ISRAEL JOURNAL OF BOTANY, Vol. 34, 1985, pp. 113–128

PHYSIOLOGICAL INVESTIGATIONS OF LEAF MUCILAGES II. THE MUCILAGE OF *TAXUS BACCATA* L. AND OF *THUJA OCCIDENTALIS* L.

HEIDRUN DISTELBARTH AND ULRICH KULL
Biologisches Institut, University of Stuttgart, West Germany

ABSTRACT

Seasonal variations of the content of leaf mucilages of *Taxus baccata* and *Thuja occidentalis* were determined. In *Taxus* the content is highest during the late winter and early spring and lowest in the summer. In *Thuja* it is highest in the winter, decreasing during spring and low in the summer months. Under the light microscope, *Taxus* mucilage was located in the vacuoles of mesophyll cells and that of *Thuja* in mucilage idioblasts. Temperature experiments show that storage of mucilage is not temperature dependent and is not reduced by prolonged darkness. *Taxus* mucilage consists of the sugars galactose, rhamnose, glucose, arabinose and xylose and contains a low percentage (<5%) of uronic acid. Furthermore, a peptidic component is found and shows annual variations from 6% to 15% of total mucilage. Seasonal variations of the sugar components are pronounced only during the spring in the period of intensive mucilage synthesis. Purified *Taxus* mucilage could not be separated into different components by gel chromatography. All fractions showed a similar composition of sugars and peptide. These findings, in addition to the IR spectra, lead to the conclusion that the mucilage is a proteoglycan and perhaps a mixture of polymers of similar molecular weights. Isolated and dried mucilage has a high water-binding capacity; at 96% relative humidity it equilibrates to 180%, and at 100% rh to 280–300% of its dry weight. From mucilage content and cell volume the mucilage concentration of the vacuoles can be estimated as being higher than 5–6%, which must give rise to a remarkable matric potential. It is suggested that the water-binding capacity of the mucilage plays an important role in stabilizing the water relations of the needles, thereby increasing frost resistance.

Mucilages are complex water-soluble polysaccharides of high molecular weights (Aspinall, 1969) which are commonly found in higher plants (Fahn, 1979). Their physiological function in most cases is unclear. They may serve as reserve substances (Franz, 1979; Sutton et al., 1981) or may contribute to water balance (Spegg, 1959; Mollenhauer, 1967; Lyshede, 1977; Clarke et al., 1979) and/or drought-resistance (Fahn, 1974; Clarke et al., 1979; Naglschmid et al., 1982). Their apparent involvement in plant water relations suggests that they may play a role in frost hardiness. A function in symbiosis, as reported by Dexheimer and Guernin (1981), seems to be an exceptional function for mucilages. However, since mucilages in many cases are located extracellularly, it might be assumed that symbiotic bacteria, pathogens or parasites may use mucilage as a recognition factor.

In the first publication of this series (Naglschmid et al., 1982) we investigated the

Received January 23, 1985

mucilage of a biennial *Verbascum* species. We have now examined the mucilage content of leaves from evergreen woody species and correlated these findings with respect to seasonal variability. *Taxus* was chosen because its mucilage is located in normal mesophyll cells and not in idioblasts (Mangin, 1893). For comparison, *Thuja occidentalis*, with its mucilage located in idioblasts, was used as a second coniferous species. A preliminary report of some of the results was published earlier (Distelbarth & Kull, 1983). We previously used similar material from *Taxus* and *Thuja* to investigate seasonal trends of storage carbohydrates, total nitrogen and energy content of the main storage products (Distelbarth et al., 1984).

MATERIALS AND METHODS

Materials

One-year-old leaves (needles) of *Taxus baccata* L. and *Thuja occidentalis* L. were used. They were harvested from approx. 50-year-old trees obtained from the former Botanical Garden of the University of Stuttgart (area now part of the "Wilhelma"). Sporadic frosts occurred in this area from November to April.

Methods

The leaves were gathered at monthly intervals throughout the year and always at the same hour. After isolation the needles were frozen and lyophilized. For experiments investigating the influence of temperature on mucilage content, twigs (about 1.5 m long) from the respective trees were held in a climate chamber using a 12-h light/dark cycle in a 24-h period. Twigs were illuminated with about 5000 lx at a relative humidity of 70% (when temperatures below 0°C were used, rh = 10%).

Viscosity measurements were made in aqueous extracts (7.5%) after filtering the raw extract through cloth in a Rotovisko RV 2 (Haake) viscosimeter using the measuring instrument head MK 50 at a constant temperature of 20°C. A flow curve was measured for each extract.

Isolation of the mucilages was performed according to Naglschmid et al. (1982). Separation of the precipitated mucilage by centrifugation resulted in some loss of material. Therefore for the gravimetric determination the mucilage was collected on a membrane filter, which was then dried over P_2O_5. Each point was measured independently at least three times. The standard deviation for measuring the mucilage content is 0.3 (as % of dry weight).

Hydrolysis of the mucilage was performed according to the method of Hillestad et al. (1977): 10 mg of mucilage were suspended in 2 ml of 1M H_2SO_4 and held in a sealed tube at 100°C for 4–10 h. The solution was then neutralized with $BaCO_3$, filtered, evaporated in vacuo and taken up in 2 ml of 60% ethanol. This solution was used for chromatographic separation of the sugars by TLC according to Naglschmid et al. (1982). In addition, some mucilage samples were hydrolyzed according to the method of Woolfe et al. (1977).

Peptide fraction of the mucilage of *Taxus* was determined in aqueous solution using the method of Bradford (1976).

Total carbohydrate content was determined by the anthrone method (Loewus, 1952), and the *total N content* of the mucilage by the Kjeldahl (1883) method.

To separate the mucilage from co-precipitating proteins repeated extractions were performed using phenol/chloroform (1/1, v/v). The aqueous phases were pooled and the mucilage precipitated with ethanol; the content of total carbohydrate and polypeptide was estimated on this phenol-extracted fraction.

Gel chromatography is often used to further characterize mucilages. Gel filtration of *Taxus* mucilage was performed using Sephadex G 200 (elution with NaCl), Ultrogel AcA 44 and Ultrogel AcA 22 (elution with tris-HCl, pH 7.2) at 20°C (Hillestad et al., 1977; Paulsen & Lund, 1979). The carbohydrate and N contents of each fraction were measured as described (cf. Jarvis et al., 1981). Different mucilage fractions were then precipitated and subsequently hydrolyzed.

IR-spectroscopy of the refined mucilage of *Taxus* was performed in KBr-pressings using a Perkin-Elmer IR-spectrometer 283.

Water-binding capacity of the *Taxus* mucilage was estimated as follows: weighed samples of purified and freshly lyophilized mucilage were maintained in vials at 20°C over saturated solutions of different salts and over H_2SO_4/H_2O mixtures of varying composition. Samples were weighed at intervals until equilibrium was reached. For control, water loss from hydrated mucilage was determined as weight loss in the same manner.

Osmotic potential was measured using a micro-cryoscope (Knauer), according to the method of Walter & Kreeb, 1970. Calibration was performed using sucrose solutions of different concentrations. Osmotic pressures of mucilage solutions and cell sap expressed from fresh needles of *Taxus* were measured.

Histological investigations. Localization of the mucilages in the leaves of *Taxus* and *Thuja* was investigated by light microscopy. Fresh leaves from various developmental stages were sectioned by hand and with a cryo-microtome (Leitz). Sections were fixed in either ethanol (96%) or lead-acetate (10%), and were stained with congo red, thionine and ruthenium red; differentiation was carried out with 60% ethanol. A superior staining method which demonstrates structures stained by both safranin and fast green was also used (Schömmer, 1949): 0.1 g fast green and 0.3 g safranin was dissolved in 100 ml ethanol (70%) to which 3 ml of acetic acid (78%) was added. The sections were stained in this solution for 5–10 min and then differentiated with isopropanol (100%). Because the staining solution has fixation properties, fresh slices could be stained directly. Mucilages show a red-orange staining which differs from the yellow staining of pectic substances.

RESULTS

Histological Investigations

Cormophytes in most cases show mucilage localization in special mucilage-secreting cells (Fahn, 1979). As early as 1893 Mangin found mucilage in mesophyll cells of *Taxus* leaves. Our staining experiments confirmed these data: the vacuoles of most cells of the palisade parenchyma and of about one third of the spongy

parenchyma cells contain mucilage. The staining effect of ruthenium red, indicating the presence of uronic acids, although very weak, is positive in the case of *Taxus* mucilage. Therefore a small amount of uronic acids may be present in the mucilage-polysaccharide.

In *Thuja*, mucilage cells were identified by Gauba (1927) in the vascular bundles. In our investigations most cells of the bundle sheath and some parenchyma cells nearby showed mucilage staining, which was also observed in mucilage-containing idioblasts in the mesophyll.

Seasonal Trends of Mucilage Contents

The results of monthly measurements of mucilage contents of one-year-old needles of *Taxus* during the course of a year are shown in Figure 1. The content varies from 2.7% to about 6% of dry weight. Viscosimetry is sometimes used to estimate mucilage contents (e.g. Molina-Cano & Conde, 1982; Kram & Franz, 1983), and accordingly we also determined the viscosity of aqueous plant extracts. Its variation during the year shows a similar curve (Fig. 1). In order that the estimation of mucilage content would be influenced by increase in cell-wall substances during maturation of the needles, the mucilage content was also examined with respect to the total N content. A comparison of the two curves (mucilage as percentage of dry weight and as percentage of total N) shows the great similarity in the seasonal variations. Especially related to total N the mucilage content is high during the late winter and early spring. The lowest content was found during the summer months.

In Figure 2, the annual variation of the mucilage content in the leaves of *Thuja* is shown. Again, the variation in viscosity is similar to that of the mucilage content. The amount of mucilage is, on average, lower than in *Taxus*. The content is high during the winter, decreasing in spring and low in the summer months.

The water content of the leaves of *Taxus* and *Thuja* is also shown in Figures 1 and 2. As is characteristic of these two species, little seasonal variation is seen. In comparison, investigations on other conifers which lack mucilages (*Juniperus* and *Larix*; Distelbarth et al., 1984) showed distinct seasonal variation.

When *Taxus* twigs were held in the dark for 10 days (with 1 h light every day) we found no significant alteration in the mucilage content of the needles.

Influence of Temperature

Since the mucilage content of *Taxus* leaves rises significantly during the late winter and that of *Thuja* during the winter months, experiments were carried out in climate chambers to investigate whether temperature variation can affect mucilage content. Twigs of *Taxus* were held at +4°C and −4°C, respectively, in May, August and December. In December twigs were also subjected to +22°C. After exposure for 2, 4 and 6 (or 7) days, needles were gathered and lyophilized. During December experimental samples were taken as above, but also after 9, 13 and 15 days of exposure.

The total nitrogen content remained constant under all conditions, with two exceptions. The water content of the needles also stayed constant in most cases, while the needles held at −4°C in the May and December experiments showed a decrease

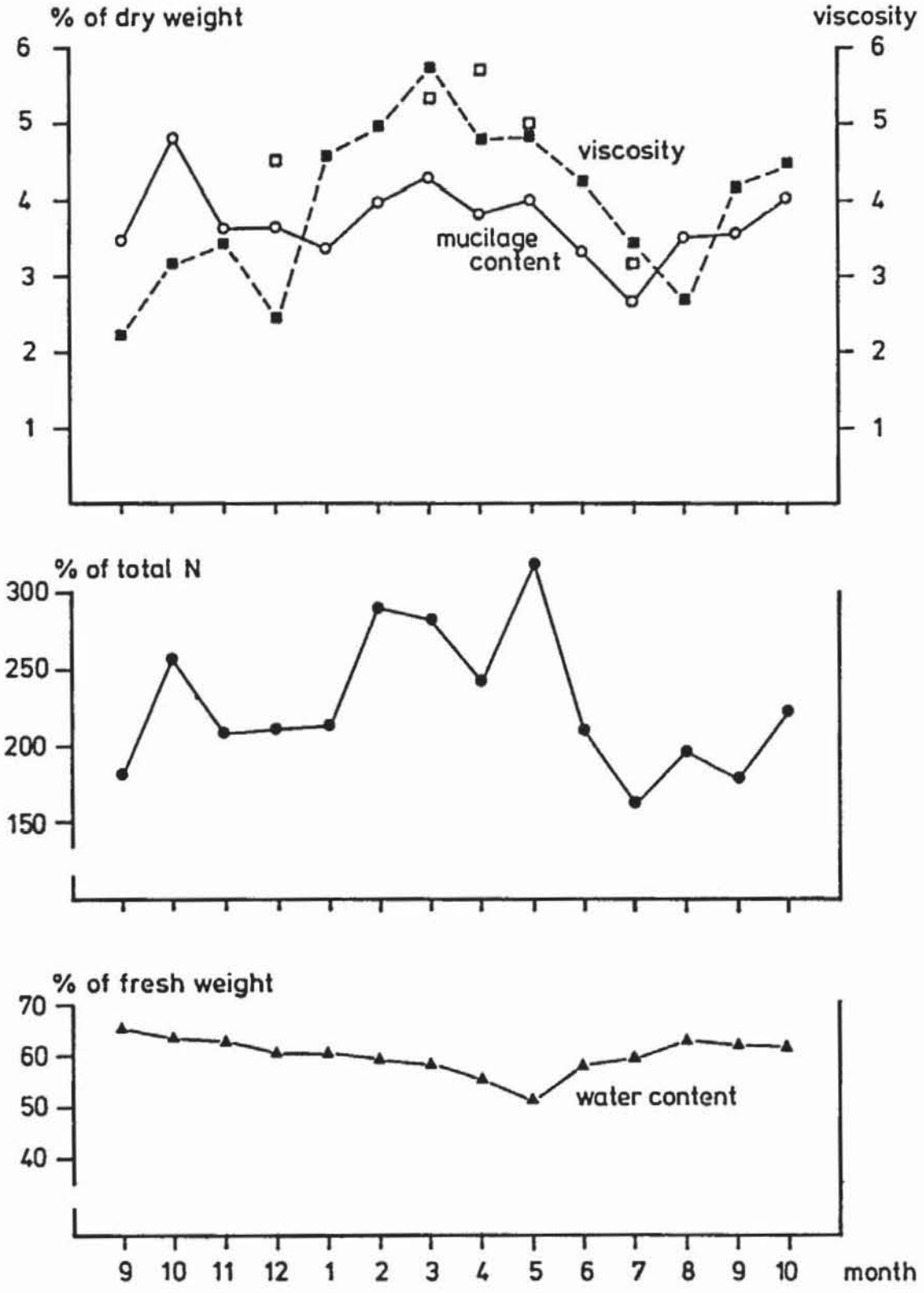

Fig. 1. *Taxus* needles. Seasonal variations of mucilage content as percentage of dry weight (upper diagram) and of total N (middle diagram). In the upper diagram, some measurements of mucilage content as percentage of dry weight from another year are shown as open squares. Viscosity of the plant extract is shown in the upper diagram, the water content as percentage of fresh weight in the bottom diagram.

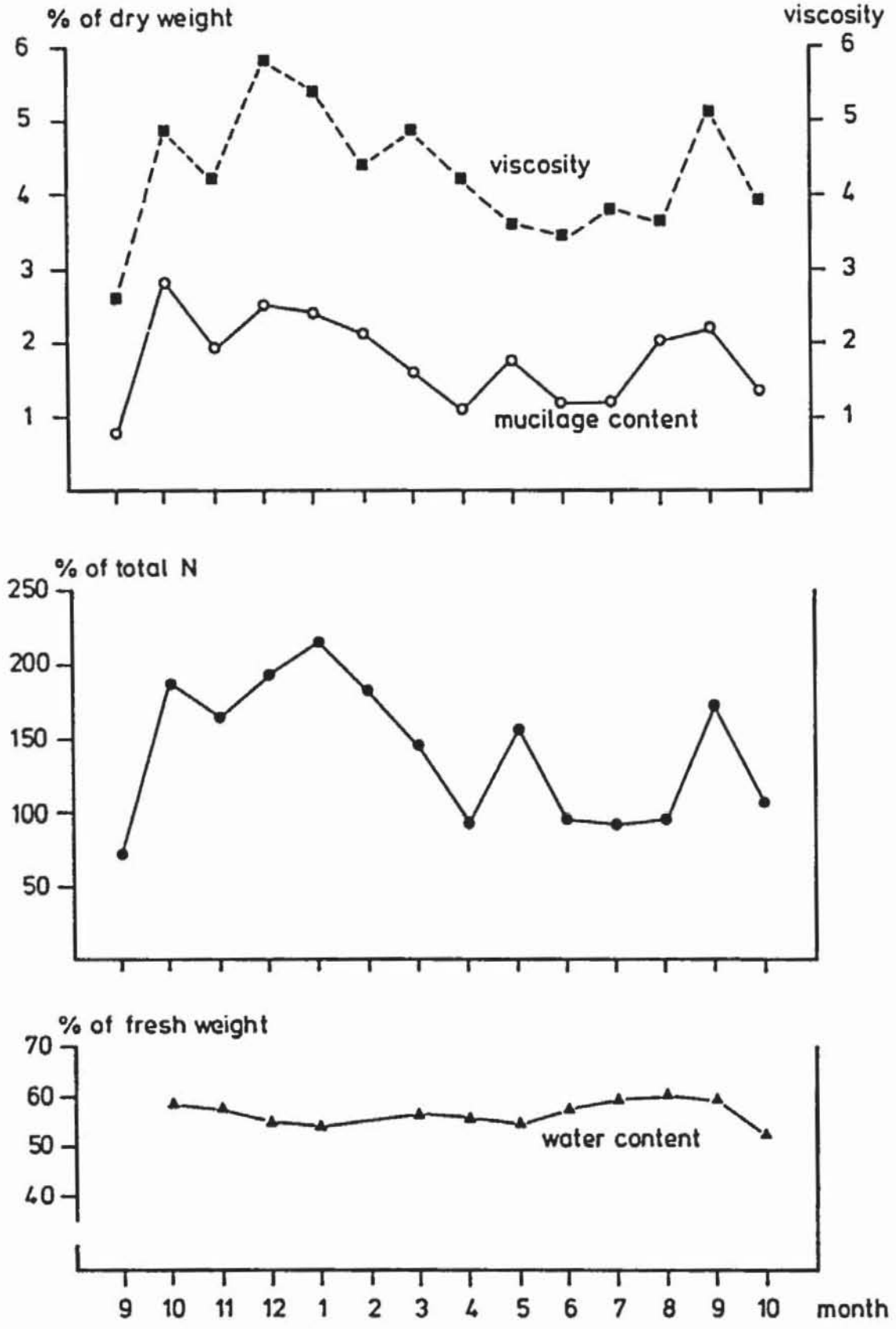

Fig. 2. *Thuja* needles. Seasonal variations of mucilage content as percentage of dry weight (upper diagram) and of total N (middle diagram). Viscosity of the plant extract is shown in the upper diagram, the water content as percentage of fresh weight in the lower diagram.

after 4 and 6 days respectively. The mucilage content showed no characteristic changes, although it did decrease significantly in the May and December experiments at −4°C after 6 and 13 days, respectively. However, in a December experiment with *Thuja* twigs (9 days), the mucilage content did not vary significantly. It can be concluded from these findings, that the storage of mucilages is not temperature dependent. A decrease in mucilage content is observed after a time lag and only at sub-zero temperatures and in cases when the water content of the needles decreases.

Characterization and Qualities of Taxus Mucilage

Composition. Determination of the total carbohydrate and peptide contents of the mucilage suggests an average proportion of 8–10% peptide component. When the mucilage is further purified by several rounds of precipitation, the proportion of carbohydrate to peptide components remains constant. Furthermore, it is not significantly altered by extraction with phenol/chloroform. From the total N content of the mucilage it can be seen that there is a higher ratio of the peptide component in the mucilage during winter and spring (maximal value, about 15% peptide) than during summer (minimal value, about 6%).

After hydrolysis of the mucilage, the sugars arabinose, xylose, glucose, galactose and rhamnose were found. In some cases the presence of very small amounts of

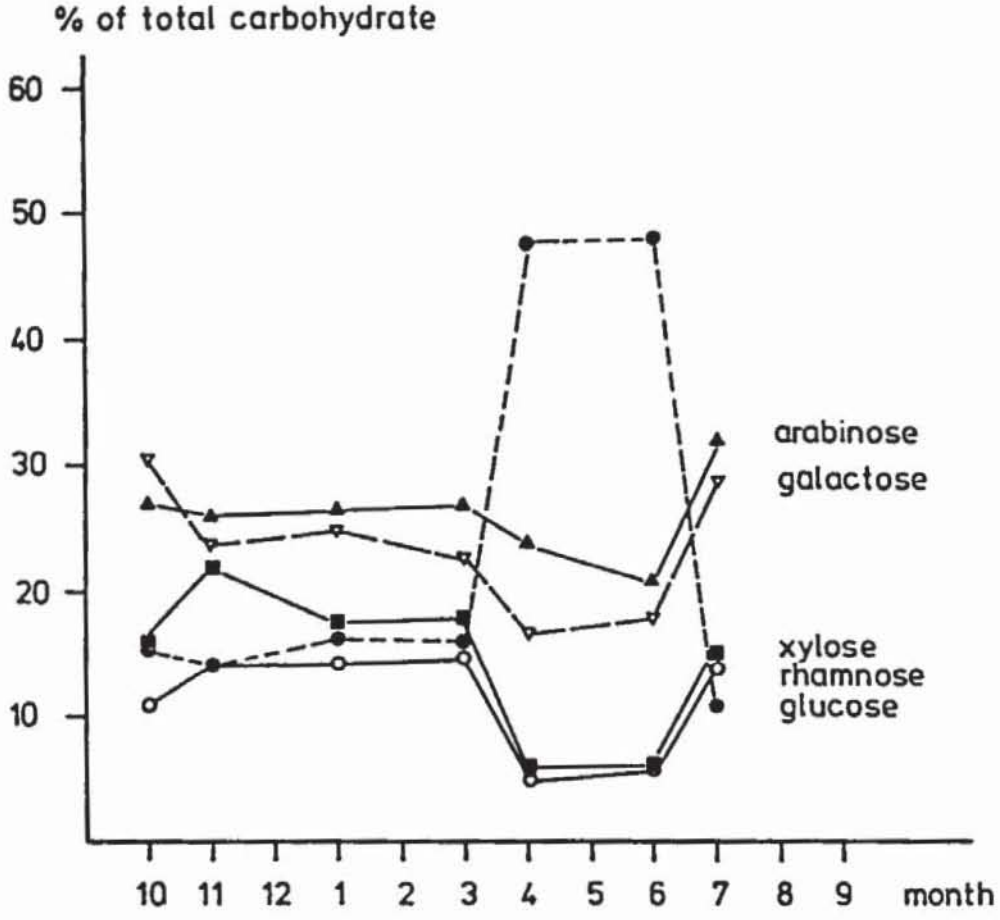

Fig. 3. *Taxus* mucilage. Seasonal variations of the sugar components, measured as percentage of total carbohydrates. The low uronic acid content (<5%) is not taken into consideration.

phenolic components was suggested, but this was not investigated further. Using the method of Blumenkrantz and Asboe-Hansen (1973), uronic acids in amounts of 3–5% of total mucilage carbohydrates could be measured. The sugar components show seasonal variations, especially from March to July (Fig. 3). These variations are most distinct with respect to glucose. The changes in glucose content during spring are opposite to those of the other sugar components, of which arabinose and galactose are especially important.

Gel chromatography. To find out whether the *Taxus* mucilage is a mixture of several polymers of different molecular weights we tried to fractionate the mucilage by gel-permeation chromatography using different gels. The experiments were performed on mucilage from leaves gathered in February.

By measuring the carbohydrate and peptide content of the eluted fractions we obtained one relatively broad peak in all cases (Fig. 4). The mucilage must therefore consist of a mixture of polymers with similar molecular weights. When mucilage was dissolved in 8M urea and again subjected to gel chromatography, no changes in the elution profile were found.

Some of the fractions obtained were hydrolyzed and subjected to sugar and N

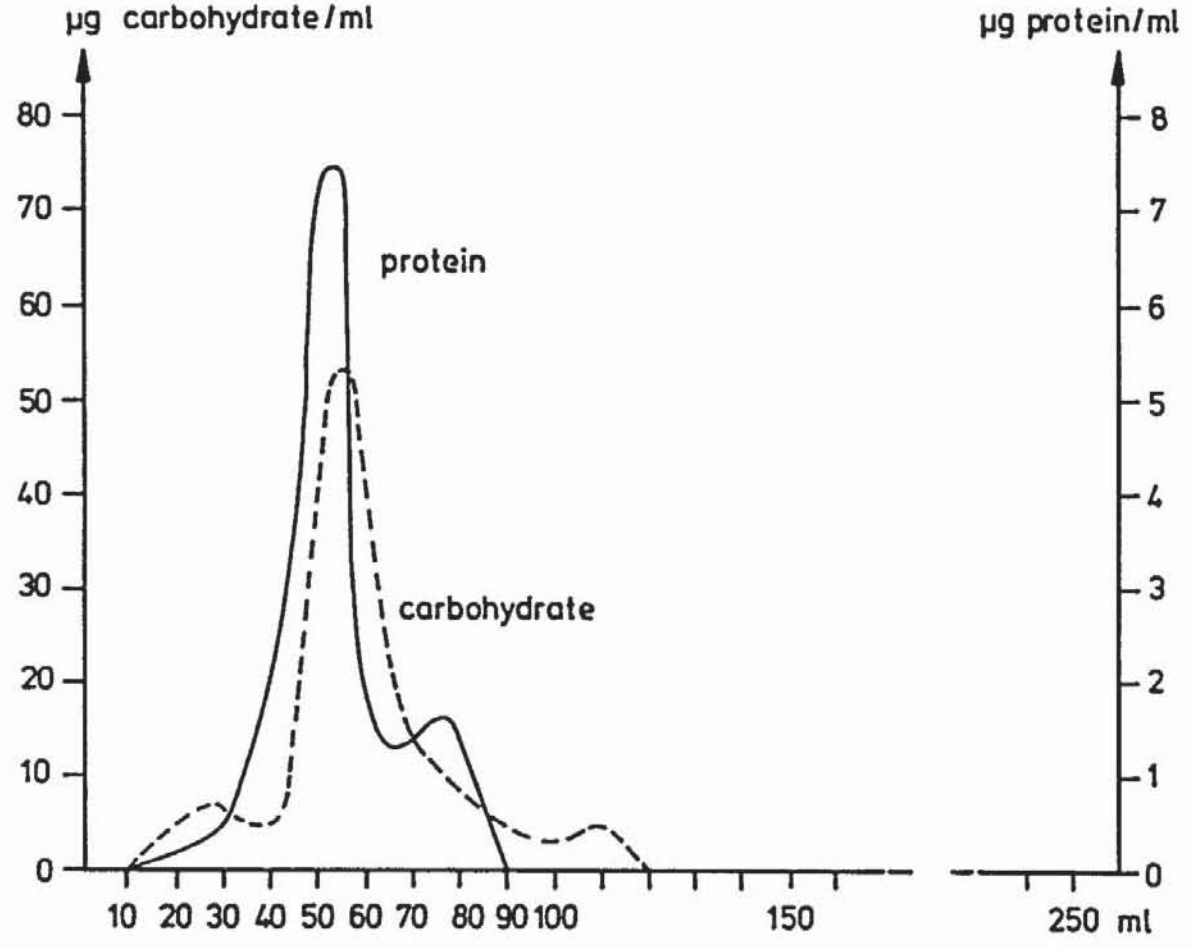

Fig. 4. Gel chromatography of *Taxus* mucilage on Ultragel AcA 22. Elution with Tris-HCl, pH 4.2 at 20°C.

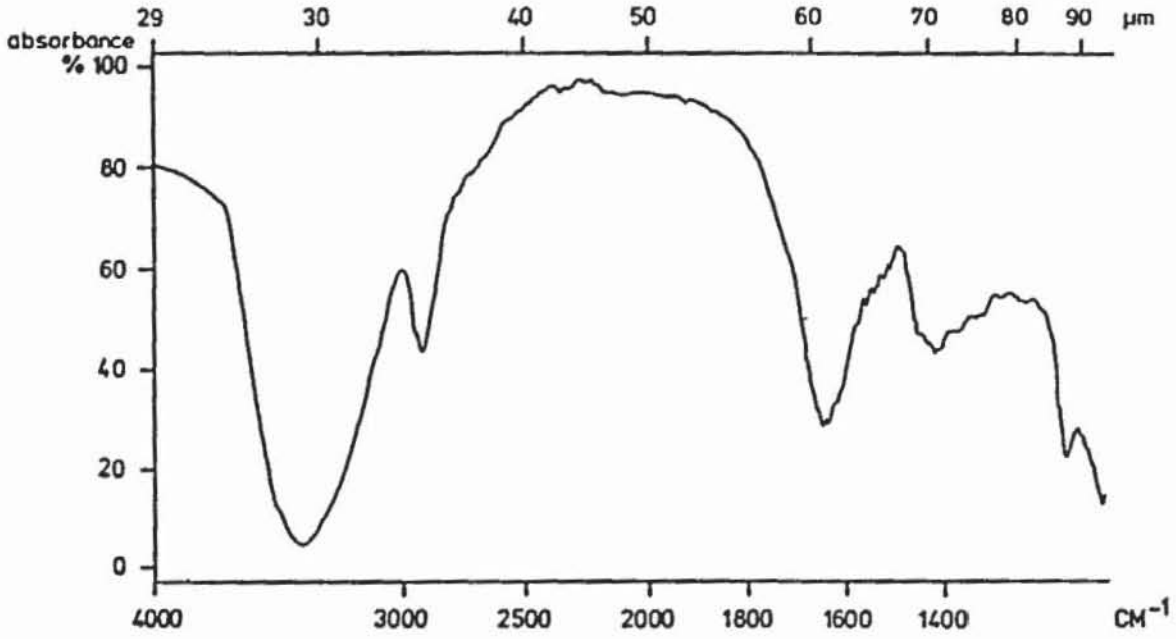

Fig. 5. IR spectrum of *Taxus* mucilage.

content determinations. In all cases the sugar composition was similar and in agreement with that obtained from the total mucilage hydrolysate. Likewise, the N content did not vary significantly.

IR-spectroscopy. IR spectra were measured from *Taxus* mucilage preparations (Fig. 5). The usual peaks of the sugars were distinct, but the peaks obtained in the ranges 1650–1620 and 1560–1510 cm^{-1} show that peptide bonds must be present. These peaks were not altered by purification of the mucilage samples.

An average of 25 spectra of the same sample was measured by a computer method, thereby diminishing the noise and allowing the identification of some weak peaks of peptide bonds in the area 1560–1510 cm^{-1}.

Water-binding capacity. Dried mucilage immediately takes up water and assumes a constant weight (water saturation) at the given humidity within 24 h. At relative humidities higher than 96% it takes a longer time to reach equilibrium. The water uptake at 20°C at different relative humidities is shown in Figure 6. In a water-saturated atmosphere mucilage has taken up 180% of its dry weight in water by 24 h. After 48 h the value is about 200% (i.e. it equilibrates to 280–300% of its dry weight). When the water-containing mucilage is brought into atmospheres of lower relative humidities, the process of water uptake is seen to be fully reversible.

Osmotic potential. The osmotic potential of *Taxus* mucilage solutions is proportional to the concentration. A solution of 0.5% (by weight) mucilage has an osmotic value of 20.10^5 Pa. In solutions with a higher mucilage concentration than 0.6% the osmotic potential could not be determined with precision using the

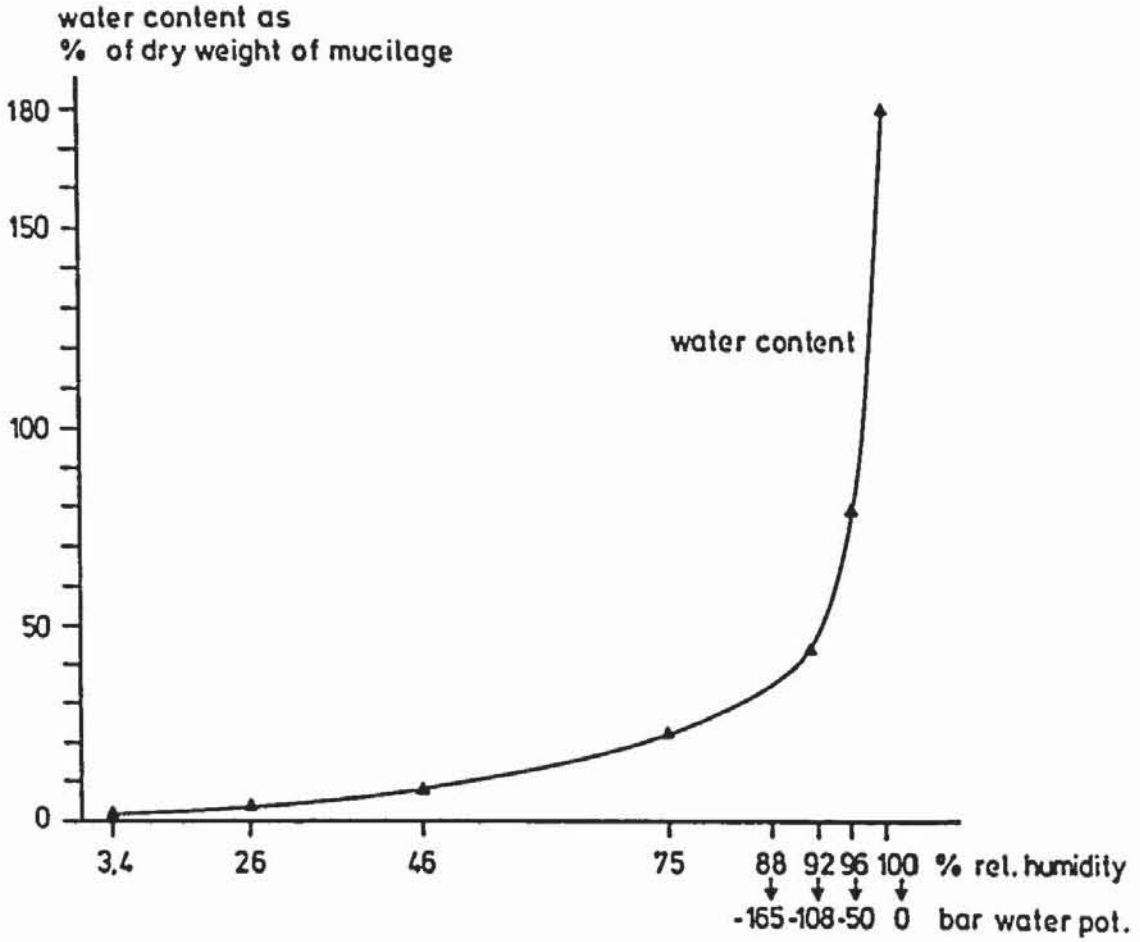

Fig. 6. Water-binding capacity of *Taxus* mucilage.

cryoscopic method because the high viscosity, despite heavy stirring, results in irregular and unequal freezing.

Measurements of osmotic potentials showed that viscosity of mucilage solutions rises markedly at low temperatures. This finding was investigated further by viscosimetry. The temperature coefficient of the increase, measured from the slope of the straight line obtained, is comparable to that observed with gum arabic (Kuhn, 1953). The concentration of Ca^{2+} ions in the solution has no influence on the viscosity and its increase at low temperatures. This may be expected for a mucilage with a very low uronic acid content.

Mucilage concentration. The results of our histological investigations, together with determinations of mucilage content, allow a calculation of mucilage concentrations. From the annual variations in mucilage content the range of the amount present in one leaf can be determined. The average weight of a fresh *Taxus* needle is about 15 mg and its volume about 0.015 ml. It contains more than 0.2–0.3 mg of mucilage. From measuring the area of mucilage-containing cells in diverse microphotographs the percentage of these cells in relation to the total volume of the leaf was found to be about 25–35%. Taking the higher value, the mucilage concentration in these cells is therefore higher than 5–6%.

DISCUSSION

Only a few investigations are concerned with mucilages of conifer leaves. The mucilage in *Thuja* was first observed by Prantl (1889) and in *Taxus* mesophyll cells by Mangin (1893). As in dicotyledons, the *Thuja* mucilage is localized in the extra-protoplastic compartment. The mucilage of *Taxus*, however, is found in vacuoles. Vacuolar mucilages have also been found in monocotyledons (Mollenhauer & Larson, 1966; Bouchet, 1982; Trachtenberg & Mayer, 1982a; Trachtenberg, 1984), mostly in specialized cells and sometimes together with oxalate crystals. The mucilage-containing cells of *Taxus*, on the other hand, are normal mesophyll cells. According to their composition, the mucilages of both *Taxus* and *Thuja* belong to the neutral mucilages which lack significant amounts of uronic acids. The results obtained from ruthenium red staining and the method of Blumenkrantz and Asboe-Hansen (1973) show the presence of a low percentage of uronic acids which are degraded to arabinose during the usual isolation procedure. The monomers arabinose, xylose, galactose and rhamnose are typical components of mucilages. Glucose was often considered to be a contamination, but several authors have shown this sugar to be a native component of mucilages (e.g. Haaland, 1969; Franz & Chladek, 1973; Woolfe et al., 1977; Paulsen & Lund, 1979; Naglschmid et al., 1982). Our data, especially the constant glucose percentage seen during several purification steps of the mucilage, as well as in diverse fractions of gel chromatography, also show that glucose is a component of *Taxus* mucilage. The N content of this mucilage must be caused by a peptide component, as confirmed by the IR spectrum. The peptide component is not separable from the carbohydrate moiety by phenol extraction or by gel chromatography. Therefore, there is most probably a covalent linkage between carbohydrate and peptide. Several findings relating to peptide components of native mucilages are known (e.g. Holzach & Fluck, 1950; Woolfe et al., 1977; Naglschmid et al., 1982) and recently data have accumulated showing that mucilages are in most cases proteoglycans. A proteoglycan structure has been established for the relatively well-investigated mucilages "gum arabic" (Akiyama et al., 1984) and that of *Linum* seeds (Heinze & Amelunxen, 1984). These proteoglycans may have lectin-like properties (Fountain, 1982), thereby perhaps producing crosslinking effects which may cause the broad peak found in our gel chromatography experiments. However, since the same sugar composition and the same proportional percentage of peptides were found in the different fractions, and since 8M urea did not alter the elution profile, we can then assume that *Taxus* mucilage is probably a mixture of polymers of similar molecular weights.

Changes in the mucilage content during the seasons or during plant ontogenesis are known from several investigations (e.g. Spegg, 1959; Franz, 1966; Breckle & Kull, 1973; Naglschmid et al., 1982). In *Taxus* leaves, the high mucilage contents in early spring and autumn coincide with blossoming and seed ripening. High amounts in spring, during flowering season, were also found by Höllwarth and Rump (1979). A high mucilage content was also measured in *Thuja* during seed ripening in autumn. This coincidence of high mucilage contents with periods of high activity and the failure to demonstrate a decrease in mucilage in the dark-treated twigs leads to the

conclusion that mucilage cannot function as a storage carbohydrate. In addition, when the seasonal variations of free sugars and starch in needles containing mucilage (*Taxus*, *Thuja*) are compared with those of species not containing mucilage in the leaves (*Juniperus*, *Larix*, Distelbarth et al., 1984), no significant deviation is found. Furthermore, in *Taxus* and *Thuja* there is no correlation between mucilage content and starch content or starch degradation, which has been observed by some other investigators (Hyde, 1970; Heinze & Amelunxen, 1984). Temperature variation has no effect on mucilage content. This is in agreement with the earlier findings on *Verbascum* (Naglschmid et al. 1982).

The water content of *Taxus* and *Thuja* needles remains remarkably constant throughout the year. In the temperature experiments the water content of *Taxus* needles remained constant for a significantly longer period than the water content of *Juniperus* needles in similar experiments. Perhaps the high water-binding capacity of *Taxus* mucilage can reduce water loss. The water-absorbing capacity reported here is significantly higher than that found in *Opuntia* mucilage by Trachtenberg and Mayer (1981). Because the water potentials (ψ) in leaf tissues are usually more positive than −60 bar (Walter, 1931; Larcher, 1980), the water-binding capacity of *Taxus* mucilage may have a physiological function in regulating the water content of the tissues. A minute variation of the water potential in the physiological range must lead to a relatively large alteration in the amount of mucilage-bound water. Therefore, as proposed earlier (Naglschmid et al., 1982), it may be concluded that the mucilage has a function as a "buffering system" in the stabilization of the water relations of the plant. The localization of *Taxus* mucilage in the vacuoles causes a significant matrix potential for the cell sap and therefore an increased resistance to water loss (cf. Williams, 1973). The potential measured for a 0.33% solution of *Taxus* mucilage is equivalent to the osmotic potential of a 0.5M sucrose solution ($14.2.10^5$Pa). The mucilage concentrations which are reached in the mesophyll cells are much higher (>5–6%).

The seasonal variations in sugar composition of *Taxus* mucilage allows the sugars to be grouped according to their behaviour: galactose with arabinose, and rhamnose with xylose. Glucose behaves independently and can be placed in a third category. This grouping is very similar to that found earlier in *Verbascum*. According to our ongoing investigations, the high glucose content of *Taxus* mucilage, particularly during spring and early summer, seems to be connected with the period of intensive mucilage synthesis. High percentages of glucose in mucilage during the period of maximum mucilage content were also observed in *Althaea* (Franz, 1966). In *Linum* seed coats, the glucose content is higher during mucilage synthesis than it is in the mucilage of the ripe seeds (Heinze & Amelunxen, 1984). These findings may also be relevant to leaf mucilages since the mechanism of mucilage secretion is apparently the same in different organs and species (Fahn, 1979); dictyosomes always appear to be involved (e.g. Schnepf, 1968; Mauseth, 1980; Trachtenberg & Fahn, 1981; Trachtenberg & Mayer, 1982b). It seems that during mucilage synthesis there is more glucose in the mucilage than in deposited material. The gel chromatography results suggest that *Taxus* mucilage is composed of polymeric forms having similar molecular weights and

containing all sugar components; however no attempts at gel chromatographic separation were made during the period of high glucose content of the mucilage.

The water balance of the leaves is connected with frost resistance of the tissue. The frost hardiness of *Taxus* leaves is high (Till, 1956; Melzack & Watts, 1982). In our experiments, hardened *Taxus* twigs held at −14°C for two weeks showed no damage of the needles by frost or dehydration. Mucilage in the tissues can apparently lead to higher cold resistance. As observed by Molisch (1897), freezing mucilage forms a network in which ice crystals are embedded. During melting, the water is taken up by the mucilage and the network disappears. We confirmed this observation on *Taxus* mucilage. Besides trapping ice crystals in a harmless state, vacuolar mucilage depresses the freezing point of cell sap and leads to a significant delay in freezing. Our experiments showed a characteristic freezing delay in 1% mucilage solutions. The mucilage concentration in *Taxus* mesophyll cells is much higher. Although ice is formed in the vacuole, the mechanical damage to the cell is reduced and retarded. Such a protective effect of mucilage was suggested by Trachtenberg and Mayer (1981). The effectiveness of mucilages as cryoprotectants may depend on their monomeric components and their polymeric structure and molecular weight (Olien & Smith, 1981). Mucilages containing high uronic acid levels show additional nonspecific colligative dilution of ions injurious to freezing tissues (Heber & Santarius, 1973). Investigations on freezing injury of winter wheat led Williams and Hope (1981) to conclude that water loss leads to a decrease in cellular volume in which lipid is lost from the membrane. This lipid loss is irretrievable, at least in the short term. Upon deplasmolysis the membrane may be damaged, thus killing the cell (Steponkus et al., 1981; Willing & Leopold, 1983; Mittelstädt & Müller-Stoll, 1984). We postulate that in *Taxus* a function of intravacuolar mucilage is to maintain cell volume, thereby avoiding losses in membrane lipids. Due to their net negative character, acid mucilages may function as "cation-buffers" (Trachtenberg & Mayer, 1982c). Acid mucilages may act as cationic sinks which modulate cation levels and prevent abrupt changes (Kloareg, 1981). However, in *Taxus* the cation content of the mucilage from different habitats did not differ significantly and was low (Höllwarth, pers. commun.), which is in agreement with its character as a neutral mucilage.

Mucilages found in rhizomes, roots and seed endosperms may act primarily as reserve substances. In contrast, our results lead to the conclusion that the leaf mucilages of *Verbascum* (Naglschmid et al., 1982) and *Taxus* are not storage carbohydrates, but probably have a primary function in the regulation of the tissue water balance. In comparison to other conifers, *Taxus* and *Thuja* leaves are less xeromorphous. This may be explained by the ability of mucilages to act as "buffering systems" which stabilize the water content, thereby increasing frost hardiness. Although such a system is genetically fixed, it is still highly dependent on the environment, thereby allowing for modifying and modulative adaptations.

ACKNOWLEDGEMENTS

We wish to thank Prof. W. Neugebauer and Dr. F. Oechssler for supplying us with plant material, Dr. K. Roessel for providing the IR spectra, Prof. K.W. Mundry for

valuable discussions, Mrs. B. Schreiter for help in manuscript preparation and Mr. U. Baechle for graphics. We gratefully acknowledge the correction of the English and thoughtful comments on the manuscript by Mr. Richard Cyr, University of California, Irvine.

REFERENCES

Akiyama, Y., S. Eda and K. Kato. 1984. Gum arabic is a kind of arabinogalactan-protein. Agric. Biol. Chem. 48: 235–237.

Aspinall, G.O. 1969. Gums and mucilages. Adv. Carbohydr. Chem. 24: 333–379.

Blumenkrantz, N. and G. Asboe-Hansen. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54: 484–489.

Bouchet, P. 1982. Etude ultrastructurale des cellules muco-oxalifères d'une Commélinacée: *Zebrina pendula* Schnizl. Bull. Soc. Bot. Fr. Actual. Bot. 129: 29–35.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Breckle, S.W. and U. Kull. 1973. Ist *Diarthron vesiculosum* (Thymelaeaceae) ein ökologisches Rätsel? II. Die Wirkung der Dürre auf Mineralstoffverhältnisse und Kohlenhydrathaushalt. Bot. Jahrb. Syst. 93: 539–561.

Clarke, A.E., R.L. Anderson and B.A. Stone. 1979. Form and function of arabinogalactans and arabinogalactan-proteins. Phytochemistry 18: 521–540.

Dexheimer, J. and F. Guenrin. 1981. Etude des modalités de la secretion de mucilage par les trichomes stipulaires de *Psychotria bacteriophila* (Rubiacées). Cytologia 46: 731–747.

Distelbarth, H. and U. Kull. 1983. Significant role of *Taxus* mucilage in the water balance. Plant Physiol. 72, Suppl. 14.

Distelbarth, H., U. Kull and K. Jeremias. 1984. Seasonal trends in energy contents of storage substances in evergreen gymnosperms growing under mild climatic conditions in Central Europe. Flora 175: 15–30.

Fahn, A. 1974. Plant Anatomy. 2nd ed. Pergamon Press, Oxford.

Fahn, A. 1979. Secretory Tissues in Plants, Academic Press, London, New York, San Francisco.

Fountain, D.W. 1982. Lectin-like properties associated with mucus and mucilage of diverse biological origin. Naturwissenschaften 69: 450–451.

Franz, G. 1966. Die Schleimpolysaccharide von *Althaea officinalis* und *Malva sylvestris*. Planta Med. 14: 89–110.

Franz, G. 1979. Metabolism of reserve polysaccharides in tubers of *Orchis morio* L. Planta Med. 36: 68–73.

Franz, G. and M. Chladek. 1973. Vergleichende Untersuchungen über die Zusammensetzung von Rohschleimen aus Kreuzungsnachkommen von *Althaea officinalis* L. × *Althaea armeniaca* Ten. Pharmazie 28: 128–129.

Gauba, E. 1927. Beiträge zur biologischen Anatomie des Koniferenblatts. II. Die Schleime und die Schleimbehälter. Biol. Gen. 3: 281–316.

Haaland, E. 1969. Water-soluble polysaccharides from the leaves of *Tussilago farfara* L. Acta Chem. Scand. 23: 2546–2548.

Heber, U. and K.A. Santarius. 1973. Cell death by cold and heat and resistance to extreme temperatures. Mechanisms of hardening and dehardening. In: H. Precht, J. Christophersen, H. Hensel and W. Larcher, eds. Temperature and Life. Berlin. pp. 232–263.

Heinze, U. and F. Amelunxen. 1984. Zur Schleimbildung in *Linum*-Samen. Elektronenmikroskopische und chemische Analysen. Ber. Dtsch. Bot. Ges. 97: 451–464.

Hillestad, A., I.K. Wold and B.S. Paulsen. 1977. Structural studies of water-soluble glycoproteins from *Cannabis sativa* L. Carbohydr. Res. 57: 135–144.

Höllwarth, M. and H.H. Rump. 1979. Beiträge zur Immissionssituation in Hessen. Inst. Naturschutz Darmstadt, Schriftenreihe 11(4): 1–107.

Holzach, O. and H. Flück, 1950. Untersuchungen über Zusammensetzung und Aufbau der Schleimstoffe von *Tamus communis* L. Pharm. Acta Helv. 25: 300–336.

Hyde, B.B. 1970. Mucilage producing cells in the seed coat of *Plantago ovata*: developmental fine structure. Am. J. Bot. 57: 1197–1206.

Jarvis, M.C., M.A. Hall, D.R. Threlfall and J. Friend. 1981. The polysaccharide structure of potato cell walls: chemical fractionation. Planta 152: 93–100.

Kjeldahl, J. 1883. Über die Stickstoffbestimmung. Z. Analyt. Chem. 22: 366–370.

Kloareg, B. 1981. Structure et rôle écophysiologique des parois des Algues littorales: contribution à la resistance aux variations de salinité. Physiol. Veg. 19: 427–441.

Kram, G. and G. Franz. 1983. Untersuchungen über die Schleimpolysaccharide aus Lindenblüten. Planta Med. 49: 149–153.

Kuhn, A. 1953. Kolloidchemisches Taschenbuch, 4th ed. Leipzig.

Larcher, W. 1980. Physiological Plant Ecology, 2nd ed. Berlin.

Loewus, F. 1952. Improvement in anthrone method for the determination of carbohydrates. Anal. Chem. 24: 219.

Lyshede, O.B. 1977. Studies on the mucilaginous cells in the leaf of *Spartocytisus filipes* W.B. Planta 133: 255–260.

Mangin, M.L. 1893. Sur les cellules mucifères et résinifères du *Taxus baccata*. Bull. Soc. Bot. Fr. 40: 313–316.

Mauseth, J.D. 1980. A stereological morphometric study of the ultrastructure of mucilage cells in *Opuntia polyacantha* (Cactaceae). Bot. Gaz. 141: 374–378.

Melzack, R.N. and D. Watts. 1982. Cold hardiness in the yew (*Taxus baccata* L.) in Britain. J. Biogeogr. 9: 231–241.

Mittelstädt, M. and W.R. Müller-Stoll. 1984. Veränderungen der Vakuolengröße in Rindenparenchymzellen von Gehölzen nach Frosteinwirkung. Flora 175: 231–242.

Molina-Cano, J.L. and J. Conde. 1982. Genetic and environmental variation of gum content in barley. J. Inst. Brew. 88: 30–33.

Molisch, H. 1897. Untersuchungen über das Erfrieren der Pflanzen. Jena.

Mollenhauer, H.H. 1967. The fine structure of mucilage secreting cells of *Hibiscus esculentus* pods. Protoplasma 63: 353–362.

Mollenhauer, H.H. and D.A. Larson. 1966. Developmental changes in raphide-forming cells of *Vanilla planifolia* and *Monstera deliciosa*. J. Ultrastruct. Res. 16: 55–70.

Naglschmid, F., U. Kull and K. Jeremias. 1982. Physiologische Untersuchungen über Blattschleime. I. Untersuchungen an *Verbascum densiflorum*. Biochem. Physiol. Pflanz. 177: 671–685.

Olien, C.R. and M.N. Smith. 1981. Protective systems that have evolved in plants. In: C.R. Olien and M.N. Smith, eds. Analysis and Improvement of Plant Cold Hardiness. C.R.C. Press, Boca Raton. pp. 61–87.

Paulsen, B.S. and P.S. Lund. 1979. Watersoluble polysaccharides of *Opuntia ficus-indica* cv "Burbank's Spineless". Phytochemistry 18: 569–571.

Prantl, K. 1889. Natürliche Pflanzenfamilien, 1st ed. Part 2. Leipzig. p. 40.

Schnepf, E. 1968. Zur Feinstruktur der schleimsezernierenden Drüsenhaare auf der Ochrea von *Rumex* und *Rheum*. Planta 79: 22–34.

Schömmer, F. 1949. Kryptogamenpraktikum. Stuttgart.

Spegg, H. 1959. Untersuchungen zur Lage, Ausbildung und Funktion schleimführender Gewebe bei Malvaceen und Tiliaceen. Planta Med. 7: 8-23.

Steponkus, P.L., J. Wolfe and M.F. Dowgert. 1981. Stresses induced by contraction and expansion during a freeze-thaw-cycle: a membrane perspective. In: G.J. Morris and A. Clarke, eds. Effects of Low Temperatures on Biological Membranes. London. pp. 307–322.

Sutton, B.G., I.P. Ting and R. Sutton. 1981. Carbohydrate metabolism of a cactus in a desert environment. Plant Physiol. 68: 784–787.

Till, O. 1956. Über die Frosthärte von Pflanzen immergrüner Laubwälder. Flora 143: 499–542.

Trachtenberg, S. 1984. Cytochemical and morphological evidence for the involvement of the

plasmamembrane and plastids in mucilage secretion in *Aloe arborescens*. Ann. Bot. 53: 227–236.

Trachtenberg, S. and A. Fahn. 1981. The mucilage cells of *Opuntia ficus-indica* (L.) Mill. Development, ultrastructure and mucilage secretion. Bot. Gaz. 142: 206–213.

Trachtenberg, S. and A.M. Mayer. 1981. Composition and properties of *Opuntia ficus-indica* mucilage. Phytochemistry 20: 2665–2668.

Trachtenberg, S. and A.M. Mayer. 1982a. The lytic compartment of disintegrating (*Opuntia ficus-indica*) and non-disintegrating (*Aloe arborescens*) mucilage cells. Biol. Cell. 43: 129–134.

Trachtenberg, S. and A.M. Mayer. 1982b. Quantitative autoradiography of mucilage secretion in *Opuntia ficus-indica* (L.) Mill. Biol. Cell. 44: 69–76.

Trachtenberg, S. and A.M. Mayer. 1982c. Biophysical properties of *Opuntia ficus-indica* mucilage. Phytochemistry 21: 2835–2843.

Walter, H. 1931. Die Hydratur der Pflanze und ihre physiologisch-ökologische Bedeutung (Untersuchungen über den osmotischen Wert). Jena.

Walter, H. and K. Kreeb. 1970. Die Hydratation und Hydratur des Protoplasmas der Pflanzen und ihre ökophysiologische Bedeutung. Protoplasmatologia IIC6, Vienna.

Williams, R.J. 1973. Osmotic properties of glycoproteins from hardy dogwood trees. Plant Physiol. 51, Suppl. 25.

Williams, R.J. and H.J. Hope. 1981. The relationship between all injury and osmotic volume reduction. III. Freezing injury and frost resistance in winter wheat. Cryobiology 18: 133–145.

Willing, R.P. and A.C. Leopold. 1983. Cellular expansion at low temperature as a cause of membrane lesions. Plant Physiol. 71: 118–121.

Woolfe, M.L., M.F. Chaplin and G. Otchere. 1977. Studies on the mucilages extracted from okra fruits (*Hibiscus esculentus* L.) and baobab leaves (*Adansonia digitata* L.). J. Sci. Food Agric. 28: 519–529.