Acta Bot. Neerl. 29 (2/3), May 1980, p. 207–222.

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON SEPTEMBER 26, 1979

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Flowering strategies in Taraxacum

Taraxacum is a rather intricate genus, in which about 2000 microspecies have been described. In our laboratory, the biosystematics and ecology of this genus are under investigation. In the Netherlands, *Taraxacum* exhibits abundant flowering in early spring. To test the hypothesis that this flowering is influenced by the amount of biomass present in the tap-root in the previous autumn, an experiment was carried out with the microspecies *T. ekmanii* Dt, *T. hollandicum* v.S. and *T. hygrophilum* v.S.

40 Tap-roots of each species were weighed and planted in a *Lolium perenne* L. lawn in November, 1978. The plants were dug up in May, 1979, and dried. The relations between the fresh weights of the tap-roots in November and some characteristics of the corresponding plants in May were analysed with linear regression and rank-correlation methods.

In all species, a significant ($p < 0.001$) regression of the dry weights of the plants in May (y) on the fresh weights of the corresponding tap-roots (x) was found:

$$y = (0.50 \pm 0.04)\, x.$$

The correlation between fresh weights of roots and number of capitula was also significant at $p < 0.001$ with $\tau = 0.4$ for all species.

However, flowering strategies differed from species to species. *T. ekmanii* and *T. hollandicum* flowered only if a tap-root with a weight exceeding 0.6 g had been planted. For *T. hygrophilum*, this threshold-value was only 0.3 g. In *T. hollandicum*, the size of the capitulum is variable (depending on the weight of the planted tap-root) and the number of capitula rather constant, whereas in *T. hygrophilum* the opposite was found. In *T. ekmanii* and *T. hygrophilum*, the ratio between the dry weights of generative and vegetative plant parts was 0.3 ± 0.1 and in *T. hollandicum* 0.6 ± 0.1. In *T. hollandicum*, flowering clearly takes place at the expense of vegetative growth but in the other two species this was not evident.

These observations are indicative of the existence of a certain degree of niche-differentiation between microspecies of *Taraxacum*.

G. LONDO *(Rijksinstituut voor Natuurbeheer, Broekhuizerwg 2, 3956 NS Leersum)*

Population dynamics and vegetation succession

In vegetation dynamics and population dynamics as well two categories can be distinguished:

1. steady state dynamics: a constancy in the population and habitat or fluctuations around an average. Also a cyclic succession can occur with species alternating with each other in time.
2. succession dynamics: the habitat is changing in a certain direction and there is a succession of species.

In the following we are dealing with succession dynamics. Some results are given of the first ten years of a grassland vegetation (where an Arrhenatheretum has developed) in an experimental nature garden. Every plant species has been mapped yearly via a grid of square meters (total surface 9 m × 5 m). Per square meter the coverage or abundance was determined according to the decimal scale (LONDO 1976). So the spatial and temporal distribution of each species is known and can be correlated with vegetation characteristics and the soil map. On the basis of the coverage values graphs are made showing the increase and decrease of species.

Investigations have given the following conclusions:

1. It is inaccurate to study only one species and to pay no attention to other species and to the total vegetation development.
2. The behaviour in space and time of each species differs from that of other species. However, there are strong correlations among several species.
3. Within one species the behaviour can differ in the various habitats (a.o. different soil types).
4. Not only the presence (and abundance or coverage) of a species is of ecological interest, but also the intensity of flowering.
5. Long-term research is required for a good knowledge of population dynamics and population ecology. Therefore it is necessary to make observations throughout the year, and if possible several times a month or weekly.

As nature reserves usually are situated far away from the universities and because of the frequent observations when studying population dynamics, it is recommended to lay out nature gardens near universities. By using various kinds of soil (see LONDO 1977), it is possible to make on ca 0.5 to 1 ha a variety of habitats where about 500 wild plant species can grow in natural conditions.

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A. H. L. HUISKES, W. DE MUNCK, P. PAALVAST and H. SCHAT *(Delta Instituut voor Hydrobiologisch Onderzoek, Vierstraat 28, 4401EA Yerseke)*

Some aspects of the population dynamics of Salicornia spp.

Salicornia europaea L. is a polymorphic species, currently divided into two other species: the diploid *S. brachystachya* with short inflorescences and rounded flower bracts and the tetraploid *S. stricta* with long inflorescences and pointed flower bracts. These species differ also in their spatial distribution on the salt marsh: *S. brachystachya* is mainly found above the Mean High Waterline while *S. stricta* generally occurs below the Mean High Waterline (KÖNIG 1960, SCHAT 1978).

Of the many biotic and abiotic factors influencing the population dynamics of *Salicornia* the influence of plant density, competition for light and herbivory were discussed briefly.

A density experiment was set up in monospecific stands of *S. stricta* on the salt marsh South of Bergen op Zoom. Various densities, ranging from 16 plants /m^2 to 4380 plants /m^2 were established by weeding in marked plots. No density-dependent mortality was observed, but this may occur in places with an even higher plant density (PAALVAST 1979). The total amount of dry matter per m^2 increased with density up to 1600 plants/m^2. In higher densities a slight decrease in total yield was observed which has also been described by DE WIT (1960) for sugar beets and oats as being caused by the phenomenon that in very dense stands mortality of certain individuals does not implement a better growth of the survivors.

Other field experiments showed that artificial shading increased the mortality in *S. stricta* populations by a factor 3 to 6. In mixed stands of *S. stricta* and *Spartina anglica* C. E. Hubbard clipping of *S. anglica* plants diminished the mortality as compared with plots with unclipped *S. anglica*. It was found that the mortality (in percentages) in the unclipped plots was positively correlated with the logarithm of the product of cover and height of the vegetation at the start of the experiment. This correlation was not found in the clipped plots and it was therefore concluded that the shading by *S. anglica* caused the higher mortality in the unclipped plots (PAALVAST 1979).

Of the herbivores grazing on *Salicornia* species *Hydrobia ulvae* Pennant (Mollusca) is most common, but has probably little effect on the population dynamics. Larvae of *Coleophora salicorniae* Wck. (Lepidoptera), however, occur in the flowering season in the inflorescences and have been observed to consume up to 28% of the seeds. Other herbivores found on *Salicornia* are larvae of *Lita salicorniae* E. Hering (Lepidoptera) and of *Baldratia salicorniae* Kffr. (Dipt., Itonididae) (DE MUNCK & BEEFTINK 1978, 1979).

KÖNIG, D. (1960): Beiträge zur Kenntnis der deutschen Salicornien. *Mitt. Flor.-Soz. Arbeitsgem.* **8**: 5–58.

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— & — (1979): Invertebrate herbivory and seed production in Salicornia. In E. K. DUURSMA (ed.), *Progress report 1978 of the Delta Institute for Hydrobiological Research:* 46–48.
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SCHAT, H. (1978): Populatiebiologie van Salicornia stricta en Salicornia brachystachya en van enkele andere soorten op de schorren ten zuiden van Bergen op Zoom. *Student Report, Delta Institute for Hydrobiological Research, Yerseke.* 218 pp.
WIT, C. T. DE (1960): On competition. *Versl. Landb. Onderz.* **66**: **8**. 82 pp.

J. A. C. VERKLEY and J. VAN ANDEL *(Biologisch Laboratorium van de Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam)*

Population differentiation in Chamaenerion angustifolium?

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

Similarities and differences in some biennials

Biennials are thought to be adapted to short-living habitats of which the occurrence is unpredictable in time and space. To test this idea the ecology of four biennials (*Cirsium palustre* (L.) Scop., *Cirsium vulgare* (Savi) Ten., *Cynoglossum officinale* L., *Echium vulgare* L.) is studied in Meijendel, a dune area north of The Hague.

Within the group of biennials (or short-living monocarps) some similarities can be observed. The life history is identical: seedlings grow into rosettes and in the second year after germination flowering stems may develop. Under field conditions flowering can be delayed for one or more years. In Meijendel biennial species were often found to grow at the same site. A comparison of the distribution of fourteen biennials in The Netherlands (data from the Rijksherbarium, Leiden) revealed that out of 91 pairs, 46 showed a significant positive and 7 a significant negative association. Biennial species in The Netherlands mainly (over 70%) occur in typically man-disturbed habitats (E. van der Meijden, pers. comm.).

In spite of these similarities a detailed study showed remarkable differences between species.

In an experiment on the longevity of the seeds of the four species, samples of seeds buried in pots were periodically tested on viability. Contrary to the other species, *Cynoglossum* does not have a buried-seed bank.

Germination in *Cynoglossum* is restricted to early spring, whereas in the other species germination in autumn as well as in spring can be observed (*Cirsium* spp.) or during the entire year, except for the winter (*Echium*).

Survival of seedlings measured during the first seven months after germination was highest in *Cynoglossum*; the other species did not show differences. In *Cirsium* spp. large differences in survival between autumn and spring cohorts were found. The risk of failure of seedling establishment is spread by this type of germination.

Comparison of species characteristics

	C. palustre	C. vulgare	C. officinale	E. vulgare
seeds/plant	300–2000	300–5000	50–800	1000–10,000
seed weight (mg)	c. 1.5	c. 2.8	c. 20.0	c. 2.7
dispersal	anemochorous		epizoochorous(?)	passive ballistic
distance (m)	> 5	> 5	< 5	c. 5
survival buried seed after one year (%)	40	50	0	80

Seeds of *C. palustre*, *C. vulgare* and *E. vulgare* are capable of reaching suitable habitas both in time and space. Seeds of *C. officinale* have to cope with the habitat near the motherplant.

So maintenance of *Cynoglossum officinale* was achieved by a different set of properties compared to the other three species: the relatively small number of seeds produced germinated during the first spring after seed shed, near the motherplant. Survival of seedlings was high. In the other species seeds may germinate depending on environmental conditions either immediately after dispersal in autumn, or in spring or summer, or even in one of the following years. Through dispersal new habitats can be reached. Survival of seedlings was lower.

It is thought that *Cirsium palustre*, *C. vulgare* and, to a lesser extent, *Echium vulgare*, survive in Meijendel by constant displacement, while *Cynoglossum officinale* has a more stationary distribution.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY ON NOVEMBER 16, 1979

J. KOEK-NOORMAN *(Instituut voor Systematische Plantkunde, Heidelberglaan 2, 3584 CS Utrecht)*

Wood anatomy and classification of Henriquezia, Platycarpum and Glaesonia

A detailed account of this study is published in this issue, p. 117–126.

H. J. PLUYMAEKERS *(Botanisch Laboratorium, Katholieke Universiteit, Toernooiveld, 6526 ED Nijmegen)*

Cell wall texture in root hairs of Limnobium stoloniferum

An abstract is published in "Ultramicroscopy".

W. VERKERKE *(Hugo de Vries Laboratorium, Plantage Middenlaan 2A, 1018 DD Amsterdam)*

Ovule and seed-coat development in some species of Polygala (Polygalaceae)

During a study of the ovule development in some *Polygala* species for the first time an outer integument was noted which is asymmetrical owing to its subdermal origin at the anti-raphal side and its fully dermal initiation at the lateral sides of the ovule. While the inner integument remains entirely dermal in all the four species examined, they exhibit diverse subdermal contributions towards their outer integuments. In *P. chamaebuxus* L. and *P. vayredae* Costa the subdermal contribution towards the outer integument (= o.i.) starts when the dermal integument primordium is 3 cells high, whereas in *P. myrtifolia* it starts when the integument is 7 cells high. In *P. vulgaris* L. subdermal cells start their mitotic activity when the integument primordium is 10 cells high and this subdermal contribution is restricted to the anti-raphal side. The mature ovule of *P. chamaebuxus* has a plurilayered o.i. with an all-sided subdermal contribution and a vascular bundle at the anti-raphal side. In *P. myrtifolia* the subdermal contribution is less pronounced at the lateral sides and no post-chalazal vascularization appears. In *P. vulgaris* the o.i., when viewed in transverse sections, is laterally 2 cell layers thick, whilst at the anti-raphal side it is 3 to 4 cell layers thick. The reduction of subdermal tissue occurring in these species of *Polygala* represents a phylogenetic dermalization of the outer integument. The differences in ovular ontogeny are reflected in the seed-coat structure, and the crescent-shaped small group of cells at the anti-raphal side in transverse sections of the testa of *P. vulgaris* can be interpreted as a rudiment of a middle layer. According to RODRIGUE (1893) the testa structure is of great taxonomic value in the *Polygalaceae* and the author emphasized the importance of the differences between the

various sections of *Polygala*. It may be concluded that the ovule of *P. chamaebuxus* is more primitive than that of *P. myrtifolia* which in its turn is more primitive than that of *P. vulgaris*. This is in full agreement with the views of CHODAT (1914) and MILBY (1976).

CHODAT, R. (1914): Die Geographische Gliederung der Polygala-Arten in Afrika. *Bot. Jahrb. Syst.* **50**: 111–123.

MILBY, T. H. (1976): Studies in the floral anatomy of Polygala (Polygalaceae). *Amer. J. Bot.* **63**: 1319–1326.

RODRIGUE, A. (1893): Recherches sur la structure du tégument séminal des Polygalacées. *Bull Herb. Boissier* **1**: 450–463, 517–541, 571–583.

L. GOOSEN-DE ROO *(Vakgroep Botanische morfogenese, Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden)*

Ultrastructure and cytokinesis in the cambial zone of Fraxinus excelsior L.

The active cambial zone i.e. the zone of cell divisions between bark and wood of the ash Fraxinus excelsior L. contains elongated fusiform cells with an average length of 250 µm.

Up till now, ultrastructural analysis of dividing fusiform cells was hampered by difficulties in obtaining uncollapsed, well-fixed fusiform cells and in orientated lengthwise sectioning of these cells. The technical procedure to overcome these problems has been presented elsewhere (GOOSEN-DE ROO & VAN SPRONSEN 1978). Sections of periclinally dividing fusiform cells indicate that when the cell plate between the two sister nuclei is completed, two lumps of cytoplasm (the so-called phragmoplasts) move with the growing cell plate to the tips of the cells.

A phragmoplast contains different zones. In a transverse section through the cell plate and the centre of the phragmoplast the following zones can be distinguished:

1. centrally near the cell plate a relatively empty zone with microtubules, Golgi vesicles, ribosomes and some endoplasmic reticulum;
2. a zone with more endoplasmic reticulum;
3. a zone with dictyosomes;
4. a peripheral zone containing mitochondria, plastids, lipid bodies, and vacuoles.

Considerations about the possible functions of these organelles in the cytokinesis lead to the tentative conclusion that they are involved in the synthesis of the cell plate materials, the formation of the vacuole in the sister fusiform cells as well as in keeping the cell plate in a central position.

GOOSEN-DE ROO, L. & P. C. VAN SPRONSEN (1978): Electron microscopy of the active cambial zone in Fraxinus excelsior L. *IAWA Bull.* **1978/4**: 59–64.

O. C. DE VOS *(Vakgroep Plantensystematiek, Biologisch Centrum, Postbus 14, 9750 AA Haren)*

Development of septa in the ovary of Onagraceae

In the lower region of the onagraceous ovary the upgrowth of the septa takes place from the floor of the ovary together with the ovary wall. In the upper region of the ovary the septa grow inward and fuse postgenitally in the centre of the ovary. Sometimes in the uppermost part they do not reach each other. The lower region is referred to as the synascidiate region and the upper region as the syncplicate region (LEINFELLNER 1950).

In some cases in tetramerous ovaries a small region can be distinguished, in which two septa grow upward and the lateral portions of the two other septa grow inward. SATTLER (1973) described this intermediate zone in *Fuchsia hybrida*, where one of the two septa grows to a slightly higher level than the other. The present author studied the septal growth in 10 onagraceous genera.

Ovaries with a synascidiate zone, covering from $\frac{1}{2}$ up to $\frac{3}{4}$ of their length are found in *Fuchsia magellanica* Lam., *Lopezia racemosa* Cav., *Circaea lutetiana* L., *Circaea cordata* Royle, *Gaura lindheimeri* Engelm. & Gray, *Oenothera biennis* L., *O. fruticosa* L., *O. perennis* L., *Epilobium hirsutum*

L. and *Zauschneria californica* Presl. The placenta covers both zones, except in the *Circaea* species and *Gaura lindheimeri*, where the only ovule or pair of ovules in each locule is attached to the top of the synascidiate part.

In *Circaea lutetiana* and *C. cordata* the periclinical divisions in the epidermal cells, characterizing post-genital fusions in general (BAUM 1948) are very abundant. Here the septa in the synplicate region are fused by a thick cell-layer of epidermal origin.

Although in *Boisduvalia densiflora* the synplicate zone is rather small, yet in this zone some ovules are born. In *Clarkia elegans* Dgl., *Ludwigia octovalvis* (Jacq.) Raven and *L. peruviana* (L.) Hara the synplicate zone is restricted to the top of the style. The placenta exclusively covers the synascidiate region.

S.E.M. photographs show the sequence of ovule initiation. In the synascidiate zone the sequence is basipetal and in the synplicate zone it is acropetal. Ovaries with placentae covering both zones, bear the oldest ovules in the central part and the youngest on both ends.

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LEINFELLNER, W. (1950): Der Bauplan des synkarpen Gynözeums. *Oesterr. bot. Z.* **97**: 403–436.
SATTLER, R. (1973): *Organogenesis of flowers.* University of Toronto Press, Toronto.

F. D. BOESEWINKEL *(Hugo de Vries-Laboratorium, Plantage Middenlaan 2A, 1018 DD Amsterdam)*

A comparative study of ovules and seed-coats in the genus Dichapetalum

The opinions concerning the taxonomic relationships of the Dichapetalaceae are by no means consistent. The various authors writing on this subject have suggested an alliance with one or more of the following orders: Geraniales, Euphorbiales, Thymelaeales, Celastrales, Polygalales, Linales and Rosales.

The ovule and seed-coat development in *Dichapetalum mombuttense* was studied in order to gain a better insight into the relationships of the Dichapetalaceae.

Both almost simultaneously initiated integuments are of dermal derivation. During ovule ontogenesis the inner integument becomes thicker than the outer one owing to periclinal division of all cells below the outer dermal cell layer. The full-grown ovule is bitegmic, anatropous and tenuinucellate. The small nucellus is ultimately completely resorbed. The inner integument forms the micropyle by becoming longer than the outer one. During the post-fertilisation stage of development the inner integument is resorbed from the inside ultimately to disappear completely. The outer integument is likewise resorbed from the inside, its remaining part developing large intercellular spaces before it is flattened. The mature seed-coat consists of an unflattened tanniniferous outer cell layer and a layer of squashed elements which contains remnants of vascular bundles. The endosperm is of the nuclear type and is only one cell layer thick. The embryo is large and has planoconvex cotyledons. Ovule and seed-coat exhibit a number of advanced features associated with the indehiscent nature of the fruit, which renders a comparison with the above mentioned groups rather difficult. The putative allies of the Dichapetalaceae have, as far as can be ascertained, usually crassinucellate ovules and a well-differentiated seed-coat. The results of the present study indicate that the Dichapetalaceae constitute a family with rather obscure taxonomic affinities.

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Flower morphology of Aristolochia species and the consequences for pollination

Aristolochia species show pollinator specificity (Brantjes, in prep.). Species sharing the same type of pollinator (e.g. fungus gnats, VOGEL 1978) have disjunct areas. The space (s) between the gyno-

stemium and the chamber wall is correlated with the thorax height (h) of the actual pollinating flies: *A. esperanzae* O. Kuntze, s = 1.3–6.0 mm, h = 1.3–3.2 mm; *A. cymbifera* Mart. & Zucc., s = 1.6–3.2 mm, h = 1.6–2.6 mm; *A. brasiliensis* Mart. & Zucc., s = 1.0–3.0 mm, h = 1.0–1.6 mm (Brantjes, Sazima & Sazima, in prep.). In this way non-pollinating visitors can be distinguished from the pollinators, e.g. the very small Phoridae, Cloropidae and Drosophilidae enter and leave freely the flowers of *A. esperanzae*, *A. cymbifera* and *A. brasiliensis* without pollinating. Also pollinators of *Aristolochia* species may be predicted in this way, e.g. the very large flowers (diameter about 30 cm) of *A. cordiflora* Mutis ex H.B.K., h = 1.5–2.6 mm, by small flies. Selective action of the specific scents is only partly responsible for the specificity. The putrescent odour of *A. brasiliensis*, *A. esperanzae* and *A. cymbifera* attracted a wider spectrum of flies than became imprisoned. The trapping devices produced additional selectivity in specific ways. In *A. esperanzae* the lower lip trapped all infalling flies, whereas only the right sized flies passed the narrowest part of the tube (diameter 6 mm, further restricted by hairs). In *A. cymbifera* (diameter 5.4 mm) and *A. brasiliensis* (diameter 7 mm) the tube diameter was not limiting. Here selection occurred in the lower lip. In *A. cymbifera* presumably because large flies were able to escape, and in *A. brasiliensis* only Sepsidae showed an oriented response towards the tube entrance. Here the hinged hairs seemed too short for trapping the other flies.

Size selection can occur on specific locations in the tube: In *A. grandiflora* Schwartz in a slit at the beginning of the tube (CAMMERLOHER 1923), and in *A. esperanzae*, *A. arcuata* Mast. and *A. fimbriata* Cham. in the basal section of the tube. In *A. lindneri* Berger (CAMMERLOHER 1933) and in *A. melastoma* Manso ex Duck. an opening in the septum between tube and chamber is the bottle neck. In sympatrically growing *A. melastoma* and *A. arcuata* differences in pollinators gave reproductive isolation. Lauxanidae and Phoridae pollinated *A. melastoma* (Brantjes, in prep.), Chloropidae and Millichiidae pollinated *A. arcuata*, which also captured Phoridae, without dusting these with pollen, however.

Identification and nomenclature of *Aristolochia* material were according to HOEHNE (1942).

CAMMERLOHER, H. (1923): Zur Biologie der Blüte von Aristolochia grandiflora Schwartz. *Oesterr. Bot. Z.* **72**: 180–198.

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VOGEL, S. (1978): Pilzmückenblumen als Pilzmimeten. *Flora* **167**: 329–398.

MEETING OF THE SECTION FOR PLANT PATHOLOGY ON NOVEMBER 22, 1979

L. J. TURKENSTEEN *(Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, 6709 PD Wageningen)*

Epidemiologic aspects of Phoma exigua var. foveata on potato

Gangrene caused by *Phoma exigua* var. *foveata* is considered as a mainly tuber borne disease in those countries where it presents a serious storage disease. In The Netherlands the pathogen is occasionally detected in samples sent for inspection.

In field trials to study transmission of contamination through inoculated and naturally infested tubers, offspring tubers grown for seed appeared to be contaminated less than the original seed, whereas tuber offspring grown for consumption which is harvested much later showed a higher level of contamination. Experimental evidence indicates that development of stem lesions in aging crops may be very important to soil and tuber infestation. In a field trial 30% of the offspring of disease free seed was found contaminated apparently due to spores generated on stem lesions on neighbouring plots. Similarly high contamination rates, 80–99 and 35% respectively, were found in pot and field trials with artificially induced stem lesions.

Further research on the aspects of tuber-borne and soil-borne phases of the pathogen with respect to their epidemiological importance is in progress. Our interest is specially directed to the significance of the development of stem lesions in late harvested field crops as they may be an additional factor to soil and crop infestation.

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Development of physiologic races of Bremia lactucae

New physiologic races in fungi may result from recombination of virulence genes during sexual reproduction or from spontaneous mutations in asexual spores.

Sexual reproduction in *Bremia lactucae* has long been thought to be of little importance. Since it has recently been found in the United Kingdom that *B. lactucae* is heterothallic, more attention is paid to this phenomenon. In the Netherlands both compatibility types occur. Although we are now able to produce oospores, we have not yet found new races originating from these experiments.

B. lactucae produces huge masses of sporangiospores per season. Spontaneous mutations will only become evident when they have an advantage over the existing races, e.g. the possibility to attack lettuce varieties which were hitherto resistent to all occurring races. This is regularly found.

In experiments it is shown that from a mixture of two races one may disappear. This is also found in nature. In greenhouse experiments it is possible to alter races which do attack certain varieties only to a limited extent into races which can attack these varieties for 100%. In this way the Dutch race NL 10 has developed in nature from NL 7.

J. D. JANSE *(Plantenziektenkundige Dienst, Geertjesweg 15, 6706 EA Wageningen)*

Symptoms on common ash caused by Pseudomonas savastanoi and several other organisms

A number of diseases of common ash (*Fraxinus excelsior*) produce symptoms which are in practice frequently confused with those caused by the bacterium *Pseudomonas savastanoi*. An attempt has therefore been made to differentiate these symptoms.

It has been shown that the bacterial disease is often confined to the bark for long periods, causing considerable swelling and formation of cork layers. When the swollen tissue bursts open, other parasites such as fungi gain entry. Following cambial death more canker-like symptoms are produced; however, the swellings due to bacterial infections remain evident. Symptoms can be found at practically any site along the branch or trunk and can result in total deformation of a tree.

Real ash canker as caused by *Nectria galligena*, shows rapid bark death and shriveling, instead of swelling. Infection is nearly always confined to leaf scars, buds, branch axils or stubs. Wound-callus formation and die-back are frequent, but cork formation is less intensive than with bacterial infection.

Boring for hibernation and maturation of the bark beetle *Leperisinus varius* consists of small ($\pm$ 2 cm), superficial, vertical galleries near rough places (mostly stubs) in the bark of healthy ash trees. These beetles return each year to the same place, so that large scab-like patches develop on trunks and older limbs. On young branches they sometimes penetrate as far as the cambium which is then stimulated and proliferations result which may resemble bacterial swellings. However, the presence of galleries is characteristic.

Early stages of bacterial infection can be confused with the small swellings which are the result of larval twig mining of the moth *Prays fraxinella*. In the absence of infection caused by *P. savastanoi* or fungi, bursting of the tissues surrounding these mines has not been observed.

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Host-parasite relationships of Botrytis cinerea on immature tomato fruits

Light and electron microscopical studies on the infection of young tomato fruits by *Botrytis cinerea* indicate that the cuticle is dissolved enzymatically. Once the penetration tube emerges from the cuticle into the cell wall, wall discolouration becomes evident and a considerable increase in host cell organelles below the penetration site is observed. When the hypha emerges from the cell wall into the cell lumen, the host cytoplasm is killed. Further hyphal extension occurs in the epidermis, leading to collapse, but not to penetration of underlying tissue. The lack of hyphal penetration into the subepidermal layer of the fruits supports the concept of the latency of *B. cinerea* in tomato fruits, as described by VERHOEFF (1970).

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Effects of synthetic onion oil on sclerotia of Sclerotium cepivorum and influence of inoculum position on disease

Previous experiments in Australia on control of white rot of onions caused by *Sclerotium cepivorum* have demonstrated that pre-sowing soil injections with synthetic onion oil significantly reduced sclerotial populations and disease. Evidence suggested that sclerotia germinated in response to alkyl sulphides diffusing from the oil, and subsequently died in the absence of roots of *Allium* spp. In this study the mode of action and other effects of onion oil on field sclerotia were examined. Under laboratory conditions, oil at concentrations of 5, 1 and 0.1% in water caused germination of 50–60% of sclerotia on the soil surface in comparison with 20% for the control. Production of secondary sclerotia was restricted to 0.5% of those which germinated. The effect of application of oil (0.5 ml of 5% conc.) at 10 cm on sclerotia at different depths in moist and dry soil was examined under controlled conditions. Germination reached a maximum after 4 weeks which confirmed the findings of the laboratory study. At depths of 3, 6, 14 and 17 cm the results from oil treatments were similar, and the mean percentage of germinated or empty sclerotia was 62%. At 0 cm values of 40 and 57% for dry and moist soils were recorded. Mean values for controls at all depths were 25 and 36% for moist and dry soil. Onion oil was applied to field plots at 5 and 0.1% in water and this reduced sclerotial numbers by between 50–70% in comparison with 20–35% for controls.

In an additional experiment the influence of root infections, from sclerotia, at three depths in soil, on plant growth was examined in the field. Weekly increases in height of plants were similar for all treatments up to 10 weeks after sowing. By week 13 growth from treatments with sclerotia at 0–2, 10–12 and 18–20 cm declined levels of 65, 48 and 32% of the controls. Dry weights of plant tops reflected similar differences between the treatments.

The reduction of sclerotial populations by treatment with 0.1% synthetic onion oil has potential as a control measure for white rot. However, outstanding questions such as the number of applications and the depths of the treatment in soils of varying sclerotial populations require investigation before practical application can be considered.

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Resistance to ridomil in Phytophthora megasperma f.sp. medicaginis

In order to evaluate the potential of fungi to develop resistance to the systemic fungicide ridomil, selection experiments were carried out with *Phytophthora megasperma* f.sp. *medicaginis* (Pmm), the causal organism of lucerne root rot. Two procedures were used:
A. Adaptation of mycelium by growing the fungus on V8-agar containing ridomil (0.5–? µg/ml) for 2–4 weeks and subculturing on medium with higher concentrations. Six isolates were obtained with a relatively low degree of resistance. Although all isolates were able to grow on V8-agar amended with 50 µg ridomil/ml, mycelial growth of the most resistant isolate was still inhibited for 68% at 5µg/ml.
B. Mass selection on V8-agar at 2 µg ridomil/ml from encysted zoospores either non-treated or treated with N'-nitro-N-nitrosoguanidine (NG). Thirteen isolates were obtained from 0.32×10^8 non-treated zoospores and 248 isolates from 1.14×10^8 NG-treated zoospores. Mycelial growth of the most resistant isolate from non-treated zoospores was inhibited for 69% at 5µg/ml, whereas the most sensitive isolate out of 6 NG-induced mutants tested, was only inhibited for 36% at 100 µg/ml. Growth of the most resistant NG-induced mutant was completely unaffected at that concentration.

The virulence and resistance *in vivo* of the isolates were determined in a damping-off test with 7-day old seedlings of the lucerne cultivar Vernal, growing in water-tight cups containing a sand-perlite (1:1, v/v) mix.

All isolates obtained by adaptation and mass selection from non-treated zoospores proved to be either less virulent or completely non pathogenic. Eighty one out of 176 NG-induced mutants tested were as virulent as the original isolate. When ridomil was applied as a soil drench to the seedlings (final concentration 20 mg a.i./kg mix), only one of the isolates obtained by adaptation was able to infect the seedlings, whereas none of the isolates obtained by mass selection from non-treated zoospores caused damping-off. Thirty three out of 176 NG-induced mutants tested were able to kill the seedlings despite the presence of ridomil.

The virulence and resistance *in vivo* of 7 of the NG-mutants showing ridomil resistance in the seedling test were also examined in a mature plant assay. Eight-week old plants growing in a peat-sand (1:1, v/v) mix were inoculated by applying a mycelial suspension to the pots, which were subjected to periodic waterlogging. After 4 weeks the root symptoms were evaluated. Six isolates were as virulent as the original isolate, the seventh was slightly less virulent. Ridomil, applied as a soil drench (final concentration 20 mg a.i./l soil) two days before inoculation completely failed to control the development of root rot with all seven isolates. The original isolate did not cause any symptom under these conditions.

Our results indicate that development of resistance to ridomil *in vitro* as well as *in vivo* can easily be induced in *P. megasperma* f.sp. *medicaginis*. Whether resistance will develop in populations of target fungi depends highly on factors such as type of pathogen, mutation rate, fitness of the resistant strains, selection pressure etc., but in view of our results one should be aware of the possibility.

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The role of an energy-dependent permeability barrier in uptake of fenarimol by Aspergillus nidulans

Uptake of the fungicide fenarimol [α-(2-chlorophenyl)-α-(4-chlorophenyl)-5-pyridinemethanol] by mycelium of wild-type and fenarimol-resistant strains of *Aspergillus nidulans* appeared to be the result of passive influx and active efflux. Uptake by the wild-type strain was characterized by a rapid initial influx during the first 10 min of incubation, followed by an efflux, which gradually increased until after about 60 min of incubation an equilibrium was attained. Low temperature, anaerobiosis, starvation of mycelium or incubation of mycelium with respiratiry inhibitors did

not affect influx while efflux was severly inhibited. Upon establishment of equilibrium under normal conditions an energy-dependent permeability barrier seemed to become operative since uptake could instantaneously be enhanced through addition of respiratory inhibitors such as N,'N-dicyclohexylcarbodiimide or oligomycin.

Uptake of fenarimol by fenarimol-resistant mutants was invariably low and almost constant in time. Influx as described for the wild-type strain could be obtained by low temperature, anaerobiosis, starvation of mycelium or incubation with respiratory inhibitors. Low uptake by these mutants can be ascribed to an energy-dependent permeability barrier to fenarimol with a higher activity than in the wild-type strain. Upon inhibition of the barrier activity, net uptake was the result of remaining passive influx only.

The results suggest that the resistant mutants are altered in their energy-dependent modulation of permeability to fenarimol; this might explain the mechanism of resistance of *A. nidulans* to fenarimol and other related fungicides.

For further information the reader is referred to:

WAARD, M. A. DE & J. G. M. VAN NISTELROOY (1979): Mechanism of resistance to fenarimol in *Aspergillus nidulans*. *Pestic. Biochem. Physiol.* **10**: 219.

— & — (1980): An energy-dependent efflux mechanism for fenarimol by a wild-type strain and fenarimol-resistant mutants of *Aspergillus nidulans*. *Pestic. Biochem. Physiol.*: in press.

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Susceptibility to virus infection: a genetic-physiological problem.

Whether virus infection results in systemic symptoms or in a localized reaction depends on the genetic constitution of both the host plant and the virus. Whereas the gene-for-gene relationship describing plant-fungus interactions is considered to involve recessive genes for virulence in the fungus and dominant genes for resistance in the host, virulence or pathogenicity of the single-stranded RNA viruses must be a positive function and resistance of the host may be either dominant or recessive, as exemplified by the interaction of bean common mosaic virus and bean. Pathogenicity of the virus can be lost by mutation, resulting in inability to spread throughout the entire plant and leading to a resistance reaction in a host plant susceptible to wild-type virus, as has been described for alfalfa mosaic virus on bean, cowpea mosaic virus on cowpea and bean, and tobacco mosaic virus on tobacco.

Viruses thus need an active factor to circumvent localization by the plant, and the existence of different strain groups that infect only host cultivars with a particular genetic constitution can be explained by assuming that only in those combinations specific recognition between a viral product and a host function takes place. In all other combinations, specific recognition would not be achieved and an aspecific localizing mechanism would be activated. Such an aspecific, yet efficient mechanism is the hypersensitive reaction, in which the virus is localized concomitant with the formation of necrotic local lesions. Independent of the infecting virus, this type of localization is basically similar and always leads to identical physological and biochemical alterations: a burst of ethylene synthesis, stimulation of the phenylpropanoid pathway, increased peroxidase activity, induction of pathogenesis-related proteins, and acquired resistance to further virus infection.

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ELISA studies on acquisition of pea enation mosaic virus by the pea aphid Acyrthosiphon pisum

To transmit circulative viruses, aphids have to acquire them in rather long feeding periods. The amounts of virus acquired and the rates of acquisition have not been measured because the techniques available were not sufficiently sensitive. The recently developed technique of enzyme-linked immunosorbent asssay (ELISA) may have the required sensitivity.

Aphids of known age were allowed to acquire virus on pea plants infected with pea enation mosaic virus. The rate of acquisition differed considerably between aphids. This variability might be due to differences in the virus content of the phloem cell penetrated. The amount of virus acquired in an access feeding period of 8 h varied less in aphids disturbed whilst feeding than in those undisturbed. The latter aphids may feed on one and the same cell during this feeding period.

After 16 h of feeding the amount of virus acquired by a group of aphids reached its maximum value. Further access did not increase the virus content which consists mainly of virus accumulated in the intestinal tract. In this situation virus acquisition and excretion are in equilibrium. Infective aphids feeding on healthy plants lose virus less rapidly than it had been acquired. This loss of virus is mainly due to excretion of virus occurring in the intestinal tract. It can be concluded from earlier studies on the transmission of PEMV that the quantities of virus acquired are magnitudes higher than those required to make aphids infectious.

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Incorporation of [^{14}C]-phenylalanine and [-^{14}C]-mevalonate in soybean phytoalexins

Soybean cotyledons respond to cell-wall preparations of *Phytophthora megasperma* var. *sojae* (PMS-elicitor) by producing the phytoalexin glyceollin (cf. ALBERSHEIM & VALENT 1978; ZÄHRINGER et al. 1978, 1979), of which four isomers (glyceollin I-IV) are known today (LYNE et al. 1976; LYNE & MULHEIRN 1978). In addition, a non-fungitoxic pterocarpan, viz. 3,6a,9-trihydroxypterocarpan, accumulates as well (LYNE & MULHEIRN 1978).

In order to compare the abilities of legume pathogens and non-pathogens to degrade pterocarpanoid phytoalexins (cf. FUCHS & HIJWEGEN 1979), radioactively labelled pterocarpans were needed, preferably ^{14}C-labelled at various positions in the molecule. In addition, for glyceollin and its alleged precursor trihydroxypterocarpan, a method was sought, by which large quantities could be readily obtained and purified. To this end, ten large petri dishes, each containing 40 superficially wounded cotyledons from 14-day-old soybean seedlings cv. Harosoy 63, treated with PMS-elicitor, were incubated at room temperature. After 3, 21, 27, 31 and 43 h, respectively, small droplets of either [^{14}C]-phenylalanine (sp. act. 486 mCi/mmole) or [-^{14}C]-mevalonate (sp. act. 8.05 mCi/mmole were applied onto the wounded surface of the cotyledons. [-^{14}C]-Phenylalanine was expected to become incorporated into the B and D-rings of both pterocarpans. [-^{14}C]-Mevalonate, on the other hand, was assumed to be incorporated via isopentenylpyrophosphate (cf. ZÄHRINGER et al. 1979) into the dimethylchromene ring, which is a part of the glyceollin, but not of the trihydroxypterocarpan molecule.

Forty eight hours after application of the PMS-elicitor the experiment was terminated by extracting each batch of cotyledons with 50 ml of ethyl acetate. The extraction was repeated three times; after combining the extracts of each batch of 40 cotyledons, they were dried down, and the residues taken up in ethanol. After separation and purification on TLC-plates (with toluene/chloroform/acetone 45:25:35 and n-hexane/ethyl acetate/methanol 40:60:5 as solvents) and on a Sephadex LH-20 column (with methanol/water 2:1 as the solvent), specific activities of the two pterocarpans were determined and compared with those of the precursor molecules. With [^{14}C]-phenylalanine as a precursor the specific activity of both glyceollin and trihydroxypterocarpan slowly decreased, from c. 7.10^{-4} mCi/mmole upon application of the radiochemical 3 h after elicitation, to c. 2.5. 10^{-4}mCi/mmole upon application after 43 h. Contrary to expectation [2-^{14}C]-mevalonate was incorporated in both pterocarpans with almost identical specific activity, which decreased from 2.5 to 1.10^{-4}mCi/mmole proportionally with the incorporation time. Apparently, instead of being incorporated as such, mevalonate was metabolized to acetate, which in its turn, via the acetate-malonate pathway, became part of the A-ring in both the pterocarpan molecules.

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Influence of the addition of fermented house hold waste material on the pathogenesis caused by Rhizoctonia solani and Pythium ultimum

Addition of organic material to the soil seems to be very promising with regard to the biological control of soil borne diseases. In this respect especially the N-content of the substrate and its C/N-ratio are extremely important.

The use of fermented house hold waste material could moreover give an answer to the problem of the shortness of organic material in horticulture. However the salt content and the contamination with heavy metals of this substrate could influence plant growth negatively.

In our experiments the influence of the addition of fermented house hold waste material to the soil is evaluated based on the development of *Rhizoctonia solani* (on *Phaseolus vulgaris*) and *Pythium ultimum* (on *Pisum sativum*).

The use of increasing quantities of fermented material results in an increase of the health index of *Phaseolus vulgaris* in substrates artificially infested with *Rhizoctonia solani*; on the contrary the health index of *Pisum sativum* with regard to *Pythium ultimum* decreases with increasing percentages of house hold waste material.

In this respect we may observe a direct influence due to the fungitoxicity of the heavy metals such as Cu^{2+}, Pb^{2+} and Zn^{2+} and an indirect one caused by the stimulation of the microbiological activity as a result of the addition of organic matter. Indeed, the number of bacteria and actinomycetes is doubled after addition of 5 to 10% house hold material, however, higher quantities reduce the biological activity.

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Ecological aspects of root nodule formation in Alnus glutinosa by Frankia spec. and Penicillium nigricans

Members of the genus *Frankia* (Actinomycetales) are involved in the root nodule symbiosis which gives rise to nitrogen fixation in *Alnus glutinosa*. The abundance of *Frankia* in the soil can be quantified to some extent by determination of the number of nodules which develop on test plants of the host species grown on soil samples. The value of this method was sometimes seriously affected by simultaneous development of root nodules caused by the fungus *Penicillium nigricans*. These "myconodules" had the same appearance as young *Frankia* nodules although part of the myconodules tends to turn brown. Preliminary microscopical investigations revealed infection of deformed root hairs followed by local intracellular invasion of the cortex region. Vascular strands penetrate into the infected region, but contrary to nodule development in *Frankia* symbiosis, further development of rhizotamnia was not observed. The results of mixed inoculation of *A. glutinosa* seedlings with *P.*

nigricans and *Frankia* spec. indicate that both organisms compete at least to a large extent for the same sites in the root system. Occasional differences in the maximum numbers of nodules produced by each of the organisms on separate host plants could be attributed to a divergent development of the number of nodules which occurs from the moment that growth differences between both nodule types are becoming obvious.

The formation of both types of nodules is approximately equally sensitive to nitrate which was supplied as the sole source of nitrogen to the nutrient solution.

Attempts to suppress myconodulation selectively by addition of pymaricine to the nutrient solution of inoculated alder seedlings or by pretreatment of inoculi with the antibiotic were unsuccessful. Both myconodulation and *Frankia* nodulation proved to be equally sensitive to pymaricine.

The widespread distribution of *P. nigricans* in the soil and the competition for infection sites with *Frankia* spec. under experimental conditions suggest that the fungus may also play a part in the establishment of *Frankia* nodulation in the field.

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The influence of the soil fertility on the occurrence and significance of endomycorrhizas in some agricultural crops

Investigation on VA-mycorrhizal infection in wheat roots in two series of experimental fields showed a significant effect of N- and P-fertilizers on the level of root infection. In the first series the amount of N-fertilizer ranged from 0–175 kg N/ha. All the plots were given 108 kg P_2O_5/ha. The root infection ranged from 55 to 17% (line intersection method). At the high infection levels the infected roots were bright yellow coloured. Many arbuscules occurred at the lower N-levels. On the high N-levels, however, few arbuscules were visible.

In the second series the effect of the P-level in the soil on the VAM infection was less pronounced. In the P-availability range of 7, 22, 30, 68, 92 mg P_2O_5/litre soil the infection rate varied from 57, 39, 38, 38, 42 per cent. The plots were given 77.5 kg N/ha.

VA-mycorrhizal infection in potato roots did not differ significantly with the amount of N-fertilizer applied.

A pot experiment with 5 weeks old maize plants infected with *Glomus mosseae* showed, that the level of infection is not necessarily correlated with its efficiency. Compared with uninoculated plants no growth stimulation was measured at 19% root infection (54 mg N/plant, 1.4 mg P_2O_5/plant) and at 59% root infection (150 mg N/plant, 49.4 mg P_2O_5/plant). But at 45% infection (78 mg N/plant, 13.4 mg P_2O_5/plant) the inoculated plants weighed about 150% more than the uninoculated plants.

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Effect of inoculum composition on infection of bean leaves by conidia of Botrytis cinerea

Inoculation of primary leaves of bean (*Phaseolus vulgaris*, cv. Dubbele Witte z. dr.) with small drops of a suspension of conidia of *Botrytis cinerea* gives rise to either spreading lesions, lesions remaining restricted in size or no visible necrosis. The type of reaction appeared to be dependent on the composition of the inoculum.

Conidia suspended in distilled water did not cause any visible necrosis, whereas a suspension of conidia in 0.1 M citric acid-phosphate buffer + 2% glucose always caused spreading lesions. The varying reactions, observed with conidal suspensions in phosphate buffer, were dependent on pH and molarity of the buffer, presence of glucose in the buffer, and concentration of conidia in the inoculum. The reactions were not influenced by the proportion of K^+ or Na^+ ions in the buffer.

From these observations, a model system is adopted to study the mechanisms underlying the

development of the resistant and susceptible reactions. In this system, inocula are used consisting of 2 × 10^6 conidia of *B. cinerea*/ml 10 or 67 mM phosphate buffer (pH 5.0) + 2% glucose, which give rise to restricted or spreading lesions, respectively.

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Biological implications of the stereochemistry of isoflavonoid phytoalexins

An account of this study is published in this issue, p. 193–197.