

Leaf Longevity and Defense Characteristics in Trees of Betulaceae

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Abstract- To make baseline data for the maintenance of forest health, net photosynthesis, leaf carbon/nitrogen (C/N) ratio, toughness, existence of trichomes, and chemical defense traits were studied in trees of 18 Betulaceae species. These included five species (*Alnus glutinos*, *A. incana*, *A. sibirica*, *Betula pendula*, and *B. verrucosa*) originating from the Eurasian continent. The amount of total phenolics showed a positive correlation with C/N ratio. Leaf longevity correlated negatively with the amount of total phenolics. Except for *Betula platyphylla*, two *Carpinus* and *Corylus heterophylla*, there was a positive correlation between the amount of condensed tannin and leaf longevity. A weak negative correlation was found between leaf lifespan and LMA (leaf mass per area). No relation between the existence of trichomes and leaf longevity was found. Successional traits of 18 Betulaceae species and leaf defense are discussed based on relations of the C/N ratio of leaves and adaptive radiation in the family.

I. Introduction

Betulaceae trees are a common species in secondary forests in northern Japan. These species show several successional traits, i.e. early-, mid- (or gap phase), and late-successional characteristics. Research has been conducted into phylogeny constraints of the pattern of leaf survival and structure of winter buds [1]. There are several studies on the growth characteristics of Betulaceae, although their defense traits have not been researched [2].

Several alders and birches were imported from western Eurasian regions, especially from northern Europe, for breeding [3]. Some of these are resistant to insect herbivores, but others are susceptible. In general, tree species with a short leaf lifespan seem to have a lower defense capacity against insect herbivores while trees with a long leaf lifespan have a higher defense capacity [4].

Based on field observations [5], alder is a typical pioneer and its leaves are grazed annually by leaf beetles (*Agelastica coerulea*). Leaf grazing, however, rarely kills these trees. In contrast, even though from the same family of Betulaceae, leaves of *Ostrya japonica* (gap phase species) are hardly grazed by insect herbivores. Why is there a difference in the susceptibility to insect herbivores among tree species of Betulaceae? Is there a relationship between leaf longevity of

Betulaceae trees and their defense characteristics? It is expected that trees with a long leaf lifespan may have a higher defense capacity but a relatively lower photosynthetic rate.

In general, contents of defense chemicals are not only affected by soil fertility, but also by the growth phase of the plant life cycle [6,7]. The defense characteristics of plants change as the seasons progress and with the effect of grazing of herbivores. However, these characteristics are strongly dependent on species-specific traits [8]. Therefore, as baseline data for keeping vital and healthy forests dominated by Betulaceae species in northern Japan, the ecological role of defense characteristics of the representative tree species should be researched.

To achieve this, we examined leaf defense characteristics of 18 successional Betulaceae species in relation to the carbon/ nitrogen (C/N) in leaves and adaptive radiation in the Betulaceae family.

II. Materials and Methods

A. Plant materials

All species were young stage trees and ca. 25-years old (*B. davurica* was 15-years old as of 2002), grown at the Hitsujigaoka Experimental Forest of FFPRI (Sapporo; 42°58'N, 141°23'E, 146 m a.s.l.). Sample leaves, with a leaf age of 60-80 days, were collected from the sun crown (mid Aug, 2000). Early successional species: *Alnus hirsuta*, *A. inokumae* (= *A. hirsuta* var. *microphylla*), *A. japonica*, *A. maximowiczii*, *Betula ermanii*, *B. obovata* (= *B. tatewakiana*; shrub), and *B. platyphylla* var. *japonica* are native to Hokkaido, Japan. Five species (*Alnus glutinos*, *A. incana*, *A. sibirica*, *Betula pendula*, and *B. verrucosa* = *B. pubescens*) originated from the Eurasian continent (northern Europe). Mid successional species: *Betula davurica*, *B. maximowicziana*, *Ostrya japonica*, *Corylus heterophylla* var. *thunbergii* (shrub), and late successional species: *Carpinus cordata* and *C. laxiflora* originated in Hokkaido. All *Alnus* species have symbiotic microorganisms (*Frankia* sp.) in their roots, which fix nitrogen directly from the air.

B. Measurement of light-saturated photosynthetic rate

The light-saturated photosynthetic rate of early and late leaves for experimental trees was determined. We used a portable system for the measurement of photosynthetic rates (LI-6400, Li-Cor, NE, USA) at a light saturation of $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a leaf temperature of 25°C , with CO_2 concentration of 350ppmV .

C. Measurement of defensive characteristics of leaves

After the photosynthesis measurement, the leaves were immediately freeze-dried (FLEXI-DRY, FTS Systems, USA) and mill-ground (TM10, Tescom, Japan) down to powder. The concentration of total phenolics in 20mg leaf powder was determined by the Folin-Ciocalteu method [8]. The concentration of condensed tannin was determined using the proanthocyanidin method [9]. The remaining sample leaves were used for the measurement of carbon and nitrogen content by a N/C analyzer (NC-900, Shimadzu, Kyoto) after being dried at 60°C for 48 hours. We also measured the toughness of fresh leaves using a push-pull gauge (CPU gauge, AIKOH, Japan). One leaf was used from each tree. Toughness was measured at three points on each leaf, and the mean value of the three measurements was adopted for that leaf. Trichomes on the upper surfaces of the leaves were observed by SEM (scanning electron microscope; Shimadzu, Kyoto, Japan).

III. Results

A. Photosynthetic characteristics

Except in several alders, there was a strong positive correlation between net photosynthetic rate per area (Pn) and nitrogen content per area. Alders (*Alnus japonica*) had high nitrogen but low Pn (Fig. 1). There was a weak negative correlation between leaf lifespan and net photosynthetic rate (Data not shown).

B. Defense traits

A clear positive correlation was found between leaf mass per area (LMA) and leaf toughness (Fig. 2). There was no clear tendency in the relationship between LMA and leaf toughness among tree species. Tree species originating from high mountain areas or high latitude regions had a high value of LMA and toughness. No relationship between growth traits and the existence of trichome was found. Most alders have a high density of trichomes on the abaxial side, especially along leaf veins. Most species, e.g. alders, some birches and *Carpinus* had rather thick trichome along the veins (Fig. 3). By contrast, most birch had very few trichomes on the abaxial side of the leaf, except for *B. maximowicziana* seedlings. *B. maximowicziana* seedlings and young trees had thick trichomes on both adaxial and abaxial sides in early and late leaves, but in older trees and adult trees there were almost no trichomes (data not shown).

No relationship between C/N ratio and the concentration

of condensed tannin was found, with the exception of *B. platyphylla*, which had an extremely high value of condensed tannin irrespective of an intermediate value of C/N ratio (data not shown). Except for three alders (*Alnus japonica*, *A. glutinosa*, *A. maximowiczii*), there was a positive relation between C/N ratio and the amount of total phenolics (Fig 4).

Leaf longevity correlated weak but positively with C/N ratio of leaves, but it correlated negatively with LMA (Fig 5). Is there any relation between leaf longevity and defense chemicals? With the exception of four species (*B. platyphylla*, *C. cordata*, *C. laxiflora* and *Corylus heterophylla*), the leaf longevity correlated positively with the amount of condensed tannin (Fig.6). By contrast, leaf longevity correlated negatively with the amount of total phenolics.

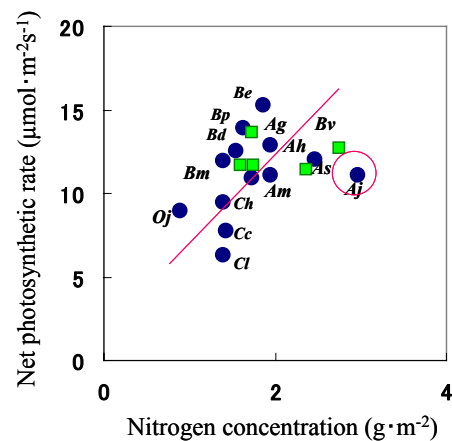


Fig. 1. Leaf nitrogen content and light saturated photosynthetic rate. Circles and squares indicate Japanese and Eurasian origins, respectively. The same as in the following figures.

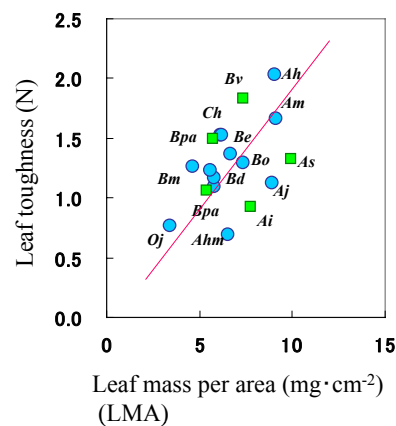
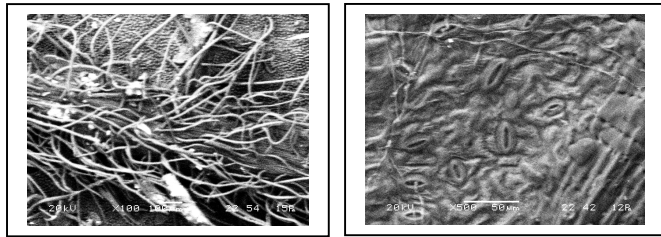
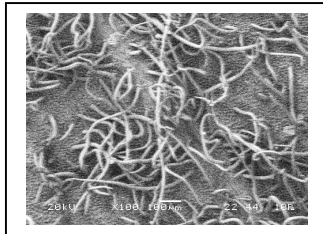


Fig. 2. A positive correlation between LMA and leaf toughness.



Alnus hirsuta

Betula pendula



Alnus sibirica

Fig. 3. Examples of trichomes on the abaxial side of leaves.

Trichomes occur along leaf veins.

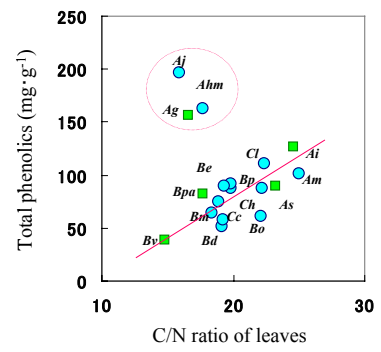


Fig. 4. Relationship between C/N ratio and the amount of total phenolics.

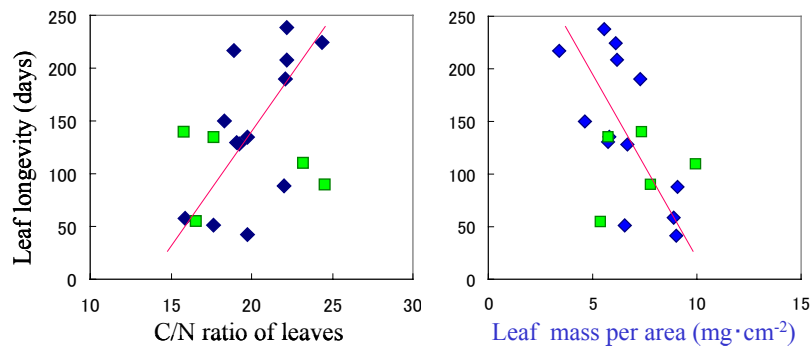


Fig. 5. Relationship between leaf longevity and C/N ratio or LMA

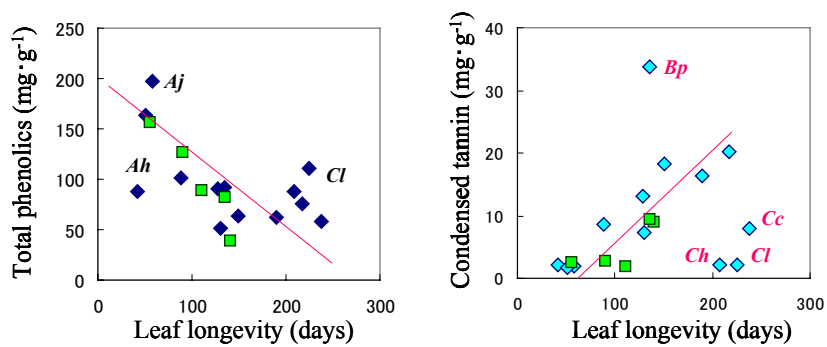


Fig. 6. Leaf longevity and total phenolics or condensed tannin

IV. Conclusion

Species with a short leaf lifespan had high N and photosynthetic rate per area with a small amount of condensed tannin. Except for three alders, there was a positive relation between C/N ratio and the amount of total phenolics in leaves. There was a negative correlation between the amount of total phenolics and condensed tannin (Spearman's rank test). Except for white birch (*B. platyphylla*), most species with long leaf lifespan have a high value of condensed tannin and generally invade forests. Most alders have a higher amount of total phenolics. By contrast, the concentration of condensed tannin in birches was higher than that of alders.

V. Discussion

In deciduous broad-leaved trees, species with long leaf longevity have a relatively lower photosynthetic rate per area [1, 2]. Most species of deciduous leaf habit with low leaf turnover rate usually have a high defense capacity [11]. Is there any relationship between leaf longevity and defense traits? Leaf mass per area (LMA) represents a physical defense, such as mechanical protection and leaf toughness. Trichomes of leaves act as a physical defense in some birch species [12, 13]. Nevertheless, it is expected that the amount of chemical defense should correlate positively with leaf longevity. However, in Betulaceae, a weak negative correlation was found between LMA and leaf lifespan (Fig. 5). Why was the negative correlation found between LMA and leaf longevity among species of Betulaceae? Throughout the six biomes, species with a high photosynthetic rate usually have a high LMA [11] because the amount of photosynthetic organs per area is large, while their leaf longevity is relatively short. Therefore, a negative correlation was found. Species with a long period of leaf emergence have a short leaf lifespan. Leaf lifespan increases as the number of bud scales increase [1]. How about chemical defense? Except for *B. platyphylla*, there was a weak positive correlation between the amount of condensed tannin and leaf lifespan. Some exceptions were observed, i.e. *B. platyphylla* had largest amount of condensed tannin but relatively lower leaf longevity. By contrast, two *Carpinus* species and *C. heterophylla* had lower amounts of condensed tannin but longer leaf lifespan.

Except in alders, the amount of total phenolics in leaves increased with increasing C/N ratio. Alders have a symbiotic relationship with nitrogen fixing microorganisms (e.g. *Frankia* sp.), however, lower C/N in their leaves did not always mean lower leaf toughness and shorter leaf longevity. In alder leaves with low C/N ratio, well-developed mesophyll and the extension of vascular bundle sheaths in leaves may maximize their mechanical protection (Koike unpublished data). The inner structure of a leaf should be analyzed from the viewpoints of function and structure. Moreover, alders may compensate for their grazed leaves by continuously producing new leaves. In this experiment, we

matched the leaf age of ca. 60-80 days, therefore, we may have missed the peak value of photosynthetic rate in alders because of their short leaf lifespan [14]. Except for *B. platyphylla*, typical early successional species have a higher photosynthetic rate and short leaf lifespan with lower condensed tannin. By contrast, two species (*Carpinus* and *O. japonica*) have lower photosynthetic rates and longer leaf lifespan with lower condensed tannin.

In general, the contents of defense chemicals are affected not only by soil fertility, but also by the growth phase of the plant life cycle. These can be understood by applying the GDB (growth/ differentiation balance) and CNB (carbon/ nutrient balance) hypotheses [6, 7]. These hypotheses also predict that the characteristics and quantity of leaves herbivores use as food are strongly affected by environmental changes and species characteristics. The defense characteristics of plants change as the season's progress and with the stimulation of grazing herbivores. However, these characteristics are strongly dependent on species-specific traits [7, 14].

Based on present results, C/N ratio in leaves is a valuable criterion for explaining the relationships between leaf longevity and total phenolics. However, there are some contradictions, e.g. total phenolics correlated negatively with leaf longevity (Fig. 6). The contents of total phenolics should be analyzed in detail, i.e. what kinds of components are included in total phenolics. To establish baseline data for keeping vital and healthy forests, we should consider the ecological role of defense characteristics of the representative tree species. For this, further analysis of plant protection is needed.

Acknowledgements

We would like to thank Dr. M. Rousi, Dr. M. Kitao, Dr. H. Tobita and Prof. N. Kamata for their constructive advice, Mr. S. Kitaoka for collecting references. Financial support in part by JSPS (to TK) is gratefully appreciated.

References

- [1] K. Kikuzawa, "Leaf survival and evolution in Betulaceae". *Annals of Botany*, Vol. 50, pp. 345-353, 1982.
- [2] T. Koike, "Leaf structure and photosynthetic performance as related to the forest succession of deciduous broad-leaved trees," *Plant Species Biology*, Vol. 3, 77-87, 1988.
- [3] M. Somego, "Cytogenetic studies in the subgenus *Alnuster* of the genus *Alnus*," *Association of Forest Tree Breeding*, Tokyo, pp. 132, 1985.
- [4] T. Koike, S. Matsuki, T. Matsumoto, K. Yamaji, H. Tobita, M. Kitao and Y. Maruyama, "Bottom-up regulation for protection and conservation of forest ecosystems in northern Japan under changing environment," *Eurasian Journal of Forest Research*, Vol. 6, pp. 177-189, 2003.
- [5] S. Matsuki, "Species biology of defense characteristics of deciduous broad-leaved trees, especially of Betulaceae," *PhD Thesis of the Graduate School of Agriculture, Hokkaido University*, Sapporo, pp. 134 (in Japanese), 2003.
- [6] N. Kamata, "Ecological traits in Siebold beech and interaction between plants and herbivories," *Proceedings of the Population*

Ecology Society, Vol. 56, 29-46, (in Japanese), 1999.

[7] J. Koricheva, S. Larsson, E. Haukioja, and M. Keinanen, "Regulation of woody plant secondary metabolism by resources availability: hypothesis testing by means of meta-analysis," *Oikos*, Vol. 83, pp.212-226, 1998.

[8] R. Julkunen-Tiitto, "Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics," *Journal of Agriculture Food and Chemistry*, Vol. 33, pp. 213-217, 1985.

[9] E. C. Bate-Smith, "Astringent tannins of *Acer* species," *Phytochemistry*, Vol. 16, pp. 1421-1426, 1977.

[10] H. A. Mooney and S. L. Gulmon, "Constraints on leaf structure and function in reference to herbivory," *BioScience*, Vol. 32, pp. 198-206, 1982.

[11] P. B. Reich, D. S. Ellsworth, M. B. Walters, J. M. Vose, C.

Greshan, J. C. Volin and W. D. Bowman, "Generality of leaf trait relationships: A test across six biome," *Ecology*, Vol. 80, pp. 1955-1969, 1999.

[12] E. Valkama, J. P. Salminen, J. Koricheva and K. Pihlaja, "Comparative analysis of leaf trichome structure and composition of epicuticular flavonoids in Finnish birch species," *Annals of Botany*, Vol. 91, pp. 643-655, 2003.

[13] S. Matsuki, Y. Sano and T. Koike, "Chemical and physical defense in the early and late leaves in three heterophyllous birch species native to northern Japan," *Annals of Botany*, Vol. 72, pp. 141-147, 2004.

[14] T. Koike, "Autumn coloring, photosynthetic performance and leaf development of deciduous broad-leaved trees in relation to forest succession," *Tree Physiology* Vol. 7, pp. 21-32, 1990.