seems not to be involved in their replication.

G. T. N. DE LEEUW, J. R. F. TH. GROESBEEK and T. VAN SCHADEWIJK *(Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn)*

Immunofluorescence and immunohistochemistry of plant mycoplasmas in situ.

The serological relationship with *Acholeplasma laidlawii* of plant mycoplasmas isolated from different plant species and kept in *Vinca rosea* plants was studied *in situ* with the aid of immunofluorescence and immunohistochemical techniques. Fresh sections (about 30$\mu$m thick) of young stems from well-developing mycoplasma-diseased *V. rosea* plants were treated with specific rabbit anti-*A. laidlawii* serum together with goat anti-rabbit IgG serum conjugated with either tetramethylrhodamineisothiocyanate (TRITC) of horseradish peroxidase (HRP). The TRITC-treated sections were examined with a Leitz Orthomat fluorescence microscope, whereas the HRP-treated sections were incubated with a substrate solution consisting of 3,3-diaminobenzidine with $H_2O_2$, and embedded in Epon for electron microscopy. Preliminary results suggest that some of the plant mycoplasmas tested are serologically related to *A. laidlawii*. Further studies, i.e. of the reaction of specific rabbit anti-*Spiroplasma citri* serum with *S. citri* in *V. rosea* plants obtained from P. G. Markham, John Innes Institute, Norwich, are in progress.