

Longevity and senescence of needles in *Pinus cembra* L.

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Summary. Needle development has been investigated in *Pinus cembra* at several locations in the Grisons. Special attention has been paid to longevity and senescence. Despite large variations from one tree to another, longevity (up to 12 years) appeared to increase with increasing altitude. With increasing age, contents of nutrients such as N, P, K and, to a lesser extent, Mg and S tend to decrease. Photosynthetic activity did not decline with increasing age of short shoots. Senescence begins in mid-August in needles of the oldest age classes. It is associated with the mobilization and withdrawal of large proportions of N, P, K and also Mg and S, but Ca appears to be immobile. The extent of yellowing is irregular in individual trees but needle losses on a large scale are followed by reduced losses in subsequent years.

Key words: *Pinus cembra* – Needle longevity – Needle senescence – Nutrient elements

Introduction

The yellowing of senescent needles of stone pine is a conspicuous phenomenon taking place in late summer between mid-August and the first half of September. Rather surprisingly, it has so far not been mentioned in the literature, although it has frequently been observed. Yet in recent years concerns about forest decline have given rise to the suspicion that the extensive yellowing and loss of needles in stone pine may represent another example of novel damage caused by air pollution. Inquiries among officers of alpine forest services in Switzerland have yielded an ambiguous result. The phenomenon has been attributed to special circumstances such as drought. Interestingly, the majority of the officers claimed that extensive

yellowing of needles has only been observed in stone pine in the past few years.

Leaves of evergreen trees have a limited life span. Hence, foliar senescence and abscission must be a natural process, which in the case of *Pinus cembra* has so far not been investigated properly. It is obvious that any reasoning about the novelty of extensive loss of needles must be based on solid knowledge about foliar development in this species. The present study has been carried out in order to fill the gap in knowledge by following some relevant parameters of needle development in trees growing under a range of different local conditions. Emphasis was placed on changes during aging of needles and particularly on the withdrawal of nutrient elements during senescence, which has been studied in several species of deciduous trees (Gäumann 1935; Tamm 1951; Oland 1963; Specht-Jürgensen 1967; Verdu et al. 1985; Millard and Thomson 1989) but never, to our knowledge, in coniferous evergreens.

Materials and methods

Selection of trees. A total of 28 trees were selected for periodic observations; 14 of them were used for sampling of needles and various determinations. Trees were selected to cover a large range of altitudes, climatic conditions and exposures as well as age and vigour. An unavoidable criterion was the accessibility of trees for sampling purposes. Trees injured by cattle or tourists were not considered. The local conditions at the sites at St. Moritz (1780–1820 m), Morteratsch (1900 m), Schindelboden/Flüela (1900–2140 m) and Stillberg/Dischma (1970–2100 m) in the Grisons, Switzerland, are detailed elsewhere (Nebel 1990). The heights ranged from 3 m to 18 m. For comparison, a tree at Birmensdorf (560 m) was included. Soils in the Grisons were acidic (pH 3–5); those at St. Moritz and Morteratsch were deep and adequately provided with nutrients; at Schindelboden and Stillberg they were shallow and levels of nutrients were suboptimal. At Morteratsch, Schindelboden and Stillberg the presence of rocks caused a pronounced heterogeneity regarding soil depth.

Sampling of shoots and needles. Parameters of needle senescence were analysed in bulk collections of material from individual trees grouped according to yellowing stages as scored subjectively. Thus, class 1 comprised presenescent needles of the oldest generation, class 2 needles at incipient senescence (indication of the start of chlorophyll loss), class 3

Table 1. Dependency of long shoot yearly increments and numbers of short shoots per age class on the altitude above sea level; mean values and ranges of all data collected from long shoots produced 1978 through 1988

Altitude (m)	Long shoots		Short shoots	
	Increments (cm)	Range	Numbers per age class ^a	Range
2050–2150	3.0 ± 0.9	1.8–4.8	22.4 ± 6.3	15–33
1950–2050	3.9 ± 1.0	1.7–5.1	21.6 ± 5.0	15–30
1900	3.8 ± 1.9	2.3–7.3	22.4 ± 6.4	9–36
1820	4.7 ± 1.5	1.0–9.3	26.8 ± 6.9	18–41
560	5.8 ± 1.4	4.0–7.7	31.1 ± 4.3	30–40

^a Numbers include scars of missing short shoots in older age classes

light yellow needles with traces of residual chlorophyll, class 4 completely yellowed but fully turgescient needles, class 5 yellowed needles with first indications of browning and desiccation, and class 6 dead and newly abscised needles picked up off the ground. For the determination of various parameters such as numbers of short shoots, weight, length, surface, contents of nutrient elements of needles etc., whole twigs of known expositions were harvested. For technical reasons all determinations concern twigs inserted at up to 2 m above ground.

Measurements. Short shoots represent developmental units characterized by a remarkable uniformity of the five needles. Individual short shoots excised at random from each of the various age classes of a long shoot were divided into two groups of two needles used for analyses and a single needle for determination of needle surface (Delta-T leaf area meter, taking the projected area as one-third of the total surface). Two needles were extracted with 80% acetone and analysed for chlorophyll content according to Lichtenthaler and Wellburn (1983). Individual pigments were separated by high performance liquid chromatography and quantified as outlined by Gut et al. (1987). The other pair of needles was employed for the determination of fresh weight, dry weight (48 h, 60° C), and total reduced nitrogen (Strauch 1965).

For the analysis of various nutrient elements needles were first washed with chloroform to remove the wax. This treatment was associated with an insignificant loss of nutrient elements such as K, Mg, Ca, P, S (<1%). The weight of wax as determined after evaporation of the solvent accounted for 3% of the total dry weight. After drying (48 h, 60° C) the needles were incinerated (12 h, 500° C) and the ash was thereafter extracted (5 ml 30% HCl per 1 g initial dry weight) for 30 min at 70° C. After addition of 2–3 ml 10% nitric acid the extracts were made up to 25 ml with distilled water and filtered. ICP emission spectrophotometry was employed for the analysis of various elements.

Gas exchange. A porometer LI-6200 (LI-COR, Lincoln, Neb., USA) was employed (Welles 1986). Segments of long shoots comprising 1–3 age classes of short shoots were enclosed in the cuvette. For proper sealing of the cuvette it was necessary to remove short shoots at the boundaries between age classes. Carbon dioxide exchange and transpiration were measured in selected twigs at intervals over 1 day. In the course of measuring periods (20 s) the loss of CO₂ in the cuvette never exceeded 50 ppm (initial concentrations were approximately 340 ppm). Measurements were normally carried out under conditions of natural illumination (8–2000 photon flux density μmol m⁻²s⁻¹). Occasionally the performance of severely shaded old short shoots was checked at light saturation employing a halogen lamp (approximately 1000 PFD μmol m⁻²s⁻¹ at the level of the cuvette). After completion of measurements the twigs were harvested for the determination of needle surfaces.

Results and discussion

Aging and longevity

Depending on the weather conditions and altitude shoots emerge between the end of May and mid-June. The short shoots reach their final length between late July and the end of August. The elongation of long shoots precedes the growth of needles. Thus, long shoots have nearly completed their growth when short shoots enter the period of rapid elongation.

It is not surprising that all variables of shoots and needles vary with altitude of the location as well as with the

Table 2. Dependencies of long shoot increments, numbers of short shoots per age class, and needle length on the orientation of twigs

	Orientation			
	South	West	North	East
Increments of long shoots (cm) ^a	4.6 ± 1.82 ^d	4.1 ± 1.53	4.1 ± 2.35	3.7 ± 1.53
Number of short shoots per age class ^a	26.5 ± 7.87 ^d	22.2 ± 7.52	24.0 ± 8.20	22.5 ± 7.93
Length of needles (cm) all trees ^b	7.4 ± 0.86	7.1 ± 0.93	6.9 ± 1.10	7.1 ± 1.20
Selected trees				
St. Moritz 1820 m	7.8	7.3	7.2	7.6
Morteratsch 1900 m	7.3	4.8 ^c	5.5 ^c	6.4
Flüela 1900 m	7.4	6.9	6.9	7.7
Flüela 2140 m	6.9	6.2	5.8	6.2
Stillberg 2140 m	6.3	6.8	6.6	6.8

^a Means 1982–1988, all trees examined

^b Average of 57 twigs from 18 trees, 1982–1989 (standard deviation)

^c Twigs severely shaded within the tree

^d Means of standard deviations calculated for each age class

Table 3. Longevity of needles as dependent on the altitude and on the orientation of the twig; maximal needle ages determined in a total of 57 twigs from 18 trees (summer 1988)

Number of age classes ^a	Altitude			
	560 m	1820–1900 m	1970–2020 m	2080–2140 m
Mean	6.2 ± 0.4	7.4 ± 1.3	8.7 ± 2.0	7.4 ± 2.3
Range	6–7	5–9	5–9	3–12
	Orientation of twig			
	South	West	North	East
Mean	7.2 ± 1.9	7.8 ± 1.5	7.8 ± 1.8	8.3 ± 2.3
Range	3–9	5–10	5–11	3–12

^a Maximal needle age = total number of age classes – 1

Table 4. Quotients of needle losses in two trees at A, St. Moritz (1820 m) and B, Flüela (2140 m) in three consecutive senescence periods

	Exposure of twigs			
	South	West	North	East
Tree A				
1987	0.95	0.85	0.90	1.05
1988	0.80	0.70	1.60	0.40
1989	0.25	0.90	0.80	ND
Tree B				
1987	1.15	1.75	2.90	3.20
1988	0.30	0.20	0.70	0.00
1989	0.70	0.80	0.60	0.10

Tree A is partially shaded by neighbouring tree except for eastern exposed twigs; B is not shaded. The quotient of needle loss is defined as the number of senesced needles divided by the average number of annually produced needles of a long shoot
ND, Not determined

orientation of twigs. Moreover, within a single location, there are marked differences from one tree to another that may be due to one or several parameters such as age of the tree, shading by neighbouring trees, local conditions in the soil and the like. Table 1 summarizes mean values and ranges of values from determinations made in 1987 and 1988. Although the mean values indicate that yearly increments of long shoots and numbers of short shoots per age class tend to decrease with increasing altitude above sea level, the variations observed within a given altitude may weaken the stringency of this result. Heterogeneity within individual trees may be caused by severe shading of twigs or by the weather conditions during growth. The grouping of the data according to the orientation of the twigs yielded values summarized in Table 2. It appears that southern oriented long shoots reach a greater length and have higher numbers of short shoots as compared to all other orientations. Southern oriented twigs also produce the longest needles, and the data obtained from selected trees suggest that needle lengths tend to decrease with increasing altitude.

The short shoots stay on the tree for up to 12 years. Longevity appears to depend on both altitude and orientation of twigs. The data compiled in Table 3 demonstrate the

large variations of longevity; thus, both minimal and maximal numbers of age classes were observed in trees at the timber line. Despite these variations the data indicate increasing longevity with increasing altitude up to 2000 m. In southern oriented twigs longevity appears, from the average of the 18 trees examined, to be lower than in eastern oriented twigs.

In the individual tree senescence and abscission may cause considerable fluctuations of numbers of age classes from one year to another. Shedding of needles occurs mainly from the oldest age classes of a twig. It is, however, not restricted to a single class but may extend over short shoots of three to six generations. Extensive yellowing and subsequent shedding have been observed repeatedly in individual trees and even in individual twigs. It is interesting to note that extensive losses were compensated by reduced sheddings in the years following such events. As a quantitative expression for corresponding changes of total needle numbers of a twig, a quotient of needles lost, over the average number of needles produced annually, was calculated. A quotient of 1 thus indicates a constant total number of age classes. In Table 4 examples of fluctuations in branches of two selected trees are compiled. In tree B extensive needle losses occurred in 1988 particularly in northern and eastern oriented twigs; in the two following vegetation periods the quotients of needle loss were markedly below average, even zero in eastern oriented twigs. As a consequence of such compensations the total number of age classes may remain constant over periods of several years.

Aging of needles

As needles grow older they acquire increasing dry weights per unit of fresh weight. Fully developed needles of the youngest age class have specific dry weights ranging from 0.32 to 0.41. These values increase progressively up to 0.39–0.52 at age 5 years and older. As a consequence of these changes, the age-dependent changes of contents of nutrient elements which were assessed on a dry weight basis are slightly underestimated. Thus, the decrease of nitrogen contents illustrated in Fig. 1 would be even more pronounced if expressed per needle instead of dry weight

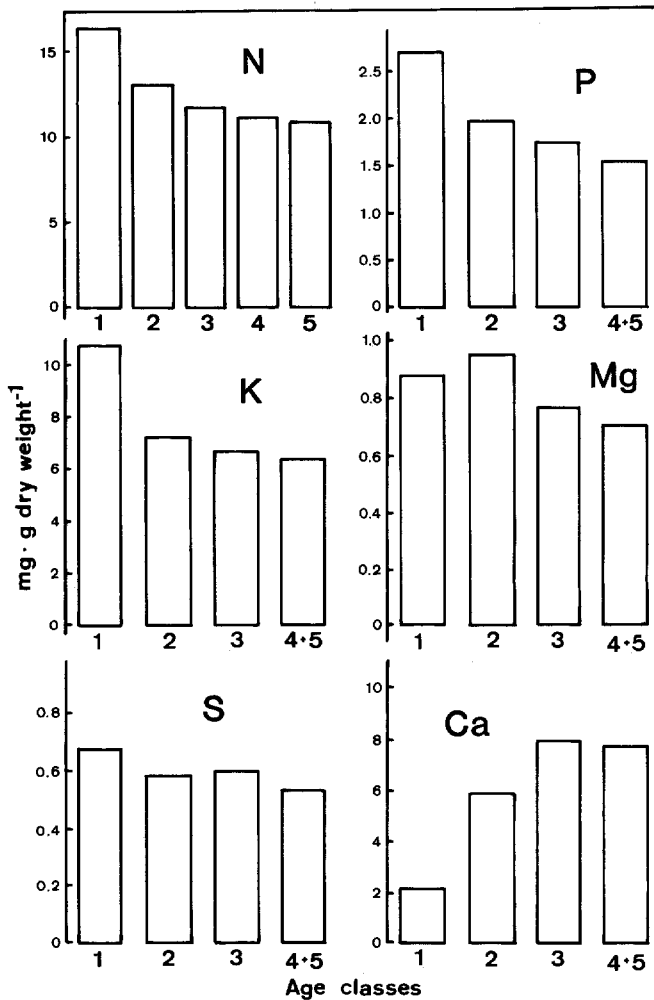


Fig. 1. Changes of contents of various nutrients in needles of different age classes. The needles were harvested on 2 September 1987 from a tree at Stillberg (2070 m)

unit. Similar age-dependent decreases were found in the case of P and K. It appears from Fig. 1 that losses were particularly marked from the 1st to the 2nd year. Age-dependent losses of Mg and S were much less pronounced than those of N, P and K. In the case of Ca the analyses indicated a dramatic increase from the first up to the third age class of needles. It should be noted that qualitatively similar changes were observed in trees from different locations despite large quantitative differences from one tree to another. Thus, the N contents present in 2- to 6-year-old needles of 11 trees ranged from 9.5 to 20.3 mg/g dry weight. Large differences were observed between trees growing under apparently similar local conditions; there are no obvious explanations for this heterogeneity.

Since the principal function of needles is photosynthesis it is logical to consider age-dependent decline of CO₂ fixation as a factor which could determine the life-span of the organ. However, the assessment of photosynthetic gas exchange in the different age classes of individual twigs did not show the expected decline with increasing age of needles. It appears from Fig. 2 that under the natural conditions of illumination fixation rates were very similar in the

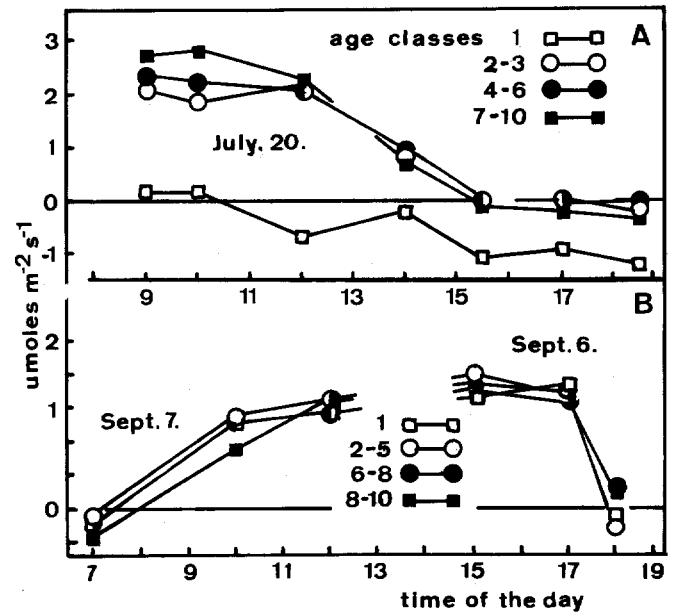


Fig. 2 A, B. Two examples of photosynthetic activities in short shoots of different age classes of a twig under natural illumination. A South-east oriented twig analysed on 20 July 1989. Decline of activities after noon is due to drop of PFD from approximately 1500 to approximately 150 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at 1400 hours and 20–60 at 1500 hours, B Southern oriented twig on 6 September (1500–1800 hours) and 7 September (700–1200 hours) 1989. Stillberg 1970 m

age classes 2–10. In July, CO₂ exchange was largely below compensation in the youngest needles which at that time were still growing, but in September it was similar to the exchange rates in the older needles. Tendencies towards reduced photosynthetic activities were clearly absent in all the twigs analysed. Hence, decline of photosynthetic capacity is unlikely to be a decisive factor responsible for the initiation of senescence and eventual abscission of old needles.

Senescence

Needles begin to turn yellow in mid-August and the senescence process is completed about 4 weeks later. The colour changes are illustrated by the progressive decline of chlorophylls and carotenoids from stages 1 to 5, which cover the entire senescence period (Fig. 3). It appears that the yellow or even golden appearance of needles at about mid-senescence is due to a comparatively high retention of carotenoids. Whereas residual chlorophyll a at stage 2 was about 10% of the content in presenescent needles, more than 20% of β -carotene was retained. This is comparable with observations in senescent needles of larch, which are characterized, like the more advanced stages in stone pine, by relatively high retention of xanthophylls (Pfeifhofer and Grill 1985). Marked changes of chlorophyll a/b ratios as, for example, observed in senescent needles of yew (Kufner et al. 1978) or leaves of *Ginkgo biloba* (Specht-Jürgensen 1967), occurred between senescence stage 2 and 3. The formation of carotenoid esters could not be observed.

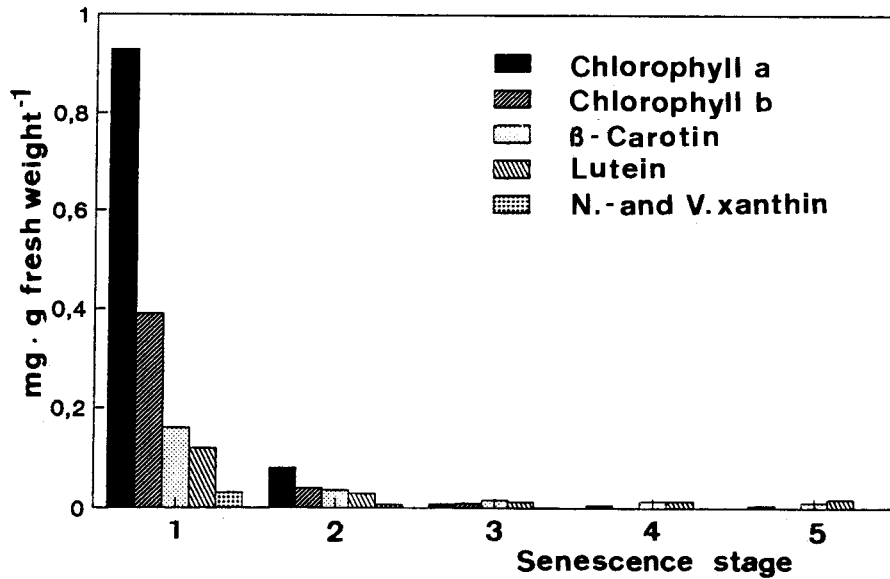


Fig. 3. Loss of chloroplast pigments in the course of needle senescence

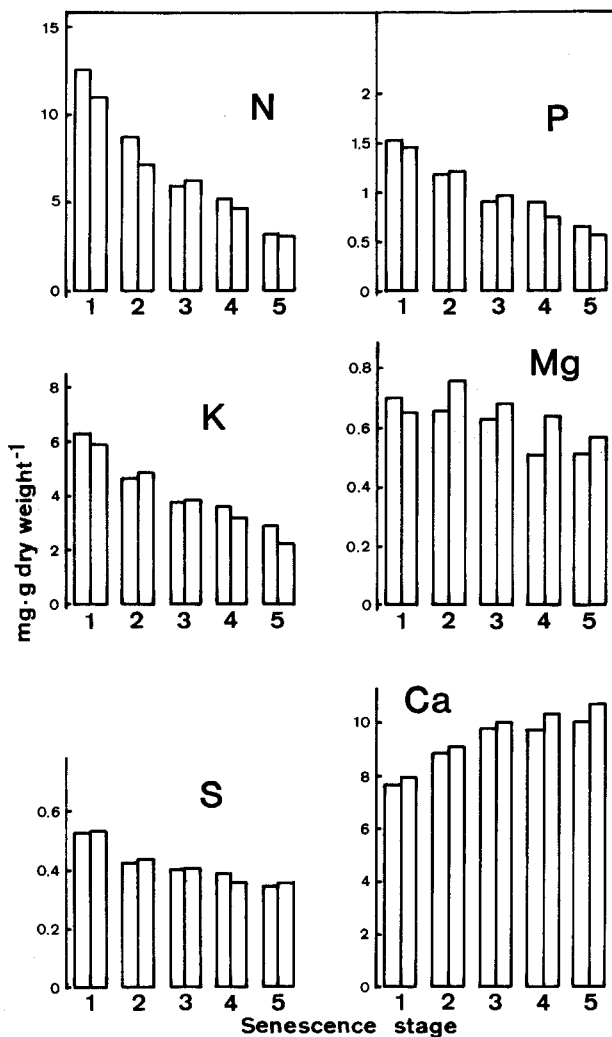


Fig. 4. Changes of contents of various nutrients in the course of needle senescence. Two samples of 3–10 g fresh weight per senescence stage were analysed. Tree at Stillberg, 2070 m

With regard to the nutrient economy of a tree the withdrawal of nutrient elements from senescent leaves is important. As illustrated by the findings compiled in Fig. 4, it is remarkably efficient in the case of such important elements as N, P and K. Since dry weights per needle decrease considerably in the course of senescence (average: 20%; range: 5–25%; calculations based on loss of dry weight as related to an average needle length of 7.4 cm), the data calculated per unit of dry weight provide an underestimation of changes or in the case of Ca, they suggest an accumulation in the course of senescence. Since Ca is likely to represent a more or less immobile element in senescent leaves (Tamm 1951; Oland 1963) its apparent changes (+23%) may be used for the calculation of the true losses of other elements. The data, corrected correspondingly, are given in Table 5. They suggest that about 70% of N, P and K are exported in the course of senescence. Hence, stone pine appears to be more efficient in the reutilization of these elements than beech (Gäumann 1935; Verdu et al. 1985), apple (Oland 1963) and birch (Tamm 1951). Withdrawal of Mg and S in stone pine needles was much less pronounced than NPK, and trace elements such as Mn and Zn appear to be as immobile as Ca (Table 5).

Although withdrawal from senescent leaves of nutrients, particularly of N (Millard and Thomson 1989), appears to be an important factor in the economy of trees, there are examples such as *Alnus* which shed leaves without prior mobilization of protein to significant extents (Bortlik et al. 1987). Apart from such examples of nutrient recycling through decomposition of litter and soil metabolism, appreciable proportions of nutrients are normally mobilized in senescent leaves and stored in the twigs and perhaps in the youngest needles to meet the requirements of growth in the subsequent vegetation period. It is conceivable, although difficult to demonstrate in stone pine, that the occasional occurrence of extensive needle senescence is due to a temporary shortage of nutrients, which is bridged by withdrawal from needles of old age classes. Despite quantitative differences of the extent, yellowing exhibits the typical features of a regulated developmental

Table 5. Percentage of nutrient elements retained in fully senescent needles of a tree at Stillberg (2070 m)

	Percent retained in yellowed needles	
	Actual values	Corrected values
N	26	20
P	40	30
K	42	32
Mg	80	60
S	66	49
Ca	133	100
Zn	113	85
Mn	131	99

The contents of elements in presenescent needles of the oldest age class are taken as 100%. The apparent accumulation of Ca, Zn and Mn is due to loss of dry weight (left column). Assuming complete retention of Ca yields corrected percentages in right hand column

process. It is interesting to note that the initiation of senescence coincides with the cessation of long shoot development and root growth (Turner and Streule 1983). Very rarely, premature and extensive yellowing was observed in individual trees. It was distinct from normal senescence as it not only began long before the initiation of senescence in the neighbouring trees but was completed much later. In a tree at Flüela (2140 m) it was clearly associated with severe insect attack. Such phenomena may be related to chlorosis and needle loss as observed in damaged Norway spruce (see Innes 1989). In stone pine chlorosis of needles may also occur as a consequence of Mg deficiency. In some cases, exceptionally late needle losses have been observed following autumnal frost. Moreover, yellowing of old needles may occasionally occur during emergence of shoots. In any case, these phenomena can easily be distinguished from normal senescence, although they have in common the colour changes and may superficially resemble each other.

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References

- Bortlik K, Gut H, Matile P (1987) Yellowing and non-yellowing trees: a comparison of protein- and chlorophyll-loss in senescent leaves. *Bot Helv* 97: 323–328
- Gäumann E (1935) Der Stoffhaushalt der Buche (*Fagus sylvatica* L.) im Laufe eines Jahres. *Ber Schweiz Bot Ges* 44: 157–334
- Gut H, Rutz C, Matile P, Thomas H (1987) Leaf senescence in a non-yellowing mutant of *Festuca pratensis*: degradation of carotenoids. *Physiol Plant* 70: 659–663
- Innes IL (1989) Rapid yellowing of needles of *Picea abies*. *Eur J For Pathol* 19: 21–28
- Kufner R, Czygan FC, Schneider L (1978) Veränderungen des Pigmentgehalts und der Ultrastruktur bei den Plastiden der Nadelblätter von *Taxus baccata* (L.) während ihrer Entwicklung. *Ber Dtsch Bot Ges* 91: 325–337
- Lichtenthaler HK, Wellburn AR (1983) Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Transact* 11: 591–592
- Millard P, Thomson CM (1989) The effect of the autumn senescence of leaves on the internal cycling of nitrogen for the spring growth of apple trees. *J Exp Bot* 40: 1285–1290
- Nebel B (1990) Lebensdauer und Seneszenz von Nadeln der Arve (*Pinus cembra* L.). Thesis, University of Zürich
- Oland K (1963) Changes in the content of dry matter and major nutrient elements of apple foliage during senescence and abscission. *Physiol Plant* 16: 682–694
- Pfeifhofer HW, Grill D (1985) Plastidenpigmente der Lärche. *Phyton* 25: 205–211
- Specht-Jürgensen I (1967) Untersuchungen über Stickstoffverbindungen und Chlorophyll während des Vergilbens der Laubblätter von *Ginkgo biloba*. I. Am Baum alternde Blätter. *Flora (Jena) Abt A* 157: 426–470
- Strauch L (1965) Ultramikro-Methode zur Bestimmung des Stickstoffs in biologischem Material. *Z Klin Chem* 3: 165–167
- Tamm CO (1951) Seasonal variation in composition of birch leaves. *Physiol Plant* 4: 461–469
- Turner H, Streule A (1983) Wurzelwachstum und Sprossenentwicklung junger Koniferen im Klimastress der alpinen Waldgrenze, mit Berücksichtigung von Mikroklima, Photosynthese und Stoffproduktion. In: *Wurzelökologie und ihre Nutzenanwendung*. Int Symp Gumpenstein 1982. Bundesanstalt Gumpenstein, A-8952 Irnding, pp 617–635
- Verdu AMC, Riba M, Roda F (1985) Nutrient retranslocation from senescing leaves of beech (*Fagus sylvatica* L.). *Orsis* 1: 91–100
- Welles J (1986) A portable photosynthesis system. In: Gensler WG (ed) *Advanced agricultural instrumentation*. Martinus Nijhoff, Dordrecht, pp 21–33