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<b>Autor:</b>	Bortlik, Karlheinz / Gut, Hans / Matile, Philippe
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# Yellowing and non-yellowing trees: a comparison of protein- and chlorophyll-loss in senescent leaves

**Karlheinz Bortlik, Hans Gut and Philippe Matile**

Department of Plant Biology, University of Zürich, Zollikerstraße 107, CH-8008 Zürich

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## Abstract

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In some species of deciduous trees, the autumnal development of leaves prior to abscission is not associated with the common yellowing. It was investigated whether this phenomenon is due to the separation of chlorophyll degradation from other senescence processes such as the mobilization of leaf protein. In the non-yellowing species, *A. glutinosa*, not only the chlorophyll turned out to be largely retained in the autumnal leaves but also the protein. Moreover, the vitality of leaves as derived from fluorescence data is preserved to some extent down to the abscission of leaves. It appears that *Alnus glutinosa* abstains largely from the salvage of nitrogen and perhaps other nutrient elements prior to leaf abscission. This may have interesting consequences with regard to the biological activity, the nitrogen cycle, and the development of the soils of *Alnus* stands.

## Introduction

Senescence is the final phase in the development of leaves and is terminated by the death of the organ. Its physiological significance can be seen primarily in the salvage of major nutrients for reuse in growing organs or reserve tissues. Indeed, the degradation and mobilization of cytoplasmic materials prior to death or abscission is a principal feature of leaf senescence. The disappearance of chlorophyll is of course the most obvious phenomenon, yet the yellowing of leaves is always accompanied by the degradation of protein as well as a number of other metabolic processes which altogether are referred to as the “senescence syndrome”. Leaf senescence appears to follow a general programme and it has not been possible so far to experimentally separate individual processes such as e.g. the degradation of chlorophyll and protein from each other. Treatments of excised leaves aimed at the modification of the rate of senescence always resulted in the hastening or retardation of the whole senescence process. (See Stoddart and Thomas 1982 for a recent review).

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Dedicated to Prof. Dr. Hans Wanner on the occasion of his 70<sup>th</sup> birthday

For practical reasons researchers have largely chosen leaves of cereals or legumes for investigating metabolism and regulation of senescence. Detached leaves kept in permanent darkness have been, and still are, rewarding experimental systems and, correspondingly, the knowledge is abundant. In contrast, the knowledge about leaf senescence in deciduous trees is very scarce. Data available on autumnal senescence in leaves of beech (Gäumann 1935), birch (Tamm 1951) and apple tree (Oland 1963) indicate that metabolism prior to abscission is associated with the salvage of nutrient elements such as N, P, K and Mg. According to Gäumann (1935) the total foliage of a 110 years old beech-tree contains ca. 4.3 kg of protein of which 2.5 kg is mobilised during senescence. Hence, about 60% of the nitrogen which in spring is incorporated into the new foliage is recuperated in fall and stored in trunk and branches for reuse during emergence and growth of the following generation of leaves.

In contrast to the incomplete mobilization of leaf protein, the catabolism of the chlorophylls is, under normal conditions, nearly exhaustive. This is rather surprising since the chlorophylls contribute very little to the nitrogen budget. The relative amount of N contained in the chlorophylls is ca. 20–30 times smaller than the amount of protein nitrogen. From an economical point of view a senescence programme aimed at a more exhaustive recuperation of protein-N would, therefore, appear to be meaningful. On the other hand, it is intelligible that a mutant genotype of meadow fescue that is unable to catabolize chlorophyll (Thomas and Stoddart 1975) is viable. It appears that the contribution of chlorophyll to the overall nitrogen economy of this grass is so small that the genetical lesion in chlorophyll catabolism can easily be compensated. A few species of deciduous trees seem to resemble the non-yellowing genotype of *Festuca pratensis* as they shed the leaves when they are still green. In the genus *Alnus* this phenomenon is quite obvious but it can also be observed in the case of *Fraxinus excelsior*. With regard to the understanding of leaf senescence it would have been important to establish natural cases characterized by an incomplete syndrome. It was anticipated that non-yellowing species are characterized by the separation of chlorophyll degradation (contributing little to the total nitrogen budget of a tree) from other senescence processes such as the degradation of protein.

## Materials and Methods

In 1984 and 1986 leaves were sampled at intervals from September through abscission at the end of October (1984) or early November (1986). The species selected were *Alnus glutinosa*, *Fraxinus excelsior* and *Tilia spec* (1984 *T. cordata*, 1986 *T. hybrida*); leaves of *Ulmus scabra* were also sampled in 1984, leaves of *Carpinus betulus* in 1986. A small stand near Uerikon (ZH) comprising all the 4 species was selected in 1984. In 1986 trees cultivated in the Botanical Garden of the University of Zürich were used. The samples were withdrawn from branches having the same exposition and care was taken to select the leaves in view of samples representative for the overall developmental state at the date of harvest. In order to obtain a decent basis for the analysis, discs with a diameter of 14 mm were cut out from intercostal areas of leaves. Data were thus expressed per unit of leaf surface. The discs were frozen and stored at –80 °C.

For the analysis of chlorophylls (as pheophytins), protein, and amino acids, 3 lots of 3 leaf discs were homogenized in 4 ml 80% aqueous acetone containing 1% oxalic acid. The pigments were partitioned in 4 ml petrol ether (40°–70°) and pheophytins determined according to Vernon (1960). Aliquots of the aqueous phase were employed for determining amino acids according to Yemm and Cocking (1955). Arginine served as the standard. The sediment obtained upon the centrifugation of the acetone extract (5 min, 1000 × g) was washed twice with 80% acetone. The pro-

teins were then dissolved in 1 ml 1 N NaOH (1 h, 60 °C) and precipitated again with TCA (3 ml 20% w/v). Finally the precipitate was sedimented (10 min, 2000 × g), dissolved in 1 ml 1 N NaOH and protein contents determined in aliquots according to Bradford (1976).

## Results and Discussion

The principal findings about the losses of chlorophyll and protein in non-yellowing *Alnus glutinosa* and yellowing *Tilia spec.* leaves are compiled in Fig. 1. Although the two sets of data were obtained with material sampled at different locations and in two

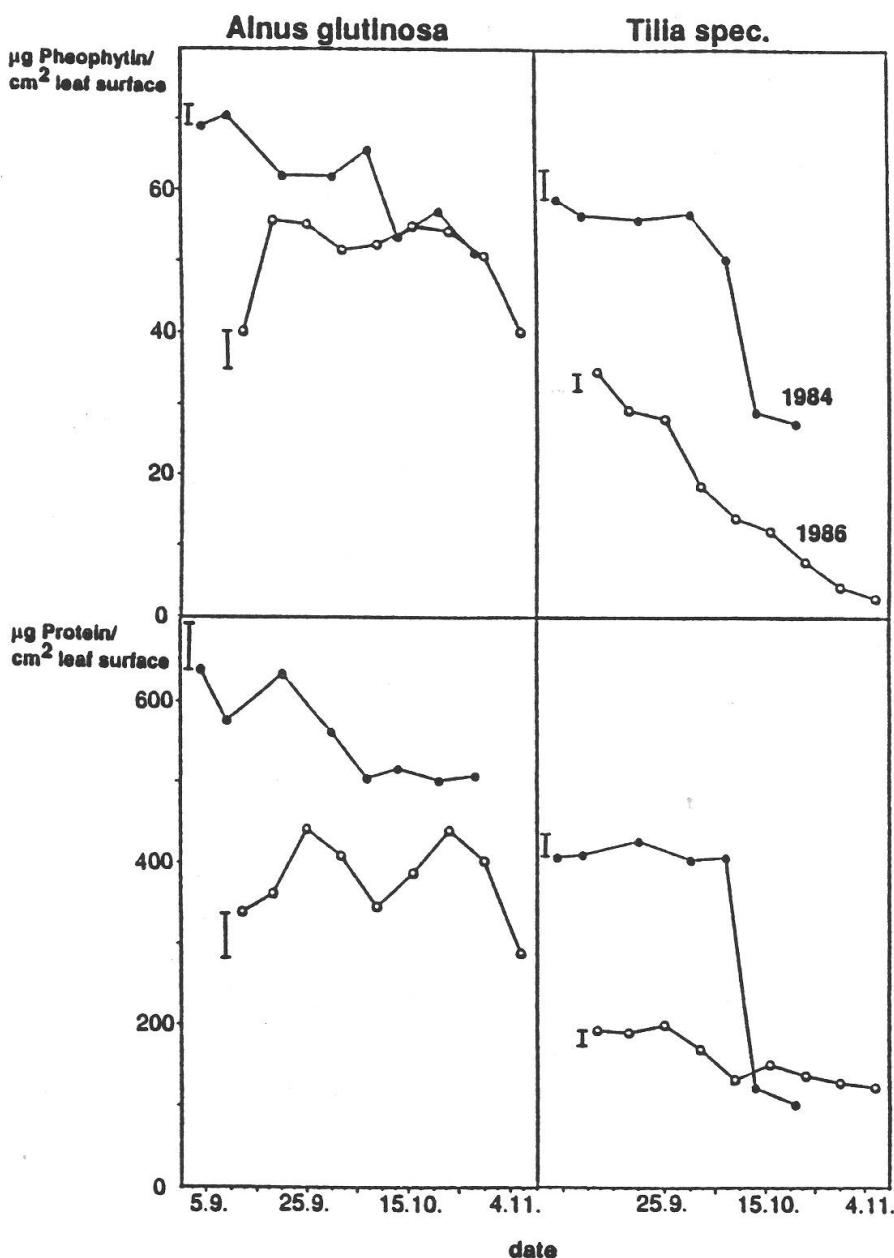


Fig. 1. Changes of contents of chlorophyll and protein in leaves of *Alnus glutinosa* and *Tilia cor-data* during autumnal senescence in 1984 (full circles) and 1986 (open circles). The data are calculated per  $\text{cm}^{-2}$  of leaf area. Chlorophyll contents are given as  $\mu\text{g}$  pheophytin  $\text{cm}^{-2}$ .

fall periods, respectively, the general patterns are similar. It appears that in *A. glutinosa* not only the chlorophyll is largely retained down to the abscission of leaves but also the total protein. In contrast, *T. spec.* leaves are characterized by their ability to degrade the chlorophyll. The protein is also clearly mobilized prior to abscission, though the extent of protein degradation was remarkably different in the two fall periods considered. In 1984 as much as 75% of leaf protein disappeared during the period of sampling, whereas in 1986 the overall loss was only 40%. Whether these differences are an expression of the genetical individuality of the two trees selected or caused by the different environmental conditions cannot be decided. The important finding must be seen in the fact, however, that the non-yellowing species, *A. glutinosa*, is distinct from the yellowing *T. spec.* not only with regard to chlorophyll but also to protein i.e. the two principal parameters usually selected to characterize leaf senescence. Hence, the hypothesis that *A. glutinosa* may represent a natural genotype having an incomplete senescence syndrome, chlorophyll breakdown being separated from other senescence parameters, appears to be clearly disproven. It should be added that similar results have been obtained with another pair of species, *Fraxinus excelsior* (1984, 1986) and *Ulmus scabra* (1984)/*Carpinus betulus* (1986), respectively. In leaves of *Fraxinus* the retention of chlorophyll was less complete than in *Alnus* but it was evident that the breakdown of chlorophyll was not separated from that of protein. It, thus, appears that there are gradual differences among the senescence programmes of individual species of deciduous trees. *Alnus* may merely follow an extreme programme which is essentially reduced to the differentiation of abscission layers responsible for the eventual shedding of leaves. In fact, newly shed *Alnus* leaves may have a completely green and viable appearance before they suddenly turn brown and dark upon the collapse of the mesophyll cells.

Another parameter which particularly in detached leaves is often used for the assessment of protein breakdown is the content of amino acids or  $\alpha$ -amino nitrogen. In attached leaves the corresponding data are less instructive as the amino acids produced are expected to be exported from the leaf. Nevertheless, the data of amino acid contents in the senescent leaves measured in fall 1986 showed a conspicuous difference between the two species. In *Alnus* the values increased markedly when the leaves approached abscission suggesting that degradation of protein occurred in actual fact; however, the leaves of this species may not export the amino acids formed, in contrast to the situation in the yellowing species, *Tilia spec.* (Fig. 2).

As the leaves of *A. glutinosa* preserve their complements of pigments and protein during autumnal senescence, it was tempting to check whether they also preserve the functional integrity of the photosynthetic system. The corresponding measurements were based on the changes of modulated chlorophyll fluorescence upon the sudden exposure to white light of dark-adapted fresh leaf discs (Oegren and Baker 1985). The fluorescence signals emitted upon the continuous illumination with white light rapidly run through a maximum and eventually become stationary within ca. 2 min. They stem from Photosystem II and reflect the electron transport which is set in motion upon the illumination of the dark-adapted leaf. The index  $R_{fd}$  calculated from the signals (Fig. 3 inset) represents a measure of oxygen evolution and thus, of photosynthetic capacity (Strasser and Sironval 1974). In the case of *Tilia* it was somewhat surprising that the  $R_{fd}$  values remained at a high level until more than half of the chlorophyll had disappeared from the leaves. Photosynthetic capacity began to decrease sharply ca. 4 weeks before leaf abscission. It was completely abolished when the leaves were about to be shed. In contrast, by the end of development, leaves of *Alnus* had retained ca. 40% of the initial

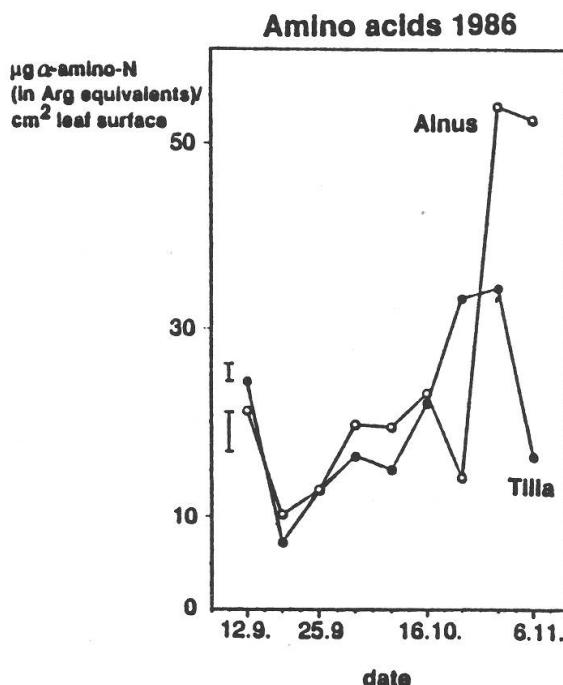


Fig. 2. Contents of amino acids ( $\alpha$ -amino-N) in leaves of *Alnus glutinosa* and *Tilia cordata* during autumnal senescence in 1986.

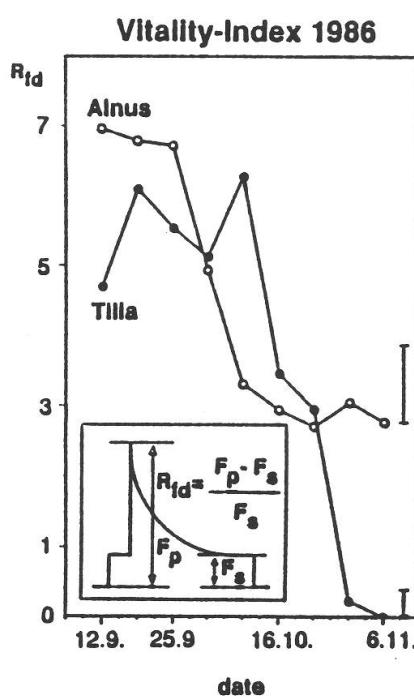


Fig. 3. Photosynthetic capacity of leaves of *Alnus glutinosa* and *Tilia cordata* during autumnal senescence in 1986. Inset: time course of fluorescence intensity upon the illumination of a dark-adapted leaf disc; calculation of vitality index  $R_{fd}$ .

capacity but a considerable loss occurred 2 weeks earlier than in *Tilia* leaves (Fig. 3). It turns out that the total photosynthetic activity in the leaves of these two species may have been about equal during the period of autumnal senescence 1986. Hence the remarkable retention of chlorophyll and protein in the leaves of *Alnus* may not have a significant advantage in terms of a prolonged and superior productivity of the chloroplasts. Rather would it seem that in *Alnus* a deterioration and loss of activity occurs in the thylakoids quite early despite the more or less unchanged chlorophyll content. On the other hand it is remarkable that in *Tilia* (as well as in *Carpinus*) a large proportion of chlorophyll is lost during the early period of autumnal senescence without a significant change in the  $R_{fd}$ -values. However, as the data are available only for autumn 1986, far reaching conclusions should not be drawn. With regard to the photosynthetic capacity, the behaviour of the non- or only partially-yellowing species *Fraxinus excelsior* resembled the behaviour of the yellowing trees rather than *Alnus*.

In conclusion, *Alnus glutinosa* appears to be characterized by a senescence syndrome which is reduced to the last item, abscission. The leaves are shed with the almost total amount of nitrogen incorporated in spring into the foliage. It can be argued, that *Alnus* does not depend on the salvage of nitrogen since it is diazotrophic. Another diazotrophic tree, *Robinia pseudacacia* has not been investigated but the yellowing of its leaves suggests that it also mobilizes protein and follows the common strategy of economical use and reuse of nitrogen. The significance of the apparent waste of nitrogen in *Alnus* may, however, remain obscure as long as only the tree and its nutrient budget is considered. In the long run the shedding of leaves containing a set of nutrients including nitrogen may be important for the stand as a whole and thus indirectly also for the tree. The specific properties of the litter of *Alnus* may provide a basis for a high biological activity in the soil and may thus be significant for soil development.

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