Acta Bot. Neerl. 30(4), August 1981, p. 313–318.

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON DECEMBER 11, 1980

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The influence of grazing on vegetation in the western coastal desert of Egypt

Within the framework of the SAMDENE (Systems Analysis of Mediterranean Desert Ecosystems in Northern Egypt) project, the influence of grazing on the vegetation upon two ecosystems characteristic for the western desert of Egypt was investigated.

One study area is situated on calcareous sandy dunes 50 km west of Alexandria; another is located in a sandy inland depression. The vegetation was sampled according to the multiple plot method. The vegetation on the sandy dunes was also analysed using the Braun-Blanquet method. Data were processed by Principal Components Analysis. The Braun-Blanquet data were also analysed by a classification programme.

In the dune area ordination and classification resulted in seven groupings each with a distinct physiographic characterization. The largest differences in vegetation composition in the inland area appeared to exist between grazed and ungrazed stands. In the dune area protection against grazing resulted in a small decrease in vegetation heterogeneity over a period of three years after fencing. A comparison of the vegetation of grazed and ungrazed stands in the inland area with the situation three years earlier, showed that factors other than grazing, such as the drought of the last three rainy seasons or the increase in phytophagous insect populations, seem to play a predominant role in determining the differences in vegetation composition.

The results of the Braun-Blanquet method appeared to be as indicative as those of the quantitative method, but time needed for collection of Braun-Blanquet data was 15 times shorter. Furthermore, transformation of Braun-Blanquet data was not necessary before processing them.

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The ecology of ferns in the "Kuinderbos" (NOP)

The following articles are dealing with this subject:

BREMER, P. (1980): *Varens in het Kuinderbos.* Mimeographed Report. Lab. of Plant Ecol., Haren (Gn), 165 p.

— (1980): The Ferns (Pteridophyta) of the Kuinderbos (The Netherlands). The establishment of 23 species in a planted forest. *Acta Bot. Neerl.* **29**: 351–357.

— (1980): Polystichum lonchitis (L.) Roth en Asplenium viride Huds. in Nederland. *Gorteria* **10** (7): 113–120.

P. SMEETS and H. SPRANGERS *(Milieukunde, Fuculteit Wiskunde en Natuurwetenschappen, Toernooiveld. Katholieke Universiteit, 6525 ED Nijmegen)*

Ecological models and Physical Planning

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Hapaxanthous species in limestone grasslands: concious savers or big spenders?

Within the limestone grassland project (WILLEMS 1980) the relationship between vegetation structure and species diversity is being studied in detail. Willems reported an increase of plant species diversity and types of regeneration strategies with a decrease of productivity in limestone grassland. One of the regeneration strategies is the hapaxanthous strategy, i.e. the strategy in which plants flower only once in their life cycle (BAKKER et al. 1966). The following questions can be put forward: 1. Is the occurrence of hapaxanthous species determined by vegetation structure? 2. Do these hapaxanthous species possess common ecological features? 3. Is there any obvious differentiation between the various hapaxanthous strategies?

These questions are being investigated in the nature reserves Gerendal and Wrackelberg, South Limburg. The following prelimininary conclusions can be drawn: In low productive stands the greater part of the total above ground phytomass is concentrated in the layer 0–5 cm, while in higher productive stands much more plant dry matter is found abouve 5 cm. The highest increase of standing crop is in May. In the low productive stands there is another important increase in plant dry matter in September.

Total above ground phytomass ("TAP") appeared to be an appropriate variable to describe vegetation structure in these grasslands. In each life phase the average dry weight per individual is less determined by the "TAP" than the number of individuals of each species in that life phase. If the presence of these species in their adult life phases is compared with the "TAP" of the vegetation in which they occur, the following series can be distinguished (from small to large "TAP"): *Carlina vulgaris – Euphrasia officinalis, Gentianella germanica, Scabiosa columbaria – Linum catharticum – Anthyllis vulneraria, Daucus carota.* Only the perennial hapaxanthous species seemed to react noticeably on "TAP" in dry weight per individual in their more mature life phases. Some species shifted their preference for "TAP" in the course of their life cycles. This shift means a waste of individuals in an older life phase in less favourable micro-habitats. The shift occurs mainly in the annual species *Euphrasia officinalis* and the biennials *Linum catharticum* and *Anthyllis vulneraria.* These species appear to be spenders, whereas species like *Gentianella germanica, Daucus carota* and *Scabiosa columbaria* appear to be savers.

BAKKER, D., S. J. TER BORG, & D. OTZEN (1966): Ecological research at the Plantecology Laboratory, State University, Groningen. III. On the life forms of hapaxants in the Dutch flora, *Wentia* **15**: 13–24.

WILLEMS, J. H. (1980): *Limestone grasslands in North-West Europa.* Thesis, State University of Utrecht, 144 pp.

J. ROZEMA *(Laboratorium voor Oecologie, Afd. Plantenoecologie, Vrije Universiteit, De Boelelaan 1087, 1071 HV Amsterdam).*

Ecology of shore-lines species of sand dune and salt marsh coasts

The shore-line habitat mainly consists of salt marsh litter and thalli of brown algae deposited along the spring tide zone. Rapid decomposition of the litter results in a locally nutrient rich environment. Soil salinity of the salt marsh shore-line habitat may greatly exceed that of seawater, while soil salinity along the sand dune coast is in the range of 0–60 mM NaCl. On Hoagland's hydroculture with NaCl added and 3.5 mM NO_3^- *Cakile maritima* appeared to be salt sensitive (growth inhibition at 60 mM NaCl), *Atriplex hastata* and *A. littoralis* showed growth stimulation at 60 and 150 mM NaCl, and the slow growing *Salsola kali* was indifferent to salt concentrations of 0–600 mM NaCl. In culture solution with a lower NO_3^- level, the salt-induced growth stimulation for *A. hastata* and *A. littoralis* was absent. Plants grown under high nitrate conditions accumulated more Na^+ (up to 750 mM Na^+), accompanied by high tissue levels of the N-containing compatible osmotic solute glycine betaine (250 µmol/g DW). The necessity of NO_3^- investment of N in glycine betaine accumulation in

order to optimalize growth under saline conditions, may explain the occurrence of both *Atriplex* species in the shore-line habitat.

Plants grown in pot-cultures showed a positive growth response to spray with seawater. This salt-spray stimulated growth was most pronounced in *Cakile maritima* and *Salsola kali*. These strand line species avoid salt spray damage by thickening of the epicuticular wax layer and minimise water-loss further by an increase of succulence. Growth stimulation by salt spray was most marked under nutrient-poor soil conditions. Possibly this stimulation is due to an observed increase of the magnesium content of the shoot. Based on these findings both *Atriplex* species may be considered "soil halophytes" and *Cakile* and *Salsola* "aerohalophytes".

MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND -ANATOMY ON DECEMBER 12, 1980

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The development of vessels in the early wood of Fraxinus excelsior L.

Investigations on cell division and vessel-element differentiation in the cambial zone of the ash (*Fraxinus excelsior* L.) indicate that growth and differentiation of the fusiform elements are not equally distributed over all radial files.

In radial files which have side-contact with rays the fusiform elements are significantly longer than in files without such contact. The index of periclinal cell divisions is also higher in radial files that have contact with the rays.

Vessel elements, however, are relatively rare in radial files that have contact with the rays. This suggests that differentiation of fusiform cells into vessel elements is likely to take place in files that are relatively isolated from the rays, which are important radial transport channels of food and other substances.

These results have been obtained mainly from cambial zones involved in the production of the summer-wood, in which the vessel elements are rather narrow. It would seem, however, that cambial zones in which the much wider spring-wood vessels differentiate, the differences between the various files should be clear.

Investigations of such cambial zones were made in transverse sections for vessel-element distribution and for periclinal cell-division index, and in tangential sections for cell length. The results are in agreement with the earlier findings. Moreover, a general tendency has been found for a decrease in cell-division index tangentially towards developing vessel elements. This supports the hypothesis of BURGGRAAF (1973), that diameter growth of the vessel elements is made possible because of differences in rate of growth and cell division in tangentially adjoining radial files.

BURGGRAAF, P. D. (1973): On the shape of developing vessel elements in Fraxinus excelsior L. *Acta Bot. Neerl.* **22**: 271–278.

M. T. M. WILLEMSE and J. C. AUDRAN *(Vakgroep Plantencytologie en -morfologie, Botanisch Laboratorium, Arboretumlaan 4, 6703 BD Wageningen)*

Autofluorescence of pollen walls

After excitation of UV light (365 nm) the autofluorescence of the pollen wall is expressed in an emission spectrum. The spectral maximum in nm and the intensity at this maximum in mV is noted. The fading in intensity at the spectral maximum is measured during a period of 30 sec.

In earlier experiments (WILLEMSE 1971, 1972) autofluorescence of pollen walls showed special characteristics between different pollen species and their ontogeny. In *Lilium* changes in autofluores-

cence were correlated to different stages of pollen wall formation (WILLEMSE 1981). In *Vicia* pollen two maxima are measured and a difference exists in the autofluorescence spectra probably depending on the genetic constitution.

The influence on the autofluorescence was studied by anther-injection. In experiments *Gasteria* and *Vicia* pollen were injected in the loculus of the anther of *Lilium*. The autofluorescence spectra of *Gasteria*, *Vicia* and *Lilium* are different. After injection different stages of *Gasteria* pollen show the same autofluorescence as *Lilium* pollen. Except at the late maturation stage of *Lilium*, the autofluorescence of the *Gasteria* pollen is different, probably due to the non-acceptance of the Pollenkitt (WILLEMSE 1981).

Anther injections with *Vicia* pollen show that the stage from tetrad till vacuolation gets the autofluorescence characteristics of lily, whereas the following stages of *Vicia* are not influenced. Pollen walls treated with KOH or acetic acid do not change their spectra after contact with locular fluid. This can be an indication that a real acceptance takes place of autofluorescent products which are built in the wall instead of a simple impregnation. The acceptance depends on the type of pollen and the developmental stage of the pollen.

WILLEMSE, M. T. M. (1971): Morphological and fluorescence microscopical investigation on sporopollenin formation at Pinus sylvestris and Gasteria verrucosa. In: J. BROOKS, P. R. GRANT, M. D. MUIR, P. VAN GIJZEL & G. SHAW (eds.), *Sporopollenin*, p. 68–107. Academic Press, London-New York.

WILLEMSE, M. T. M. (1972): Changes in the autofluorescence of the pollen wall during microsporogenesis and chemical treatments. *Acta Bot. Neerl.* **21** (1): 1–16.

WILLEMSE, M. T. M. (1981): Autofluorescence of pollen wall of Lilium and changes in pollen wall of Gasteria in Lilium anther. *Acta. Soc. Bot. Pol.* **59**, in press.

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Cell wall regeneration in plasmolyzed pollen tubes of lily and tobacco.

Cell wall regeneration after plasmolysis has been reported for unicellular and filamentous algae, multicellular hairs and root hairs (cf. SCHRÖTER & SIEVERS 1971). There are two reports about light-microscope plasmolysis studies on pollen tubes. According to SCHOCH-BODMER (1945) growing pollen tubes of two *Veronica* species, *Luzula campestris* and *Plantago lanceolata* could not be plasmolyzed. Only if tube growth had stopped could plasmolysis be obtained. In contrast, IWANAMI (1959) described for growing lily pollen tubes plasmolysis and cell wall formation around the contracted cytoplasm. On the basis of these contradictory reports we started a combined light- and electron-microscopic study on plasmolyzed pollen tubes of *Lilium longiflorum* and *Nicotiana tabacum* to get information about deposition, composition and structure of newly formed cell wall material. Growing lily and tobacco pollen tubes transferred from the growth medium (10% sucrose, 0.01% boric acid) into a medium with higher sucrose content (20%, 40% or successively 20% and 40%) showed plasmolysis starting at the tip. After 10 min of plasmolysis in either of these sucrose concentrations the tubes could still be deplasmolyzed. Longer plasmolysis resulted in the formation of a thick, often layered cell wall, deposited on the inside of the utmost tube tip. Often, a cell wall also formed on the tip side of the retracted cytoplasm some distance from the original tip. After deplasmolysis the extending cytoplasm could not break through either new cell wall and sometimes plasmoptysis occurred. Ultrastructural studies indicated that the tip of the retracted cytoplasm no longer had an accumulation of golgi vesicles. This was also observed for plasmolyzed root hairs by SCHRÖTER & SIEVERS (1971). The newly formed wall material had a flocculent appearance. Treatment of these plasmolyzed pollen tubes either with EDTA or with a mixture of H_2O_2/CH_3COOH (1:1) revealed a skeleton of microfibrils in the new cell walls. According to HERTH et al. (1974) the fibrillar skeleton of lily pollen tube walls consists of a mixture of β-1,3- and β-1.4 glucans, while that of tobacco tube walls is assumed to consist of a β-1,4 glucan (KROH & KNUIMAN 1981). Studies on the nature of the fibrillar and matrix material formed in plasmolyzed pollen tubes are in progress.

SCHRÖTER, K. & A. SIEVERS (1971): Wirkung der Turgorreduktion auf den Golgi-Apparat und die Bildung der Zellwand bei Wurzelhaaren. *Protoplasma* **72**: 203–211.
SCHOCH-BODMER, H. (1945): Über das Spitzenwachstum der Pollenschläuche. *Ber. Schweiz. Bot. Ges*, **55**: 154–168.
IWANAMI, Y. (1959): Physiological studies of pollen, *J. Yokohama Municipal Univ.* **116**: 1–137.
HERTH,W., W. W. FRANKE, H. BITTIGER, A. KUPPEL & G. KEILICH (1974): Alkali-resistant fibrils of β-1,3- and β-1,4-glucans: structural polysaccharides in the pollen tube wall of Lilium longiflorum. *Cytobiology* **9**: 344–367.
KROH, M. & B. KNUIMAN (1981): Submicroscopic morphology of cell walls of tobacco pollen tubes after selective extraction of polysaccharides. *Acta Soc. Bot. Pol.* **50** (in press).

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Ultrastructural aspects of fertilization in Spinacia oleracea L.

A detailed account of this study is given in *Acta Bot. Neerl*, **30** (1/2):
a. Ultrastructure of the developing embryosac of spinach: 75–99.
b. Pollen tube penetration and fertilization in spinach: 101–122.

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Mechanical aspects of the corolla, a quantitative and functional approach

The forces needed to operate explosive or hinged structures of flowers have been quantified with a torsion-based force meter with a sensitivity range between 0.002 and 20 grams (for a description see BRANTJES 1981a). *Salvia glutinosa* needed a pressure of 0.7 g on the sterile part of its anther to lower the fertile part. For the lifting of the upperlip-hood of *Phlomis fruticosa* a force of 9.4 g had to be applied at the hood-side, the point where the visiting bumblebees started their action. The counterforce against lifting was mainly produced by the friction of the stiff filaments over the hood-innerside (BRANTJES 1981a). Similarly, in *Polygala chamaebuxus* the total force on the carina fringes, needed for lowering the carina, could be differentiated into: a friction force over the stiff style (0.5 g), the tearing apart of the adhering carina rims (3.5 g, only on the first opening) and the resistance by the hinge (0.4 g). *Linaria* spp. differed greatly in the forces needed to lower the underlip: *L. vulgaris* from 0.08 g (initial separation of the lips) to 0.19 g (total lowering, as it is done by bumblebees), *L. dalmatica* from 0.30 to 0.50 g, *L. repens* from 0.08 to 0.17 g. Occasionally Syrphids landed on the underlip of *L. repens*, which bent down a little if the fly had sufficient weight and was in the right position. Through the narrow slit between the lips, the Syrphids then ate pollen from the exposed anthers (BRANTJES 1980, 1981b). On *Epipactus palustris* the epichile only lowers under a pressure of 0.03 g. This explains why small-sized Syrphids do not lower the epichile and therefore do pollinate, whereas the converse happens with larger Syrphids (BRANTJES 1981c, d). The necessary forces in each particular flower species correlated well with the body weight of its pollinators. Bumblebees (fresh weight 0.1 to 0.4 g) had to produce 0.1 to 8.0 g to open and pollinate bumblebee-flowers. Honeybees (0.08 to 0.15 g) had to apply 0.02 to 0.2 g on flowers that they visited and pollinated. The Syrphid flies *Helophilus* spp. (0.04 to 0.11 g), *Eristalis* spp. (0.03 to 0.09 g) and *Rhingia campestris* (0.03 to 0.07 g) caused movements in *Epipactus palustris* (0.03 g) and by lever-action in *Linaria repens* (0.17) g), whereas lighter species such as *Syritta pipiens* (0.01 to 0.02 g) and *Neoascia podagrica* (0.003 to 0.004 g) did not.

BRANTJES, N. B. M. (1980): Stuifmeeldiefstal en bestuiving bij het gestreepte leeuwebekje. *De Levende Natuur* **82**: 126–128.
BRANTJES, N. B. M. (1981a): Floral mechanics in Phlomis (Lamiaceae) pollinated by strong bees (Apidae, Hymenoptera). *Ann. Bot.* **47** (in press).

BRANTJES, N. B. M. (1981b): Scharnierkracht en insectengewicht bij het gestreepte leeuwebekje. *De Levende Natuur* **83** (in press).

BRANTJES, N. B. M. (1981c): Ant, bee and fly pollination in Epipactis palustris (L.) Cranz, (Orchidaceae). *Acta Bot. Neerl.* **30**: 59–68.

BRANTJES, N. B. M. (1981d): Mieren, luizen, zweefvliegen en de bestuiving van de moeraswespenorchis. *De Levende Natuur* **83**: (in press).

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The development of Coffea canephora embryoids in vitro

The multiplication of selected plant material plays an important part in the improvement of crops. Especially in woody crops and forest trees propagation problems could be a limitation to the screening, the introduction and the distribution of improved clones. Tissue culture methods have already proven their value and are applied on an industrial scale in the propagation of herbaceous ornamentals. In vitro propagation of woody species is more difficult and in general still in an experimental stage. However, *Coffea* spp. seem to be an exception and various ways of in vitro propagation were successful.

Isolation of shoot tips and subsequently their multiplication in vitro forms the most reliable method for clonal propagation. A more sophisticated process with interesting implications for developmental studies is the initiation, culture and multiplication of embryoids in vitro.

Earlier experiments started with the induction of callus tissue on stem explants from internodes of orthotropic branches. In 2–3 weeks enough callus is developed for transfer to solid or liquid embryo induction media. After 8–9 weeks of incubation the first globular embryoids appear, on solid media in clusters and in liquid media dispersed in the solution. The synchrony in the initial development of the embryoids is soon lost on solid media, probably because of nutrient competition, but can be maintained in liquid media. In the latter the subsequent development of embryoid generations is possible through careful separation of cell and embryoid fractions during transfer. Embryoids often multiply by "budding" at the root end. The complete process from incubation of stem explants to fully grown embryoids of the size of zygotic embryos in mature seeds takes about 16 weeks.

In later experiments is was possible to reduce the callus interphase to a minimum. Plantlets grown from embryoids develop on all their parts embryoids without preceding callus formation. Discs punched from young leaves of mature trees also produce directly embryoids. The process takes only 8 weeks and the embryoids could be subcultured and multiplied in liquid medium.

The first steps to a morphological study are made and NASSUTH et al. (1980) observed proembryo-like structures in callus of stem explants after 14 days of incubation. The developmental studies on embryoid formation will be continued.

NASSUTH, A., T. M. WORMER, F. BOUMAN & G. STARITSKY (1980): The histogenesis of callus in Coffea canephora stem explants and the discovery of early embryoid formation. *Acta Bot. Neerl.* **29**: 49–54.