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MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 9 FEBRUARY 1994*

Ammonium and Nitrate in Heathland and Heathland-related Vegetations: Preferences for Nitrogen Source and Ammonium Toxicity

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A number of species-rich vegetation types (e.g. *Violion caninae* and *Cirsio-Molinietum* communities) are present in Dutch heathlands and heathland-related ecosystems, besides the species-poor communities, such as the *Ericion tetralicis* and the *Calluna-Genistion pilosae*. The major causes for the variation in vegetation types are differences in soil nutrient content, soil acidity, in hydrology and in succession stage. Under nutrient-poor conditions acidity-related factors as soil pH, aluminium/calcium ratio and nitrogen form (NH_4^+ or NO_3^-) are discriminating factors. A field survey showed that vegetation types, such as the *Cirsio-Molinietum* and the *Violion caninae* were only found on sites varying in pH from 4·5 to 6·5, where both NH_4^+ and NO_3^- were present and where the aluminium/calcium ratio was relatively low (generally below 5). In contrast to this, *Ericion tetralicis* and *Calluna-Genistion pilosae* communities were found on more acidic soils (pH<4·5), where NH_4^+ was the major nitrogen form and aluminium/calcium ratios were higher. Furthermore, NO_3^- was often not detectable.

Growth and development of *Cirsium dissectum* (*Cirsio-Molinietum*), *Arnica montana* (*Violion caninae*) and *Calluna vulgaris* (*Calluno genistion pilosae*) were studied on hydro cultures with different nitrogen forms and NH_4^+ concentrations. *C. dissectum* and *A. montana* developed best in the absence of ammonium. When ammonium was added, both species showed signs of ammonium intoxication and mortality increased with increasing ammonium concentration. *C. vulgaris* developed best with ammonium as the nitrogen source. Even in the presence of high ammonium concentrations (up to 1000 μmol^{-1}) *C. vulgaris* developed morphologically normal, with only a small reduction in biomass compared with growth on low ammonium concentrations.

In a pot experiment it was demonstrated that *A. montana* was more sensitive to drought with ammonium as nitrogen source than in the absense of ammonium, whether nitrate was present or not. *C. dissectum* showed increased sensitivity to drought resistance when ammonium was the only nitrogen source.

This study indicates that the decline of *A. montana* and *C. dissectum* in The Netherlands might be caused by increased ammonium concentrations in the soil, due to high atmospheric ammonium input and/or a reduction in nitrification rate as a result of soil acidification.

Restoration of the Littorellion Vegetation of Shallow, Slightly Buffered, Oligotrophic Moorland Pools

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In the last few decades the presence of many species of the phytosociological alliance Littorellion has been greatly reduced as a result of acidification and eutrophication processes (Roelofs 1983, *Aquat. Bot.* 17: 139–155). Representatives of this plant community, e.g. *Littorella uniflora* (L.) Aschers. and *Lobelia dortmanna* L., are characteristic of shallow, slightly buffered, oligotrophic moorland pools in The Netherlands. Several laboratory and field experiments have been carried out to study the restoration possibilities of the vegetation of these pools. It was shown that *Juncus bulbosus* L. strongly increased after liming of the water layer during culture experiments and glasshouse experiments. After liming without removal of the organic sapropelium layer *J. bulbosus* outcompeted most other species of the Littorellion.

As a result of previous studies these moorland pools have been characterized according to the following criteria: (i) the original and present water chemistry; (ii) vegetation of the surface waters; (iii) the original buffer capacity of the water, and (iv) the cause of the deterioration of these surface waters

*The meeting was organized in cooperation with the Department of Plant Ecology and Evolutionary Biology, University of Utrecht.

(Bellemakers *et al.* 1993. Report of the 1st phase: restoration management against acidification and eutrophication in fresh water systems, Nijmegen).

To restore the original vegetation of the moorland pools, different control measures against the negative effects of acidification and eutrophication are necessary, depending on these criteria.

It has been demonstrated that, after liming of the water layer, mass, development of *J. bulbosus* took place, particularly when the sapropelium layer was not removed. After removal of the sapropelium layer and liming of the water layer or inlet of buffered surface water, many characteristic species of the Littorellion developed successfully.

It is concluded that restoration of many moorland pools is possible, particularly the former eutrophic ones, because of the more persistent seedbank in these pools.

Seed Bank Studies Within the Scope of the Dutch Nature Conservation Policy Plan (Het Natuurbeleidsplan)

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The study of seed bank dynamics is part of an integrated research program in cooperation with the AB-DLO, SC-DLO and IBN-DLO institutes, to predict the best chances and scenarios for further nature development in The Netherlands. From the climatological aspects onto the level of landscape and vegetation, existing models will be connected and extended for this purpose. The study on seed bank dynamics will resort in a small part of the complex model.

This study consists of experiments to discover the relation between the established vegetation and the soil seed bank, and its potential for nature development. It is not unusual that the established vegetation is not reflected by the composition of the seed bank flora. The soil seed bank, e.g. of an *Ericetum tetralicis* shows almost the same pattern of species as the seed bank of an abandoned *Cirsio-Molinietum*. The dominant species in the seed bank of both vegetation types are *Erica tetralix* and *Juncus* sp., also the layer distribution of the seeds is very similar. Seeds of *Gentiana pneumonanthe* and *Drosera rotundifolia* were, however, present under the *Ericetum tetralicis* but not under the abandoned *Cirsio-Molinietum*. This suggests that *G. pneumonanthe* and *D. rotundifolia* have a transient seed bank whereas *E. tetralix* and *Juncus squarrosus* have a more persistent seed bank. Even *E. tetralix* and *J. squarrosus* are lacking in the seed bank under an improved pasture where in former times a *Cirsio-Molinietum* occurred, which represents the present goal of restoration management. The differences in longevity of the soil seed bank have important consequences for restoration management (Bakker, J.P. (1989): *Nature Management by Grazing and Cutting*. Kluwer, Dordrecht).

A method to sample the seed bank has been developed. The 10-quadrat sampling method implies the sampling of 100 cores, in 10 quadrats per homogeneous plant community. It is a seedling germination method after the concentration of seeds out of bulk samples by washing them on a fine 0·212 mm sieve. Concentration implies reduction of the bulk samples by 50–80% and hence the space needed for germination. Moreover, more species and individuals emerged in a shorter period of time after concentration as compared to unconcentrated samples. The remainder after seedling emergence in the concentration method revealed no living seeds by a binocular test.

The Distribution of Helophytes in "Het Wageningse Binnenveld" in Relation to Their Dispersal Characteristics

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The contribution of ditches to the ecological infrastructure of an agricultural landscape is studied with respect to seven selected helophytes (*Alisma plantago-aquatica*, *Carex vesicaria*, *Iris pseudacorus*, *Oenanthe aquatica*, *Ranunculus flammula*, *Rumex hydrolapathum*, *Veronica catenata*). A combination of pattern-analysis and dispersal characteristics has provided the basis for calculating the connectivity between (sub)populations of the selected helophytes.

GIS-based pattern-analysis revealed the different spatial relationships for the selected species and diaspore floating experiments show them all capable of dispersal via water. The size and form of the diaspores of the various species was highly variable, as was the floating time. The species in the study area can be divided into two groups. The short floaters (*V. catenata*, *O. aquatica*) had a relatively small number of populations and were more clustered. The long floaters (*R. flammula*, *A. plantago-aquatica*, *I. pseudacorus*) had relatively more populations and were more randomly distributed. *C. vesicaria* and *R. hydrolapathum* could not be assigned to any of these categories.

From the patterns of distribution of the species, it seems clear that there are enough growing places for these helophytes in the study area and that they are all potentially capable of exchanging individual seeds between populations by means of hydrochory.

Because only 10–15% of the ditches contain streaming water, water dispersal is now of restricted importance. From the results it can be inferred that appropriate management of the ditches may improve migration and hence reduce the risk of extinction.

Hay-making Machinery: A Moving Ecological Infrastructure?

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Along the rivulet 'Anlöer diepje', restoration management by hay-making without fertilizer application is carried out. This results in a predictable vegetation succession (Olff H. & Bakker J.P. (1991): *J. Appl. Ecol.* **28:** 1040–1052), despite the fact that dispersal of most plant species by natural vectors is thought to be limited. Hay-making machines, which move over long distances, might play an important role as a dispersal agent. An experiment was done to estimate this importance.

In the first part of the experiment the mowing machinery was cleaned before entering the field. Samples of transported plant material were taken after mowing. This procedure was repeated four times in fields with different vegetation composition. In the samples seeds of many species were found in high numbers, up to hundreds of seeds per gram, which means total numbers of up to a million seeds on the machine. There was a significant positive correlation between species abundances in the vegetation and the seed numbers.

Most of the material accumulated at two different parts of the machinery. On the first part, only contributing to the dispersal within fields, tall species were over-represented. The second part, which contributes to dispersal between fields as well as within, accumulated more seeds of lower species.

In the second part of the experiment the machinery mowed three fields without cleaning. The fields were chosen to have different vegetation composition. Samples were taken each time the machine entered a new field. This revealed that part of the material from one field was indeed deposited in the next. It also showed that diaspores were carried through more than one field.

For many species, hay-making machinery could play a very important role as a dispersal agent. This could affect the succession in the study area in several ways, e.g. (i) acceleration by improving dispersal, and (ii) altering the direction by favouring species setting seed at the time of mowing. If proper mowing schemes are used, hay-making machinery could serve as a management tool to improve dispersal between later and earlier successional stages.

Do Chalk Grassland Bryophytes Compete?

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The bryophyte layer of chalk grasslands consists of a fine-grained mixture of many species with high small-scale shoot dynamics. Although their biomass may be locally high (over $200\,g^{-2}$ dry weight), field studies on replacement rates did not yield evidence for interspecific competition.

The aim of the present study is to test experimentally whether subordinate species are really not outcompeted by a dominant species.

One way to do this is to carry out a pulse perturbation in which one species in a plot is reduced to a minimum cover. After this the responses of all species are recorded and compared to control plots.

In this study the most abundant bryophyte species (*Calliergonella cuspidata* and *Ctenidium molluscum*) in the field were perturbated in separate plots. Each plot was divided into a grid of subplots. The cover of each bryophate species in a plot was determined per subplot. This was done before, immediately after and 1 year after the perturbation.

We checked whether the other species could benefit from the reduction of the most abundant species by comparing their cover in pulse plots with that in control plots, and by testing whether they preferentially increased in subplots where the most abundant species was reduced.

One year after the perturbation, only a few species showed a significantly higher cover in the pulse plots. At subplot scale, few significant replacements of species had taken place and species 'winning' in one plot were found to be 'losing' in another plot. In the pulse plots there was no consistent replacement of *Calliergonella* or *Ctenidium* by one or more other species.

These results indicate that some competitive interactions take place between the bryophytes in chalk grasslands but that there is no clear dominance of any species and no competition hierarchy.

The results of this pulse perturbation will be compared to those of other competition experiments with chalk grassland bryophytes, for example implantation experiments.

The Fourth National Forest Inventory

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The Fourth National Forest Inventory was the first Dutch forest census to take into account nature

conservancy aspects of forest use (Dirkse, G.M. & Thalen, D.C.P. (1987): De natuurfunctie van het Nederlandse bos, enkele resultaten van de Vierde Bosstatistiek. *Nederlands Bosbouwtijdschrift* **59**: 116–127). It was carried out by the State Forestry Service (SBB) and the Central Bureau of Statistics (CBS), in cooperation with the Research Institute for Nature Management (RIN). Figures related to forest ecology were obtained by restratifying an existing stratified random sample of 3400 forest stands according to the forest type classed by management objective, former land use, and forest age. The field work was carried out in 1984 and 1985 by 12 observers who worked in pairs. In total, 1914 stands were described. About 600 species of higher plants were recorded of which, according to the estimate, 92% occurs in less than 5% of the forested area. It is of interest to note that among the most frequently occurring species there are nine exotics and several weeds, such as *Galeopsis* cf. *tetrahit* as well as *Stellaria media*, whose occurrence most probably has increased during the last decade.

The floristic composition of the understorey was recorded and the data were analysed with the computer programs TWINSPAN and DECORANA. The first division yielded two groups. The former group, indicated by *Deschampsia flexuosa* and *Molina caerulea*, is present in 82% of all the forest stands and occurs on poor, more or less acid Pleistocene sands. The latter group is indicated by *Urtica dioica*, *Galium aparine*, and *Poa trivialis*. This group, which is present in only 18% of the stands, occurs on dry or wet, fertile, slightly acid Holocene soils (Dirkse, G.M. (1993). *Bostypen in Nederland*. Wetenschappelijke Mededeling KNNV nr 208, Utrecht). The main floristic variation in the understorey of Dutch forests is correlated with weighted means of indices for nitrogen supply and acidity. This correlation is probably due to the strong influence of air pollution, intensive livestock farming and fertilization practices in arable fields adjacent to forests.

Secondary Succession in a Tropical Montane Cloud Forest in Costa Rica

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Secondary succession and forest recovery following clearing of a tropical montane cloud forests are studied near 3000 m altitude in the Costa Rican Talamanca Mountain Range. Special attention has been paid to floristic composition, structure and species diversity of terrestrial vascular plants. Data were collected in twelve 0·1-ha plots along a successional chronosequence covering *c.* 30 years of recovery.

With the help of TWINSPAN, an Early Secondary *Fuchsia–Abatia* Forest (ESF), a Late Secondary *Quercus* Forest (LSF) and a Primary *Quercus* Forest (PF) were distinguished. In total, 176 terrestrial vascular plant species were found, distributed over 52 trees, 52 herbs, 34 ferns, 19 shrubs, 16 climbers, 2 fern-allies and 1 bamboo. Herbs and climbers prevailed in ESF, while trees and ferns dominated in LSF and PF. Highest Family Importance Values were found for Asteraceae in ESF and Fagaceae in both LSF and PF.

Alpha diversity decreased along the successional gradient, ESF and LSF being significantly more diverse than PF. This is the result of down-slope migration of herb species naturally found in tree-less tropical alpine (páramo) vegetation above 3400 m. These herb species rapidly invade cleared sites in the montane forest belt at lower elevations and act locally as pioneers, speeding up the early stages of secondary succession.

Stem density (stems $\geqslant 3$ cm d.b.h.) was significantly higher in LSF than in PF with a maximum of 3270 stems ha^{-1}, while basal area was significantly higher in PF than in ESF and LSF with a maximum of 64·7 m^2 ha^{-1}. Height and d.b.h. showed a significant logarithmic regression for all forest phases. Density of seedlings and saplings <3 cm d.b.h. was highest in ESF.

On the bases of the relationships between species diversity, basal area and recovery time for secondary forests with ages between 5 and 35 years, a period of 75–100 years was estimated as the theoretical minimum time required for a patch of secondary forest to reach maturity. However, as growth rates tend to decrease with forest age, probably a period >150 years will be needed in order to achieve full maturity.

Restoration Management of Chalk Grassland

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Species-rich chalk grasslands (*Mesobrometum erecti*) occur in The Netherlands only in the southernmost part of the province of Limburg. At present, 20 sites covered by this vegetation remain, varying in area from less than 0·001 ha to more than 5 ha and totalling about 25 ha. The majority of the sites have been set aside as Nature Reserves and are consequently managed to preserve their biological richness, either by autumn mowing or by sheep grazing. Nature conservancy policy aims at development of potential chalk grassland sites and enlarging existing ones.

Restoration management is performed if former abandoned chalk grassland sites receive the status of Nature Reserve. Those areas are always characterized by the absolute dominance of the grass *Brachypodium pinnatum*, of which the litter may be more than 80% of the total above-ground phytomass. Autumn mowing and removal of the hay, followed by sheep grazing in a grassland abandoned for at least 40 years (St. Pietersberg), showed an increase of phanerogam number from 18 to 37 in a permanent plot (2 × 2 m) within an 8-year period. Since the absence of chalk grassland species in the soil seed bank, the newly established species originated very likely from a nearby spot (*c.* 20 m distance), where an assortment of chalk grassland species survived in a former, small chalk quarry. Restoration attempts at a similar site (Bemelerberg), where neither nearby seed sources, nor seeds in the soil of characteristic chalk grassland species were available, revealed only a slight increase in species number 2 years after mowing with different frequency (1, 2 and 4 times per year).

The introduction of sheep grazing in an abandoned chalk grassland (Zure Dries) clearly hampered the dispersal of the chalk grassland species present after enlarging the open area by shrub removal. At this site only species capable of reproducing in a vegetative way by rhizomes or stolons spread over a distance of only a few meters during the 10-year observation period.

The importance of adjacent seed sources became obvious too, in the process of succession of chalk grassland on former arable fields (Wrakelberg). Thirty years of mowing regime resulted in an extended area (5 ha) of species-rich chalk grassland on old fields. Elsewhere in the region no chalk grassland belonging to the *Mesobrometum erecti* originated on former arable fields (Wylre akkers) during a 30-year period and equal mowing conditions, because of the absence of adjacent seed sources of the greater part of species characteristic of this plant community.

Restoration attempts benefit from research on the initial stage of the situation, including recent history of the site and of the soil seed bank. Restoration processes have to be followed by monitoring activities in order to control vegetation development.

Since seed sources seem to be a limiting factor in restoration attempts, seed production of the remaining characteristic species have to be stimulated by mowing after seed set of those species. This temporary management must be followed by sheep grazing, which was of old the anthropogenic factor responsible for the long-term existence of chalk grasslands in this part of their distribution area.

MEETING OF THE SECTION FOR FERTILIZATION RESEARCH IN PLANTS AND THE SECTION FOR MORPHOLOGY, ANATOMY AND CYTOLOGY ON 25 FEBRUARY 1994

The Initiation and Development of Iris flowers: Permeability Changes in the Apex Symplasm

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The transition from vegetative to reproductive growth involves significant changes in morphology, cell number, cell cycle times and gene expression of the apical meristem (AM). Recent studies have related flower morphogenesis to specific patterns of gene expression. The expression of homeotic genes was demonstrated to correlate closely to major developmental changes. For instance, the meristem identity genes *Leafy* and *Floricaula*, homologues isolated from *Arabidopsis* and *Antirrhinum*, respectively, were expressed during the transition from inflorescence meristem (IM) to floral meristem. *In situ* hybridizations showed specific spatial expression patterns for this gene in the AM. The nature of the mechanism which restricts homeotic gene expression to specified groups of cells in the AM is unknown. In the IM of *Cruciferae*, the expression of several floral meristem identity genes seems to be restricted to the peripheral or central zones, and correlates with cell cycle rates in both zones. An aberrant supply of floral or other signals is thought to result in changes in cytohistological zonation and in ectopic expression of homeotic genes. Both phenomena express altered positional effects on cell groups within the AM. Changes in cell–cell coupling in the AM play an important role, since positional signalling is partly mediated by plasmodesmata. Indeed, microinjection evidenced transient closure of plasmodesmata at certain locations within the developing flower. Similarly, in the AM of potato a central symplasmic domain corresponds to the central zone of the cytohistological zonation model. Taken together, this suggests a relation between cytohistological zonation, differences in cell cycling times, expression domains of homeotic genes and symplasmic domains.

The idea that transient permeability changes in the apex symplasm prelude organ formation and, in

mutual interplay with differential gene expression, control development is investigated in the bulbous plant *Iris hollandica* cv. *Prof. Blaauw*. Temperature has a major effect on flower development in *Iris*. The morphological changes during flower formation have been recorded by scanning electron microscopy. During postharvest storage at 30°C, the vegetative apex is a relatively small and flat dome which continues to produce regular leaves. After transfer to 9°C the AM starts to produce spatheleaves. After an 8-week period at 9°C and 1 week at 17°C, two spatheleaves enclose the then formed IM, which has produced two differentially developing floral meristems.

To relate the changes in symplasmic permeability of the apex to morphogenesis, microinjection experiments are currently performed. In the earliest stage of development of the IM all apical cells are dye coupled, with the exception of a small band of lateral cells at the side of the developing spatheleaf. In a later stage, the entire spatheleaf primordium is symplasically isolated from the AM. Additionally, the symplasm in the AM is broken down into several domains. We are investigating the possibility that the symplasmic structure which emerges during the development of the AM is related to specific patterns of gene expression.

In situ hybridizations with *Iris*-homologues of the meristem identity genes *Leafy* and *Floricaula* will be carried out to compare the expression patterns of the genes with the patterns of the dye coupling.

Ovule Identity in Petunia is Determined by a Novel Class of MADS Box Genes

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During the past few years it has been shown that MADS box genes serve an important role in the determination of the developmental fate of the various meristematic cell groups present during the formation of reproductive organs and tissues. From Petunia floral organs MADS box cDNA clones have been isolated that can be divided into three distinct classes. Class I MADS box genes are involved in the determination of the identity of the floral meristem. Class II MADS box genes determine organ identity. In this presentation the identification of a novel class of MADS box genes will be shown which regulates the identity of the ovules.

Two genes of this class III were isolated (*fbp7* and *fbp11*) and characterized by *in situ* hybridization and reverse genetic methods. Both *fbp7* and *fbp11* were only expressed in ovules. This expression was already detectable before ovule primordia were visible, suggesting a role of these genes in ovule primordia development. To analyse the function of *fbp11*, transgenic plants were generated in which *fbp11* was inhibited by co-suppression. In a number of the generated transgenic plants ovule formation was highly aberrant. Instead of ovules, spaghetti-shaped structures were formed appeared to be that style/stigma structures. Taken together, these homeotic transformations observed in *fbp11* transgenic plants suggest that this new set of MADS box genes specifies the identity of ovules within the pistil.

Isolation and Characterization of the Microspore-specific Gene NTM19 in Tobacco

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The aim of this research is to study genes involved in early pollen development. A cDNA library has been synthesized of mRNA from *Nicotiana tabacum* microspores (NTM) and differentially screened. By northern blot analysis it has been shown that clone NTM19 is microspore-specific: no signal was detectable during later stages of pollen development and in other gametophytic and sporophytic tissues. A much weaker signal was obtained when total anthers were used, suggesting a localization in the microspore and absence in the tapetum.

This clone of 490 nt represents a mRNA with a length of 610 nt. The corresponding genomic clone has been isolated and sequenced. This information could be used to complete the entire cDNA and analyse the promoter. No homologies could be found with known sequences. A putative TATA box has been localized and the transcription start determined.

The predicted protein exists of 116 amino acids, which has a molecular weight of 13·8 kDa, a pI of 7·82, a secretory signal and a phosphorylated serine residue. The protein contains transmembrane spanning elements resulting in a membrane-bound positioning.

In situ localization experiments have been carried out with total anthers of different length, corresponding to developmental stages. There was only a hybridization signal with microspores.

A southern blot with genomic DNA of different species probed with NTM19 shows only homology within the Solanaceae plant family.

The NTM19 gene can be classified as a unique gene which is specifically expressed during the microspore stage.

The Ontogeny of the Pollen Tube Wall

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The construction of the cell of pollen tubes of tobacco, lily and petunia was studied using various microscopical techniques. Cryo-fixation showed a distinct morphology of dictyosomes, which includes a specific pre-vesicular cluster resembling a trans-golgi network (TGN). Pollen tubes only form one type of secretory vesicles (SV) as variations in staining behaviour of vesicles proved to be an artefact. Moreover, specific staining provided the first evidence that the vegetative cell is responsible for wall formation around the generative cell.

Vesicles fuse in a restricted, lenticular-shaped area of the pollen tube apex. In the extreme pollen tube tip the initial pectic and cellulosic wall layers are separating, presumably as a result of phase shifting. The pectic layer of the primary wall may readily dissolve, especially in the tubular parts. Its presence in the tube tip might be necessary for maintaining tip shape. This layer may be involved in signal transduction as well. The cellulosic layer contains 5–7 strata of cellulose microfibrils (CMF). The formed texture in apex and tube are similar, indicating that no reorientation of CMF occurs. The secondary wall, or callosic layer is formed directly behind the tip and mainly consists of callose, which, however, embeds an oriented framework of cellulose. It is assumed that terminal complexes, initially producing CMF in the tip, are not retrieved but switch to callose formation in the tubular part, which would explain the increasing thickness, and the presence of cellulose in the callosic layer towards the tube base.

Callose plugs are initiated and formed at distinct sites in the tube as a result of local intensified callose formation. The plugs will only mature if a surplus of redundant SV are present. These SV act as individual wall formation units within the tube and will merge at these sites contributing to plug formation. Callose formation is essential during pollen germination but not required for pollen tube growth. The callosic layer seems to be involved in maintaining a proper osmotic environment and as a regulatory element in nutrient or signal transport.