

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

SYMPOSIUM: FLOWER BIOLOGY, ON MARCH 17, 1978

G. VAN DER VELDE, TH. C. M. BROCK, M. HEINE AND P. M. P. M. PEETERS
(Laboratory of Aquatic Ecology, Nijmegen)

Flowers of Dutch Nymphaeaceae as a habitat for insects.

To study the relations between flowers of Nymphaeaceae and insects investigations have been carried out in two Dutch waters viz. the "Oude Waal" near Nijmegen, where *Nymphaea alba* L. occurs, and the "Haarsteegse Wiel" near Vlijmen where *Nymphaea candida* Presl occurs, while *Nuphar lutea* (L.) Sm. is present in both waters.

The flowers of these Nymphaeaceae are attractive to certain insect species in their search for food, which is provided directly by the soft parts of the flower, stigmatic exudate, pollen and nectar or indirectly as prey in the form of other animals present in the flower. By this behaviour of the insects pollination is favoured. The flowers of *Nymphaea candida* are not really suitable for insect pollination as they are often half-submerged. Consequently very few seeds per fruit have been found in comparison with *Nymphaea alba*.

Species	Number of seeds per fruit			number of fruits examined
	minimum	maximum	average	
<i>Nuphar lutea</i>	127	261	195	30
<i>Nymphaea alba</i>	224	1402	872	17
<i>Nymphaea candida</i>	1	110	30	8

The fauna in and on flowers was caught with a net and by collecting flowers and washing them out in alcohol 70%. In this way respectively 24, 14 and 44 species of insects, and 4, 3 and 4 species of spiders have been caught from the flowers of *Nymphaea alba*, *Nymphaea candida* and *Nuphar lutea*. A flight-pattern from flower to flower was observed for the bees *Apis mellifica* L. and *Bombus terrestris* L. (both not for *Nymphaea candida*) and some of the most common syrphids as *Anasimyia lunulata* Mg., *Eristalinus sepulchralis* L., *Eristalis arbustorum* L., *Eurimyia lineata* F., *Syrphus balteatus* DeG., *Syrphus corollae* F. and *Syrphus vitripennis* Mg. The following species were abundant or found very frequently in flowers and are very characteristic for nymphaeids: the flies *Notiphila brunnipes* R.D. (in all 3 species) and *Hydromyza livens* Fall. (only in *Nuphar*), the beetle *Donacia crassipes* F. (frequent in *Nuphar*, infrequent in *Nymphaea alba*, absent in *Nymphaea candida*) and the aphid *Rhopalosiphum nymphaeae* (L.) (in all species). *Rhopalosiphum nymphaeae* plays no role as pollinator. It can be very numerous in the flowers, but when the spider *Pirata piraticus* (Cl.) was present no or few *Rhopalosiphum* were found. *Donacia crassipes* is known as pollinator. Special attention has been paid to the autecology and possible role as pollinators of the flies *Hydromyza livens* and *Notiphila brunnipes*. The fly *H. livens* is characteristic for *Nuphar lutea*; the larva mines in the floating leaves and petioles of this species. Often these flies can be found in the flowers of *Nuphar* where they prey on soft-skinned insects or on *Notiphila*-eggs. As the flies are very numerous it is supposed that they can play an important role as pollinator of this plant species. *Notiphila brunnipes* must be very important as a pollinator of the Nymphaeaceae mentioned and has a preference for laying its eggs in rows on the bases of petals. It can be found in numbers in the flowers, feeding on stigmatic exudate, pollen and nectar (only *Nuphar lutea* possesses nectaria), depositing eggs, seeking shelter, copulating and sunning. A population study carried out in the "Oude Waal" has proved that it is most numerous on the floating leaves when flowering has reached its maximum. The higher temperatures measured in the flowers on sunny days due to solar radiation optimised the conditions for the hatching of the larvae, as was shown in some

laboratory experiments. The eggs can respire in aerated water via a plastron. Although adults, eggs and recently hatched larvae are found mostly on the Nymphaeaceae, older larvae and pupae occur only on the roots of *Nymphoides peltata* (Gmel.) O. Kuntze, *Acorus calamus* L. and *Typha angustifolia* L.

A. D. J. MEEUSE (*Hugo de Vries-Laboratorium, Amsterdam*)

The advent of flower-insect mutualisms and the role of nectarial secretion

The role of nectarial exudates as attractants in the flowers of recent zoophilous Angiosperms is an obvious adaptation, but this element of the "standard" zoophilous syndrome does not reveal the origin of nectariferous sites during the evolution of the flower, and the how? and why? of it.

Secretion of sacchariferous fluids occurs in intrafloral sites (here referred to as "floral nectaries" even if the secretion is diffuse) but also in extrafloral (or extranuptial) nectaries. About the function of the latter the last word has not been spoken yet, but any anthecological function can certainly be excluded. It is, primarily, rather some ecophysiological requirement that necessitates the excretion. Intrafloral nectaries are fairly generally supposed to have an anthecological significance associated with the attraction of animal visitors which may act as (potential) pollen-vectors. I do not endorse this view unquestioningly and accept the possibility of an alternative (ecophysiological) function in at least some cases (see below).

One is wont to consider pollen to be the primary source of food for anthophilous insects, but this is most probably an unwarranted starting point for phylogenetic speculations concerning early flower-insect mutualisms. It is known that a so-called pollination droplet is exuded from the ovular micropyles of many gymnosperms. The primary function of the droplet is clearly the catching of pollen grains (and possibly the transportation, by capillary suction, of the pollen grains to the pollen chamber when the droplet starts drying up), because there was no relation with insects. It has been known for quite some time, however, that the pollination droplets of the Gnetatae (*Chlamydospermae*) contain appreciable amounts of mono- and/or oligosaccharides, which results in insect visits (and at least in some cases to an effective pollen transfer by some of the visiting insects). The conditions in these highly advanced gymnosperms are in so far rather singular that they – in contrast to practically all other recent and fossil gymnosperms – exhibit a manifest tendency towards monoclony. Male specimens bear some to many, albeit usually infertile, ovules provided with a pollination droplet (and the female ones sometimes also male reproductive structures). This means that insects eager for liquid food (with sucking or licking mouth parts) can also be served by male plants and may become contaminated with pollen; a subsequent visit to a female plant may thus result in an effective entomogamy. It follows that already before full-fledged Flowering Plants existed a mutualism between certain advanced gymnosperms and insects originated. This relation did not lead to an effective beetle pollination or cantharophily but rather to a mutualism with Diptera or Hymenoptera.

As a consequence of the advent of angiospermy the primary source of liquid, sugar-containing food (the pollination droplets) dried up almost in a literary sense owing to the "covering up" of the ovules by the gynoeceal wall. This suggests that the situation was so thoroughly changed that the relations between flowers and insects originated anew, but this is only relatively true because the novel situation did not arise from one day to the next. There are at least four devices which led to a new mutualistic relation between angiospermous blossoms and insects. In the *first place* some incipiently monoclinal, early angiospermous groups could have become secondarily anemogamous again (but I do not think that this was important). *Secondly*, insects consuming both pollen and nectar (Diptera at once coming to mind) could adapt themselves to already more or less clearly monoclinal, nectariferous forms. Especially among more primitive groups of dilleniid dicots, but also among the Magnoliales, there are taxa with "pollen flowers" never producing any floral nectar. In the *third place* other attractants (scents or unpleasant odours, semaphylls) could induce insects to land on flowers; in an evolutionary sense this could easily lead to forms of mimetic cheating when flowers started to emanate the smell of carrion, dead fish, feces, decaying vegetable matter, fermenting fruit, etc., thus baiting not primarily anthophilous groups of insects; in such syndromes nectaries only enter the scene on rare occasions (and this presumably represents a secondary event). *Finally*, nectaries could have originated *de novo*, and this is a crucial point. What came first: nectarial secretion in the flower or a relation with insects? In view of the feeding habits of certain groups of anthophilous insects (and I have particularly the Hymenoptera with

sucking mouthparts in mind), regular visiting by such animals could only come about if nectar was already available and not, in a Lamarckistic way of reasoning, because floral nectaries originated on account of selective pressure by visiting insects; if there was a mutualism at all it involved animals interested in pollen and not in nectar, so that they could not select for nectarial organs. According to most anthecologists, in this early phase of the angiosperm-insect mutualism beetles were the most important visitors. This, I believe, is questionable also for other reasons that will not be discussed here in detail. Even if some early cantharophily originated, this could never have stimulated nectar secretion in the flower for the simple reason that at least the older groups of anthophilous Coleoptera were pollen-consumers. There must, therefore, have been some other causal explanation of the advent of nectarial secretion in the floral region. Nectaries outside the reproductive zone are even known from ferns, and among the angiosperms extrafloral (or extranuptial) nectaries are widespread. Whatever function extrafloral nectaries may have, for the pollination biology they have no real significance: although insects such as ants may exploit the exudate of such nectaries this does not result in a consistent visiting of the flowers, let alone in an effective pollination. I, accordingly, believe that the primal cause of nectar secretion within the floral region was the same as in the case of extranuptial nectaries – let us assume, an ecophysiological requirement possibly associated with osmotic balances and turgor tensions in tissues. It has sometimes been suggested that the opening of the pollen sacs and the extrusion of the desiccating pollen masses are initiated, or at least facilitated, by the activities of nectaries in more or less close proximity. Assuming that there is some truth in it, one may expect the incidence of nectaries in anemophilous and diclinous taxa (at least in their staminate flowers). This proves to be of rather frequent occurrence: a nectarial or diffuse excretion of a sugar-containing liquid has been observed in the flowers of, e.g., various palms, grasses, and Urticales. The hitherto most current interpretation of this phenomenon is that the monoecious or dioecious anemophiles concerned are descendants of entomogamous and monoclinal precursors and, after the change of their anthecological syndrome, still retained some atavistic characteristics such as the presence of nectaries and visits by insects. This last observation is of course irrelevant because insects exploit all sorts of sources of food (such as extrafloral nectaries) and does not provide a cogent argument; it does, moreover, not solve our problem (did the floral nectaries originate first and did the insects come later, or the other way around?). It seems plausible to accept an ecophysiological necessity as the cause of the origin of floral nectaries (as must have happened secondarily in groups with, originally, a “pollen flower” strategy). The insects, then, came of themselves, as it were, but could, generally speaking, only become effective and regular pollen vectors in (incipiently) monoclinal taxa.

In this way the significance of floral nectaries in connection with the origin of the insect-flower mutualism becomes manifest. The first question to be answered concerns the ecophysiological function of the excretion within the floral region. It seems as if studies to be undertaken to resolve the problem must be focussed on anemophilous and diclinous taxa where the production of nectar undoubtedly came first and insect visits later. Plant physiologists working on problems of sugar secretion would render students of floral biology a good turn by also paying a sufficient deal of attention to the anthecological aspect.

J. J. PETTINGA (*Proefbijenstand* “*Ambrosiushoeve*”, *Hilvarenbeek*)

Some examples of the use of pollinators in cultivated crops

Pollination is the most critical stage in fruit crops. Due to the self-incompatibility of most fruit varieties, the pollen must not only be transferred from one flower to another or from one tree to the neighbouring tree but from one special variety to another variety. Most fruit varieties do not set fruit with their own pollen owing to a number of mostly genetical factors. In some cases the pollen grains or egg cells are sterile because of irregularities in their karyotype (e.g. in triploids). In some fruit crops such as sweet cherries and apple cross-incompatibility is common as well. On the other hand, parthenocarpy may be present, as e.g. in the pear variety *Conference*, especially after damaging by spring frosts. For successful fruit crops the transport of pollen is therefore very important. Most fruit trees, except nut producing species, are entomophilous. The greater part in pollen transport is played by honey bees; in former days bumble bees and solitary bees were also used as effective pollinators. The foraging honeybee is strictly limited to one kind of flowers: this is favourable for pollen transport. On the other

hand, the foraging area of one bee is very restricted in case of full bloom. This limited area is unfavourable for cross pollination, which in most situations (except for the self-compatible varieties) is a condition for good fruit setting and a good crop. Therefore the distribution of pollinator-trees in an orchard is very important.

For the cultivation of pickling cucumbers in glass-houses there can also be a pollination problem: most growers use varieties with only female flowers. For obtaining fruits pollination is necessary. The pollen can be provided by bisexual plants; one pollinator produces sufficient pollen for the fertilisation of nine female plants. However, this amount of pollen is not sufficient for the production of brood (larvae) of the pollinators, i.e. of the honeybees living in hives inside the glass houses. Therefore the hives have to be changed every three weeks for "fresh", well-equipped ones. Pesticides can be applied under special conditions inside the glass houses without risks for the bee population.

J. T. WIEBES (*Afdeling systematische dierkunde en evolutiebiologie, Leiden*)

Figs and fig wasps

Figs (*Ficus* spp.) and their pollinating wasps (Agaonidae), as well as several other groups of wasps parasitic on this obligate symbiosis, are currently being classified: comparison of their classification leads to a reconstruction of the co-evolution of figs and fig wasps.

An important feature of the symbiosis is the way in which pollinating wasps carry pollen from the mature to the young syconia. In recent publications by Galil and co-workers, three modes of pollen transport are being reported viz., in pockets on the ventral surface of the thorax, in so-called corbiculae in the coxae of the fore legs, and in intersegmental and pleural invaginations that form in the shrunken body of the wasp following loss of water. Only in the last-mentioned process are the pollen collection and deposition passive acts, dependent on the physical conditions of the old and young fig-receptacle, respectively ("topocentric pollination"). When other modes of transport are employed, the pollination process has been called by Galil: "ethodynamic pollination".

The study of the various pollination-syndromes may lead to suggestions concerning the evolutionary pathways, which should be tested against other transformation series obtained in the phylogenetic analysis of fig and wasp groups. It appears that neither the usual classification of the Agaonidae, nor a classification based exclusively on the absence or presence of pollen-pockets and corbiculae, runs parallel to the recent classification of *Ficus* by Corner. I will try to integrate all information on as many character-series as can possibly be included. My aim is to arrive at a more evenly balanced classification of the symbiosis than now seems possible to construct for the separate groups of symbionts.

JOINT MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY WITH BELGIAN BOTANISTS IN GENT, ON MARCH 22, 1978.

J. KOSTER (*Rijksherbarium, Leiden*)

Leaf anatomy of the Malesian Myristicaceae – a light- and scanning electron microscopical study

In Malesia the Myristicaceae are represented by four genera: *Gymnacranthera*, *Horsfieldia*, *Knema* and *Myristica*. Leaf anatomical characters of 56 species have been investigated. General features are: lamina dorsiventral; oil cells present in the mesophyll; stomata confined to abaxial epidermis, paracytic, with the guard cells embedded in the subsidiary cells; hairs branched, uniseriate; and midrib vasculature with the phloem in small, separate groups, which are also present in the medullary region, and here sometimes accompanied by xylem, and with sclerenchyma strands surrounding the vascular system, connected with phloem groups.

The most striking characters varying on the genus level can be summarized as follows. 1. Hairs. These are composed of cells which are supplied with arms except the basal cells. In *Gymnacranthera* the cells have usually two arms differing in length. In *Myristica* the cells have two arms of equal length. In *Horsfieldia* and *Knema* the cells have one arm only. In species of *Knema* the hairs vary in size, in

thickness of the cell walls and in length and width of the different parts of the constituting cells.

2. Structure of the abaxial cuticle. After washing in ether, chloroform, alcohol or boiling water a layer consisting of lamellae or knobs is often visible on the cuticle, usually covering the whole epidermis including the stomatal apertures except the cells over the major veins. Similar structures, referred to as alveolar material, probably of cutin-like nature have been reported before by BAILEY & NAST (1944) in Winteraceae and were thoroughly studied by BONGERS (1973). In Myristicaceae alveolar material is present in *Gymnacranthera*, *Knema* and, varying in appearance, in most of the species studied of *Myristica*. It is absent from *Horsfieldia*.

3. Stomata. These are slightly sunken in *Gymnacranthera*, distinctly sunken in *Knema* and *Myristica* and in level with the other epidermal cells in *Horsfieldia*. In *Knema* the neighbouring cells have more or less horizontally directed, nipple-shaped papillae overarching the stomatal complex and leaving a star-shaped opening. In most species of *Myristica* the neighbouring cells have more or less upright, blunt papillae forming a ring above the stomatal complex of variable height.

4. The Vascular system of the midrib. In *Gymnacranthera* the midrib is supplied with an arc-shaped collateral bundle on the abaxial side. In *Horsfieldia*, *Knema* and *Myristica* there is, moreover, a flat adaxial bundle.

On the basis of these leaf anatomical characters the genera can easily be separated. Considering the reticulate distribution pattern of the differential characters, it is, however, difficult to suggest a grouping of genera reflecting affinity. On the species level the problems are similar: some species are recognizable on leaf anatomical features only, but relationship patterns cannot be clearly indicated. A detailed, illustrated account of the leaf anatomy of the Malesian Myristicaceae will be published elsewhere (KOSTER & BAAS, in preparation).

BAILY, I. W. & C. G. NAST (1944): The comparative morphology of the Winteraceae V. Foliar epidermis and sclerenchyma. *J. Arnold Arbor.* 25: 342-348.

BONGERS, J. M. (1973): Epidermal leaf characters of the Winteraceae. *Blumea* 21: 381-411.

KOSTER, J. & P. BAAS (in preparation): Leaf Anatomy of the Malesian Myristicaceae. *Blumea*.

P. GOETGHEBEUR* (*Laboratorium voor Morfologie, Systematiek en Oekologie der Planten, Ledeganckstraat 35, Gent, Belgium*)

Inflorescence morphology in Cyperaceae

During this study, evidence has accumulated that the so-called spikelets and flowers of *Cyperaceae* are not homologous throughout the whole family (GOETGHEBEUR 1978, in press). The primitive inflorescence unit very probably is represented by an axis with a terminal pistil and lateral monandrous flowers, each in the axil of a glume. reduction yields the typical trimerous bisexual cyperoid "flower" or anthoid (MEEUSE 1975).

The lateral morphological inflorescence units in *Volkiella* are composed of a bract, a prophyll and perpendicular on these scales a few distichous bracts; each of these hold a highly reduced 1-flowered spikelet, with a 2-keeled prophyll, a glume and a bisexual "flower".

The two genera *Actinoschoenus* and *Arthrostylis*, sometimes considered as synonyms, differ from each other by embryo characters and inflorescence structure; both genera bear "heads" of spikelets but the *Arthrostylis* inflorescence is a very complex one, showing prophyll branching, completely differing from the simple *Actinoschoenus* inflorescence.

The inflorescence of African *Lagenocarpeae* are examples of the tandem branching system: several superposed branches in the axil of a single bract, with (*Microdracoides*, *Coleochloa setifera*) or without prophylls (*Afrotrilepis pilosa*). Their spikelet prophyll, show a remarkable degree of variation. *Microdracoides* with an ordinary 2-keeled prophyll, *C. setifera* with a prophyll of 2 lateral scales, *A. pilosa* with an asymmetrically developed 1-keeled prophyll and finally *A. jaegeri* with a whole range of variously shaped prophylls (1-2-3 scales!).

Embryological (Van der Linden 1971, unpubl.) and anatomical observations (KOYAMA 1976) have made the supposed close relationship between *Scleria* and *Diplacrum* very improbable. Inflorescence morphology yields further evidence: a bisexual *Scleria* spikelet has a lateral female "flower" in the axil of a lower glume, followed by few to several 3-staminate male "flowers", each in the axil of a glume. In *Diplacrum* not even a single trace of rudimentary glumes or rachilla could be found at the fruit base

(other genera with pseudoterminal female flowers do possess remnants in almost every spikelet, e. g. *Oreobolus*, *Lagenocarpus*, *Scleria* female spikelets, ...); the *Diplacrum* inflorescence thus very probably shows a terminal female flower, surrounded by a few male spikelets, composed of a few monandrous flowers, each in the axil of a glume.

KOYAMA, T. (1967): The systematic significance of leaf structure in the tribe Sclerieae (Cyperaceae). *Mem. N. Y. Bot. Garden* 16: 46-70.

MEUSE, A. D. J. (1975): Interpretative floral morphology of the Cyperaceae on the basis of the antheroid concept. *Acta Bot. Neerl.* 24: 291-304.

(*) Aspirant of the Belgian "Nationaal Fonds voor Wetenschappelijk Onderzoek".

J. P. VERBELEN, E. SPRUYT, E. MOEREELS and J. A. DE GREEF

(*Departement Biologie, Universitaire Instelling Antwerpen, Universiteitsplein 1, 2610, Wilrijk, Belgium*)

Morphological similarities and differences in bean leaves

During former experiments the role of light in the development of primary leaves of *Phaseolus vulgaris* L. cv. Limburg was determined qualitatively and quantitatively. Phytochrome plays the major role in the photomorphogenic control of leaf development, during cell division as well as during cell expansion activity.

Thus it should be possible to change the adult leaf architecture just by changing the light regime during leaf growth. For that purpose bean seeds were sown in moist vermiculite and kept in darkness during three or nine days. Afterwards both series of plants were illuminated continuously during nine days at an intensity of 12.000 Lux. At that moment primary leaves were sampled in order to measure different morphological and physiological parameters.

Leaves of both batches were similar on the macromorphological level, they had the same surface area and the same fresh weight. On tissue level however a pronounced difference existed between both series.

The younger leaves had more but smaller cells as well in the palisade parenchyma as in both epidermal cell layers. Notwithstanding the fact that the difference in composition of the latter tissues existed both in guard cell number and in normal epidermal cell number, the stomatal index was not changed by the light treatment.

Concomitant with these morphological differences, the diffusion resistance of the younger leaves was much lower than that of the older series, as well in the upper- as in the lower epidermis. Asetiolation does exist in the natural environment, and quality and quantity of the sunlight are not constant, the analogy of morphologically similar leaves is questionable even in *natural conditions*.

F. D. BOESEWINKEL

(*Hugo de Vries-Laboratorium, Amsterdam*)

Ovule initiation and seed-coat development in some representatives of the Rutaceae

The ovule primordia of the Rutaceae are trizonate. The ovules are anatropous, bitegmic and crassinucellate. Only within the genus *Glycosmis* of the Aurantoideae unitegmy occurs. The inner integument is always of dermal derivation. The outer integument in some genera is exclusively of dermal origin, but in other genera partly of subdermal derivation. The mature seed-coats are well-differentiated, vary appreciably in structure, and contain in general remains of outer integument, inner integument and nucellus. The Aurantoideae constitute the only subfamily of the Rutaceae in which the genera show a clear uniformity in the structure of their seed-coat. The mechanical layer is always a derivative of the outer epidermis of the outer integument and contains crystals in the cells of its layers. In the Aurantoideae *Glycosmis* is the only aberrant genus and characterized by the development of a pronounced pachychalaza in the post-fertilization development of the ovule. As regards its ovule and seed characters *Glycosmis* can be interpreted as a more advanced genus within the Rutaceae-Aurantoideae.

R. VIANE and W. VAN COTTHEM (*Laboratorium voor Morfologie, Systematiek en Oekologie der Planten, Ledeganckstraat 35, Gent, Belgium*)

Spore morphology and stomatal types in the fern genera *Asplenium*, *Ceterach* and *Phyllitis* (Aspleniaceae)

Perispore characters are becoming more widely used in taxonomic studies to delimit species and genera, and to trace evolutionary relationships. In the Aspleniaceae the study of both perispore and stomatal types leads to useful information.

So far we have examined more than 60 taxa, showing a (large) morphological continuum between the extreme types of outer perispore sculpturing and a gradual changing stomatal type from anomocytic to polycytic.

In the study of the perispore anatomy, we have found that in the Aspleniaceae it consists of three elements:

- a. an inner (thin) perispore or foot layer, closely surrounding the smooth exospore,
- b. the middle perispore, i.e. columellae arising from the inner perispore and supporting (c),
- c. the outer perispore or tectum which is responsible for the over-all external morphology of the spore.

In *Asplenium* s.l. we are able to discern several perispore "types": costate, echinate, reticulate among others, bearing in mind the morphological continuum between them.

A certain perispore type (or even an intermediate type) often corresponds with a particular stomatal type: the "costate" group of species around *Asplenium aethiopicum* (Burm.f.) Becherer has anomocytic stomata, the "echinate" taxa possess polycytic stomata. Species with a resembling perispore and resembling stomata are often closely related (*A. aethiopicum* complex, *A. adiantum-nigrum* L. complex).

An interesting feature is found in species with a reticulate perispore, showing a remarkable divergence in geographical distribution: *Asplenium friesiorum* C.Chr. in central Africa and Madagascar, *Asplenium lucidum* Forst. from New Zealand and *Asplenium harpeodes* Kunze from Mexico to Bolivia and Brazil.

Considering the perispore features in the genera *Ceterach* and *Phyllitis*, which are almost indistinguishable from those in certain *Asplenium* spp., we can support the view of Copeland, Vida, Lovis, Jermy and others, and submerge these genera as subgenera or sections within *Asplenium*.

M. T. M. WILLEMSE and J. BEDNARA (*Botanisch Laboratorium, Landbouwhogeschool, Wageningen*)

Polarity during megasporogenesis of *Gasteria verrucosa*

The megasporogenesis in *Gasteria* conforms to the Polygonum type (WILLEMSE & FRANSSSEN-VERHEIJEN 1978). During diplotene the formation of a callosic wall, visible after fluorescent staining, starts at the micropylar side of the megasporocyte. This wall does not enclose the megasporocyte totally; the chalazal part of the cell lacks this wall. After the tetrad stage the callose wall disappears. The callosic wall is formed by the megasporocyte and is probably dissolved by the surrounding tissue. This wall is affected by a solution of snail digestive juice of 10.000 units ml⁻¹ at room temperature after about 40 minutes, but differs in nature compared with the young cell plate formed after mitosis because the latter is quickly attacked and disappears after about five minutes. The polarity and nature of the callosic wall can be related with the position of the future megaspore.

During diakinesis a polarity of vacuoles and dictyosomes can be noted in the developing meiocyte. A preference of localisation of glycolipid granules, demonstrated by berberine sulphate and the PAS reaction, is observed in the tetrad stage. A shift of the presence of these granules takes place from the tetrad cells at the micropylar side to the megaspore at the chalazal side. This chalazal part of the meiocyte and of the megaspore has numerous plasmodesmata.

On the base of the number of some cell organelles and ribosomes, an unequal meiotic cell division occurs.

Staining of the ovule with ninhydrin and sudan III shows a local preference of proteins, aminoacids and lipids on the chalazal part of the dyad and tetrad. Acid and basic phosphatase and peroxidase

show such polarity too. No special presence of carbohydrates and esterases is observed.

After induction of meiosis the meiocyte shows a special localisation of some of its cell organelles. The formation of the callosic wall by the meiocyte shows a polarity too.

Around the chalazal part of the dyad and tetrad some lipophylic proteins or aminoacids are present. This may mean an accumulation of nutrients from the nucellus or an excretion of products of the megaspore or both.

For polarity two components should be considered: a meiocytic component and a nucellar one. The sequence of their interaction is in study.

WILLEMSE, M. T. M. & M. A. W. FRANSSEN-VERHEIJEN (1978): Cell organelle changes during megasporogenesis and megagametogenesis in *Gasteria verrucosa* (Mill.) Haw. (in press).

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON MAY 17, 1978: AIR POLLUTION AND PLANT GROWTH

A. C. POSTHUMUS (*Instituut voor Plantenziektenkundig Onderzoek, Wageningen*)

General aspects of air pollution in relation to plants

Air pollution is defined as the occurrence of one or more compounds in the air in concentrations that may be inconvenient or noxious by the effects produced on men, animals, plants or materials. Air pollution has always been present, firstly only of natural origin, later also man-made, and may be of a biological, chemical or physical nature. Natural air pollution consists of hydrocarbons and H_2S produced by vegetation and surface waters, ash, dust and SO_2 produced by volcanic activity, salt sprays of the seas, smoke of natural fires and others. Man-made air pollution has been rapidly increasing during the last century, especially the number of pollutants, containing for example SO_2 , NO_x ($NO + NO_2$), CO , hydrocarbons, HF , Cl_2 , HCl , NH_3 , and many others. In addition to these primary air pollutants, numerous secondary pollutants may arise as reaction products of the primary ones, for example the photochemical oxidants O_3 and peroxyacetyl nitrate (PAN). Air pollutants may be divided in gaseous, fluid and particulate compounds, 90% being in the gaseous phase.

Several types of air pollution may cause effects on plants, as is known already for more than 100 years. Plants are more sensitive to many air pollutants than men, animals and materials and may be used to indicate the presence of air pollution. On the basis of the present phytotoxicological knowledge in this field one may discern different types of direct effects of air pollutants on plants: acute effects of relatively high concentrations of pollutants during short time periods, and chronic long term effects of low concentrations. Most attention has been paid till now to the clearly visible acute effects on higher plants, but it is questionable if the more hidden, chronic effects will not be more important in the long run. Besides one may distinguish positive and negative effects, and reversible and irreversible effects.

The effects of air pollution on plants are produced at different levels of organization in the plant (molecular, microscopical, macroscopical, or whole plant) and show different symptoms (e.g. disfunction of special structures or components of the cell, cell and tissue disintegration, leaf chlorosis and necrosis, leaf drop, plant death).

SO_2 in high concentrations causes acute leaf necrosis (interveinal) and in low concentration produces chronic effects as growth retardation and yield reduction. HF is very phytotoxic for special plant species, for example the monocotyledonous ornamental plants freesia, tulip and gladiolus, and some conifer species and stone fruit trees. It has also clear chronic effects and accumulates very strongly in special plant parts (leaf tips and rims). O_3 and PAN cause more or less specific symptoms on a number of plant species, consisting of respectively fine, necrotic speckles at the leaf upper side (f.e. on tobacco Bel W_3) and band forming necrosis at the leaf under side (f.e. on little stinging nettle). NO_2 has the same type of acute effects on plants as SO_2 (interveinal leaf necrosis), but at much higher (4–10 times) concentrations. C_2H_4 as an air pollutant may influence several sensitive plants in flower formation and causes also reversible effects as epinastic curvatures. NH_3 has appeared to be noxious for some shrubs and trees, especially in winter time, and to produce special leaf necrosis at the under side of leaves of cauliflower. Compounds as HCl and Cl_2 may cause damages by leaf necrosis on several plant species.

In all effects of air pollution on plants, besides concentration, exposure time, and specific sensitivity of the species or variety, also the conditions have clear influences. Both internal conditions of the plants, as age, state of nutrition, physiological activity and stage of development, and external factors as wind speed, light, temperature and relative humidity of the air are important. These play a great role in the uptake of the air pollutants by the plants and may influence the effects intensity.

Between the effects of different air pollutants being present simultaneously, there are possible interactions other than additive. For example synergism and antagonism may occur. For example $O_3 + SO_2$ and $SO_2 + NO_2$ have been shown to work synergistically. Also interactions between air pollutants and other pollutants and phytopathogens have been found.

The discussion about the use of higher plants as indicators or accumulators of air pollution and some examples of these in practice illustrate the importance of the study of effects of air pollution on plants, although the use of vegetation as a filtering body has been disappointing. Much basic, experimental research has still to be done, also on the ecotoxicological aspects of the influence of air pollution on plants.

A. VAN RAAIJ (*Instituut voor Plantenziektenkundig Onderzoek, Wageningen*)

Damage by air pollution in agriculture, horticulture and forestry

The effects of air pollution on plants in agriculture, horticulture and forestry can manifest themselves in a chlorosis or a necrosis of the assimilating leaf surfaces or a harmful effect on enzymatic processes which all can influence the vitality and the development of plants. These harmful effects can become so large that injury may lead to crop damage or a less commercial value of the products in question.

The principal air polluting components which can play a role in this are mentioned and for instance the importance of their threat to the agricultural, horticultural or forestry practice is pointed out.

Attention is not only paid to the situation in the Netherlands, but also in some other highly industrialized countries like the U.S.A. or the Federal Republic of Germany. In these cases it can be established that although all the mentioned air polluting components are found in The Netherlands, their occurrence is of such a kind, that their damaging effect on agricultural, horticultural or forestry crops can be called small in comparison with those in some other industrialized countries. The reason for this may be found in climatological (much wind) and geographical (flat land) circumstances, besides the limited size of the country and, so far as the industrial air pollution is concerned, in an effective control of the pollution by the factories.

TOKE DE WIT (*Rijksinstituut voor Natuurbeheer, Leersum*)

Epiphytic lichens and air pollution

The WHEN workgroup has made an inventory of the epiphytic lichens on freestanding trees in The Netherlands, using a 5 km square-grid as a basis. On the basis of the number of lichen species per tree species per square, the squares were divided into six classes of epiphyte richness. The data for the west of the country were analysed in more detail, resulting in a zone map (cf. DE WIT 1976).

It appeared possible to construct a descriptive model of the correlation between SO_2 concentrations and epiphyte richness. At median winter SO_2 concentrations higher than $100 \mu g/m^3$ only a few insensitive species occur. At lower concentrations winter peak values (98 percentile) are more important than median ones.

The extent to which a species is restricted to epiphyte-rich squares is used as a measure for ranking the species according to sensitivity. The more sensitive species have disappeared from large areas of The Netherlands. Comparison of the present situation with data from around 1950 reveals a decline almost everywhere in the country. The southern and western parts of the country are impoverished, the area between Rotterdam and Den Haag and the area between Arnhem and Nijmegen in particular have become very poor. A further decline of the epiphytic lichen vegetation, in particular of the relatively rich areas, is expected.

In fumigation experiments, using HF, SO_2 , C_2H_4 , O_3 and O_3 combined with SO_2 at realistic concentrations and prolonged exposition it was found that these air pollutants cause (under glass-

house conditions) significant morphological damage to all or some of the tested lichen species.

WIT, T. DE (1976): Epiphytic Lichens and Air Pollution in The Netherlands. *Verhandeling Rijksinstituut voor Natuurbeheer/Bibl. Lichenologica* 5, Vaduz, Cramer.

W. ERNST (*Biologisch Laboratorium, Vrije Universiteit, Amsterdam*)

Physiological and genetical effects of airborne heavy metals on plants

Metal pollution is no longer considered as an isolated phenomenon but as an extremely pervasive syndrome in our environments, even if the problem is restricted to airborne heavy metals, being disseminated as "anti-knock" lead compounds in car exhaust or as metal particles from downwash of power lines, from furnace ash of power stations or from metal mining and metal melting. Plants may accumulate heavy metals either from foliar deposits or via the reaction of airborne metals with the soil by uptake through their roots. The way airborne heavy metals affect the plant's functioning at the physiological level depends not only on the metal concentration, but especially on the speciation of the metals. The state of complexation influences the uptake, transport and the toxicity of these elements. A comparison of synthetic chelating agents and naturally occurring ligands has been presented.

Evidence is presented that in response to both acute and chronic pollution the early stages of selection are operating in members of species and have given rise to highly resistant populations, mostly morphologically indistinguishable from normal populations. A model of such genetical effects is provided by the evolution in relation to airborne metals. The physiological basis of metal resistance is metal specific, but frequently involves cellular sequestering of the toxic elements. The tolerance mechanism may enable us to assess the genetical effects of specific pollutants in the field.

P. DOELMAN (*Rijksinstituut voor Natuurbeheer, Arnhem*)

Effects of lead pollution on the soil microflora

The natural lead content of soils averages 15 ppm. However, due to anthropogenic redistribution and accumulation, areas with concentrations of 30 to 100 times higher than 15 ppm are nowadays no exception. Even, concentrations of 500 times as high have been measured.

The question was raised whether these high lead concentrations may affect the basic biological functions of the soil, e.g. the decomposition of organic matter, which leads to the recycling of elements. Since this cycling of elements mainly depends on microbial activities, the effects of lead pollution on the soil microflora must be known before a complete picture of the toxic action of the element can be obtained.

A concentration of 500 ppm lead ($PbCl_2$) inhibited the respiration of sandy soil by approximately 15%, while at 2000 ppm the inhibition was approximately 50%. Three years after the addition of lead, the inhibition in this sandy soil was still approximately 30%. In clay and peat soils the inhibition was less.

Lead considerably reduced the maximum oxidation rate of defined organic substrates, such as glutamic acid. Two years after the addition of lead, a retarded decomposition of glutamic acid could still be demonstrated when this compound was added to lead-amended soil. Repeated addition of glutamic acid to the lead-polluted soil shortened the time until the maximum oxidation rate occurred, but it did not cause the level of the maximum oxidation rate to increase.

Subsequently, the sensitivity of soil bacteria to lead was investigated. When pure cultures of the bacteria isolated from the soils were tested in liquid media for lead tolerance, a higher proportion of tolerant strains was found in lead-containing soils. Among strains of Gram-negative bacteria isolated from these soils a higher proportion of tolerant strains was found than in coryneform bacteria. A shift in lead-polluted soils towards a higher proportion of Gram-negative rods may be of an indirect nature, due to an uneven distribution of the various groups of microorganisms in soil aggregates.