

## Seasonal variability of chlorophyll content and the changes of the acidity and buffer capacity in the needles of *Larix decidua* and *Larix leptolepis* grown in different air pollution conditions

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### Abstract

The needles of *Larix decidua* Mill. and *Larix leptolepis* showed different pattern of seasonal chlorophyll variability, the level of pigments in *L. decidua* was more influenced by zinc-plant emissions than that in *L. leptolepis*. The concentrations of chlorophyll *a* and *b* and their changes were markedly different from those estimated for the non-deciduous species. The seasonal variability of acidity of aqueous homogenates of the considered larch needles appeared to be less characteristic for the species and also for the pollution influence than the changes in buffer capacity.

### INTRODUCTION

Estimation of seasonal changes in chlorophyll *a* and *b* concentration in the needles of larches seems particularly interesting because *Larix* species, as deciduous trees, are very efficient in dry matter production and accumulate equivalent amounts of it over a shorter period than evergreen conifers do. Some representatives of the latter, the three pine species, were also investigated by the authors (Czuchajowska, Przybylski, 1978; Czuchajowska, Niemtur, 1978), this making comparison of the results even more promising. Although the larch needles were investigated for the content of several glucosides (Medvedeva, Tyukavkina, Ivanova, 1971) and carbohydrates (Gira, Kaverzina, 1975), the problem of pigment concentration, especially interesting with respect to their seasonal changes, did not attract any particular attention.

Certainly, the discussion limited to the changes in chlorophyll level,

not connected with examination of photosynthesis efficiency, could not be complete. Unfortunately, the results of the latter concerning larch are not known at the moment, except the work of Fry and Phillips, 1977, therefore the authors present additionally the data of seasonal variability in pH and buffer capacity of the aqueous homogenates of the needles, believing that even if they could not be of any direct use in the interpretation of the changes of pigments, they would make the general characteristics of the processes more thorough. The studies performed on *Pinus silvestris* (Czuchajowska, Przybylski, 1978a) were encouraging, particularly with respect to the buffer capacity, the magnitude so strictly connected with plant ability to preserve the most favourable pH range for the life processes, i.e. for photosynthesis in which chlorophyll plays such an important role.

#### MATERIAL AND METHODS

The material was collected in the middle of every month in 1976 from the two experimental stands founded by the Department of Forest Management in Industrial Regions of the Institute of Forestry Research, IBL, in Katowice. The Żyglinek stand, representing the high degree of air pollution, is 0.5 km apart of the zinc-plant at Miasteczko Śląskie in the Upper Silesia, the Brynica stand is in the distance of 2.5 km, the degree of pollution is much lower, in spite of the close neighbourhood. Both areas are localized in forestial stands of similar edaphic conditions. The needles were taken from 100 specimens of the two larch species: *Larix decidua* Mill., and *Larix leptolepis* (Sieb. and Zucc.), 6-7 year-old, from the middle part of tree crown. The leaves on the short shoots — denoted as the ss-leaves — were collected from the middle part of previous year increments, the leaves on the long shoots, the ls-leaves, were taken from the middle part of the fresh, current year increments. The respective periods of needle collection were: May-October and August-October. The mode of collecting, transportation and pigment analysis was described previously (Czuchajowska, Przybylski, 1978a), the spectrophotometric analysis of the extracts in 100 pc acetone followed the standardized method adopted to eco-physiological investigations (Linder, 1974).

The acidity of aqueous homogenates of the needles was measured and the buffer capacity was evaluated in the way already described (Czuchajowska, Przybylski, 1978a). The latter was expressed as buffer capacity against acid,  $bc_a$ , and against base,  $bc_b$ ; the values of  $bc_a$  and  $bc_b$  give the volume of 0.025 N  $H_2SO_4$  or 0.025 N NaOH solutions, necessary — respectively — to decrease or to increase the initial pH value of homogenate by 0.5 pH unit.

## RESULTS

Seasonal changes in the content of chlorophyll *a* and *b* in the two types of leaves for *L. decidua* and *L. leptolepis* from Brynica and Żyglinek are shown in Fig. 1. Chlorophyll *a* concentration in the ss-leaves of *L. decidua*, highest in May, 2.5 mg/g d.w., decreases by 20 pc in June

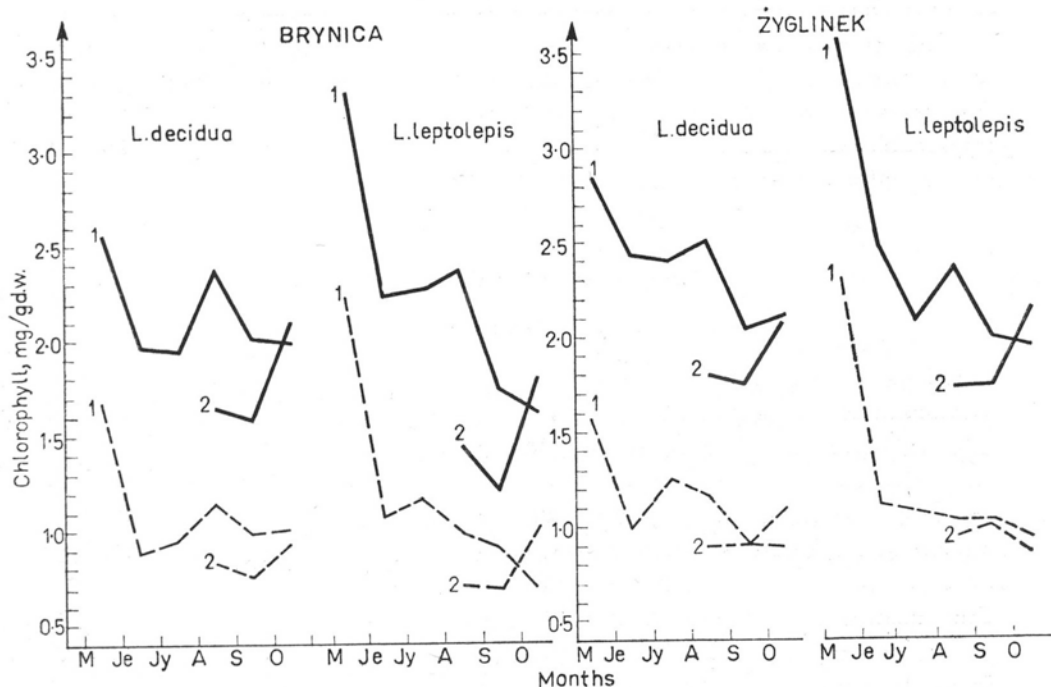


Fig. 1. Changes of the content of chlorophyll *a* (continuous line) and of chlorophyll *b* (dashed line) in the leaves on short shoots (1) and those on the long ones (2) of *L. decidua* and *L. leptolepis* from the stands at Brynica and Żyglinek; the letter denote the successive months: M — May, Je — June, Jy — July, A — August, S — September, O — October

and is maintained on this level till late autumn with exception of August when a temporary increase of concentration occurs. Chlorophyll *a* content of the newly formed ls-leaves amounts in August to 70 pc of that in ss-leaves and increases only in October. The chlorophyll *b* content changes very similarly. The starting chlorophyll *a* level in ss-leaves of *L. leptolepis*, 3.35 mg/g d.w., is by 30 pc higher than that of *L. decidua*, it decreases stronger in June and preserves the newly reached level till the end of August, then drops further to 50 pc of the starting value. The changes in chlorophyll *a* in the ls-leaves are very similar to those noticed for *L. decidua*. Chlorophyll *b* concentration in ss-leaves, also by 30 pc higher than in *L. decidua*, decreases more than by a half

to the June-September level and then continues to decrease, reaching in October 30 pc of the starting concentration. Chlorophyll *b* content in newly formed ls-leaves equals to 70 pc of that in ss-leaves.

Table 1

Significance of differences—with respect to time—in the mean values of pigment contents (mg/g d.w.) calculated for the two stands jointly

<i>Larix decidua</i>					<i>Larix leptolepis</i>			
	chl. <i>a</i>		chl. <i>b</i>		chl. <i>a</i>		chl. <i>b</i>	
	ss-	ls-	ss-	ls-	ss-	ls-	ss-	ls-
	leaves		leaves		leaves		leaves	
Level of significance	*		**		**		**	
Brynica	2.141	1.773	1.105	0.834	2.260	1.496	1.174	0.809
Żyglinek	2.367	1.925	1.139	0.977	2.415	1.874	1.250	0.944

\* significance level of 0.05; \*\* significance level of 0.01.

Intensification of air pollution examined on the samples collected from Żyglinek stand is reflected to a very limited extent in the seasonal changes of chlorophyll *a* and *b* content in ss-leaves of *L. leptolepis*, however, in *L. decidua* leaves it causes an increase of chlorophyll *a* level by 25 pc in June-July and a similar increase in chlorophyll *b* concentration. The ls-leaves of the both larch species seem to be slightly more sensitive.

The significance of differences in chlorophyll *a* and *b* content was calculated (Snedecor, 1956) for the considered period of time and

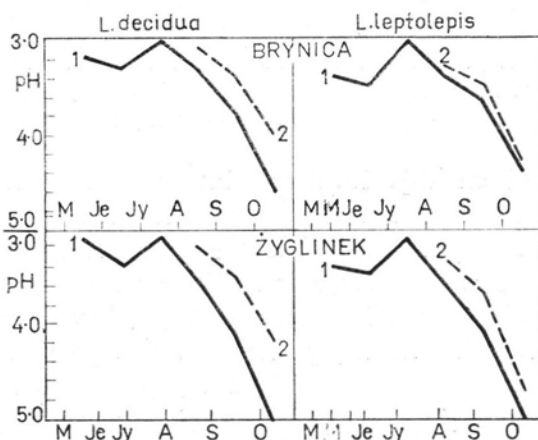


Fig. 2. Changes of pH in aqueous homogenates of the leaves on short shoots (1) and those on long ones (2) of *L. decidua* and *L. leptolepis* from Brynica and Żyglinek

for the two stands. The significance has been confirmed both for chlorophyll *a* and *b* in the leaves on the short shoots. The corresponding data are presented in Table 1.

The seasonal acidity of changes of aqueous homogenates of the ss- and ls-leaves of the two species are shown in Fig. 2. As one can see, acidity of *L. decidua* ss-leaves from Brynica maintains from May till August almost the same level near pH 3.2, except for pH 3.0 in July, and decreases later reaching pH 4.6 in October. The ss-leaves of *L. leptolepis* are slightly less acidic during the first fourmonth period, what makes their maximum in August more distinguished, the acidity decreases to pH 4.4 in October. The ls-leaves of *L. decidua* and *L. leptolepis* are, respectively, by 0.3-0.6 and ca 0.1 pH unit more acidic than the corresponding ss-leaves. Air pollution intensification — so typical for Żyglinek stand-influences, first of all, the acidity of the last two months of leaf life, it decreases the acidity by 0.5-0.6 and 0.2-0.4 pH unit, respectively, as far as the ss- and ls-leaves are concerned, those of *L. leptolepis* being more sensitive.

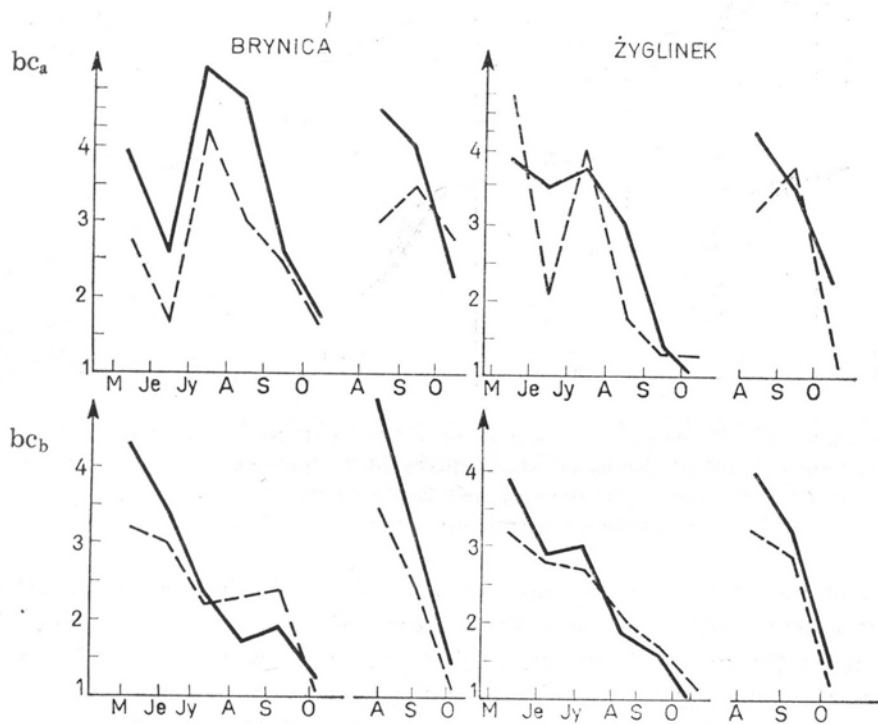


Fig. 3. Changes of buffer capacity against acid,  $bc_a$ , and buffer capacity against base,  $bc_b$ , in ml of 0.025 N  $H_2SO_4$  or NaOH per 0.5 pH unit, of aqueous homogenates of the leaves on short shoots (M-O) and on long shoots (A-O) of *L. decidua* (continuous line) and *L. leptolepis* (dashed line) from Brynica and Żyglinek

The changes in buffer capacities:  $bc_a$  and  $bc_b$  are shown in Fig. 3. It can be seen that comparatively high starting  $bc_a$  value of the ss-leaves of the two larch species in May, reaches — after a decrease in June — a very dominant maximum in July-August and then decreases, becoming lowest just before leaves fall. The increase in air pollution changes the  $bc_a$  value of ss-leaves of *L. decidua* more extensively than that of *L. leptolepis*.

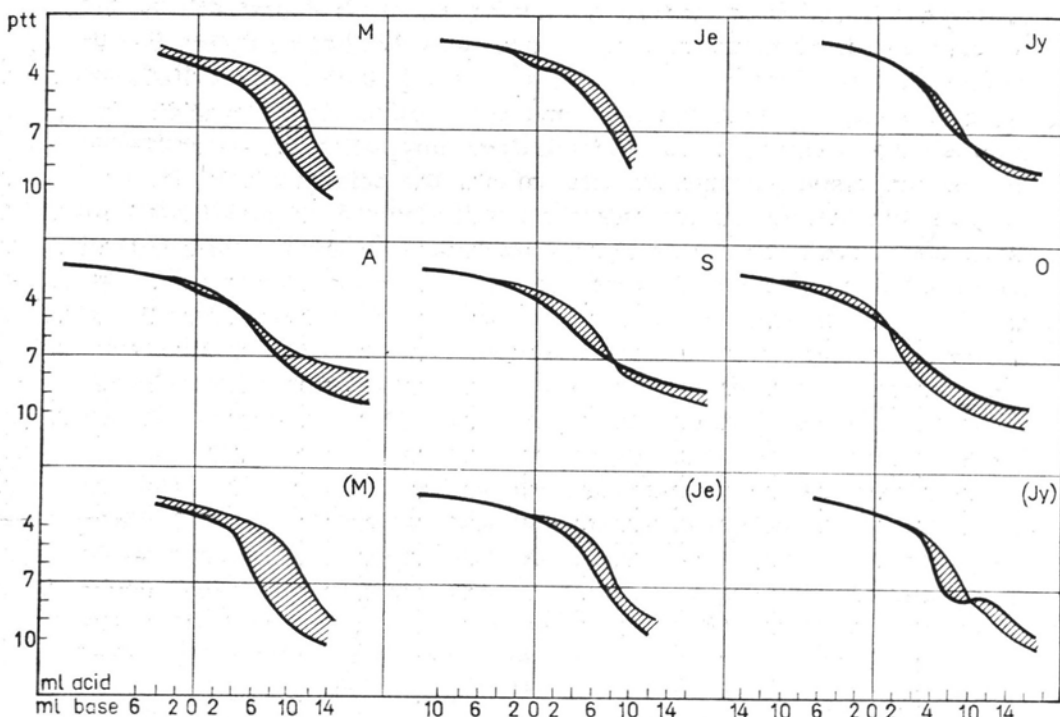


Fig. 4. Plots of pH versus the volume of  $H_2SO_4$  and NaOH solution added to aqueous homogenates of leaves on short shoots of *L. leptolepis* (broader line) and *L. decidua* (thinner line) from Brynica and Żygliniek; for the latter stand the abbreviations of month are given in brackets

Variations of the buffer capacity against alkali,  $bc_b$ , show for the ss-leaves a constant decrease with an exception of a stabilization period occurring in the period from June till September, depending on the *Larix* species, the ls-leaves are characterized by a sharp decrease occurring in both cases. Intensification of air pollution is reflected in appearing of an additional level of stabilization, higher than the former one being noticed in June and July. Although the presented  $bc_a$  and  $bc_b$  values seem to be the good basis for the discussion, the charts describing the dependence of pH versus volume of the added acid or base solution

are also presented, see Fig. 4. Seasonal changes of the area contained between the curve describing *L. leptolepis* and that of *L. decidua* show a very typical trend consisting of area decrease in the acidic range from May till August.

## DISCUSSION

It is remarkable that the newly-formed ss-leaves have the highest chlorophyll *a* and *b* concentration, it is never so high later during the season. This feature distinguishes larch from the pine species (Czuchajowska, Przybylski, 1978). Taking into account that the results of pigment contents are expressed in mg of chlorophyll per gram of dry weight, one should not treat the observed concentration decrease in *Larix* as equivalent to chlorophyll decomposition or even to smaller efficiency of its formation, still there is no doubt that its synthesis becomes less favoured as compared with other components of leaf biomass, their presence being reflected in the dry matter. The latter shows the most advanced increase just in the period of the strong decrease of chlorophyll level, pointing to the fact that the interpretation of the phenomenon would need examination of seasonal changes in photosynthesis efficiency and those of respiratory processes, the latter contributing to the total decrease of biomass. The problem, especially when regarded in comparison with the pine species, is highly involved also because of the reason that although overall photosynthesis rates of larch are higher during summer than those of any non-deciduous conifers, it is not known whether it originates from greater photosynthesis efficiency or from differences in leaf area between *Larix* and evergreen conifers, however the first view seems to prevail (Sweet, Wareign, 1968; Fry, Phillips, 1977).

The striking fact is that pattern of chlorophyll changes in *Larix* ls-leaves is quite different than ss-leaves. Considering small differentiation of the two types of leaves stated by the authors in the dry matter production, reflecting to some extent the biomass production, the comparison of pigment levels of the ss- and ls-leaves certainly proves the difference in the equilibrium: chlorophyll synthesis  $\rightleftharpoons$  decomposition.

The seasonal changes of acidity of aqueous homogenates of leaves do not changes in their chlorophyll content *Larix* species in such a degree as the changes in their chlorophyll content. The changes in acidity correspond well to those estimated for the current year needles of *Pinus silvestris* (Czuchajowska, Przybylski, 1978a) and seem to be typical, including acidity decrease in autumn, for the coniferous species in general. The fact that the leaves maintain an almost equal level of pH from May till September makes the discussion of buffer capacity changes more trustworthy. Identical  $bc_a$  and  $bc_b$  values of the

young ss-leaves point to the fact of equal protection of leaves against increase or decrease of acidity, the changes of which are always connected with the disturbance of biomechanisms. However, in summer i.e. during full expansion of life processes, the characteristic differentiation of buffer capacity occurs — with  $bc_a$  increasing and reaching the maximal value in July/August and the values of  $bc_b$  decreasing. This convinces of better ability of buffering by *Larix* the increase of acidity than its decrease. The ls-leaves behave also in this respect in a different manner, proving the occurrence of processes which need to be buffered differently by plant than those in the ss-leaves.

Intensification of air pollution, causing only small modifications in seasonal pH values, is strongly reflected in the changes of buffer capacity of the two species, of which *L. leptolepis* is generally known as more resistant. The increase of chlorophyll *a* and *b* concentration in the needles of the latter species appearing in June and July could be connected with a more limited decrease in  $bc_a$  and  $bc_b$  values, it particularly concerns  $bc_a$  in July. The reaction of leaves revealed by the increase of the buffer capacity, counteracts the influence of  $SO_2$  and  $HSO_3^{-1}$  by the increase of  $bc_a$ , and — on the other hand — opposes the action of some Zn and Pb salts by  $bc_b$  increase.

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Sezonowa zmienność zawartości chlorofilu oraz zmiany kwasowości i pojemności buforowej w igłach *Larix decidua* i *Larix leptolepis* rosnących w różnych warunkach zanieczyszczenia powietrza

Streszczenie

Oznaczono sezonowe zmiany zawartości chlorofilu *a* i *b* w dwóch typach igieł *Larix decidua* Mill. oraz *Larix leptolepis*, rosnących na stanowiskach charakteryzujących się różnym stopniem zanieczyszczenia powietrza wywołanym sąsiedztwem huty cynku. Oba gatunki modrzewia wykazały odmienny charakter zmian stężenia chlorofilu, poziom tych barwników u *L. decidua* ulegał bardziej wpływowi emisji niż u *L. leptolepis* (rys. 1). Oznaczone stężenia oraz przebieg ich zmian okazały się zasadniczo odmienne od wyznaczonych dla sosny.

Sezonowa zmienność kwasowości wodnych homogenizatów badanych igieł modrzewia (rys. 2), okazała się mniej charakterystyczna dla gatunku modrzewia oraz dla wpływu zanieczyszczeń niż pojemność buforowa. Tę ostatnio wyrażono jako pojemność buforową względem kwasu,  $bc_a$ , oraz względem zasady,  $bc_b$  (rys. 3) w sposób umożliwiający wyraźne odróżnienie obydwu gatunków modrzewia oraz obu typów szpilek: szpilek na krótkich i długich pędach. Modrzew zdolny jest skuteczniej buforować wzrost kwasowości niż jej spadek, dotyczy to okresu lata. Pola powierzchni zawartej pomiędzy krzywymi charakteryzującymi zmianę pH w zależności od objętości dodanego 0.025 N  $N_2SO_4$  i NaOH dla obydwu gatunków wykazały charakterystyczny trend zmian, przedstawiony na rys. 4.