Comparison of Foliar Defense by Chemical Analysis and Bioassay in Betulaceae Seedlings

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Abstract – Chemical analysis and bioassay test were carried out to clarify the specific differences in leaf quality and in growth of the gypsy moth on six Betulaceae species. We found significant differences in leaf quality and in pupal mass across species. However, the difference in growth was not explained by levels of defensive compounds or by the water and nitrogen content of leaves. Long and thick hairs on leaf surface were observed in non-palatable species.

I. Introduction

The gypsy moth (*Lymantria dispar*) is a typical polyphagous feeder on deciduous trees and exhibits cyclical outbreaks over extensive regions of Japan [1, 2]. From 2000 to 2003 we found an outbreak of the gypsy moth in the Nakagawa Experimental Forest (in the northern part of Hokkaido island). In this forest, variation in the intensity of feeding by the gypsy moth was observed. Although it was reported that the gypsy moth preferred larch and birch species [3], its order of preference of various Betulaceae species is still unknown. The Betulaceae species distributed in Hokkaido thrive at different stages of forest establishment. *Alnus hirsuta* and *Betula* sp. are typical pioneer species, showing high growth and photosynthetic rate under high light condition, indeterminant growth pattern and a short leaf lifespan. In contrast, *Carpinus cordata* thrives at the relatively shady forest floor, showing determinant growth pattern and a long leaf lifespan. *Ostrya japonica* shows an intermediate pattern of growth between these extremes. The growth / differentiation hypothesis [4] and the cost-benefit hypothesis [5] suggest that fast growing species should be more palatable to herbivores than slow growing species, because defense is costly and competes with productivity. Phenolic compounds (e.g. total phenolics and condensed tannin) are typical defensive compounds against herbivores. Toughness and hairs (non-glandular trichome) are also effective at preventing feeding by herbivores. High water and nitrogen content in leaves have a positive effect on the growth of herbivores. Larvae of the gypsy moth were grown with leaves of six Betulaceae species, and defensive parameters, together with water and nitrogen content of the leaves, were analyzed.

II. Materials and Methods

Gypsy moths (*Lymantria dispar*) were grown from eggs to pupae with leaves of six 3-year-old Betulaceae seedlings (*Alnus hirsuta*=Ah, *Betula platyphylla* var. japonica=Bp, *B. ermanii*=Be, *B. maximowicziana*=Bm, *Ostrya japonica*=Oj and *Carpinus cordata*=Cc). This took from mid May to mid June in 2002. The fresh masses of pupae were measured. Egg mass was sampled from the Nakagawa Experimental Forest in spring 2002. The seedlings were grown at the nursery of the Sapporo Experimental Forest from spring 2001. A total of 15 larvae were grown on each species. Total phenolics [6, 7], condensed tannin [8], toughness, and water and nitrogen content of leaves as sampled in 29 May were all measured. Hairs on leaf surfaces were observed via scanning electron microscopy (SEM). The mass of each pupa was measured in mid July.

III. Results

A. Bioassay test

Most gypsy moth larvae successfully reached the pupal stage with the leaves of the six Betulacea species (2, 3, 1 and 3 larvae died on Ah, Bp, Bm and Cc respectively). A difference was found in the mass of pupae (Fig. 1) across the species. Masses of pupae grown on Ah and Bp, which are fast growing species, were relatively high. Masses of pupae grown on Oj and Cc, which are slow growing species, were relatively low. Masses of pupae grown on Bm were the lowest.

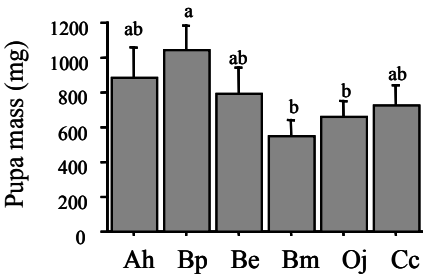

Fig. 1 Pupa mass on each species

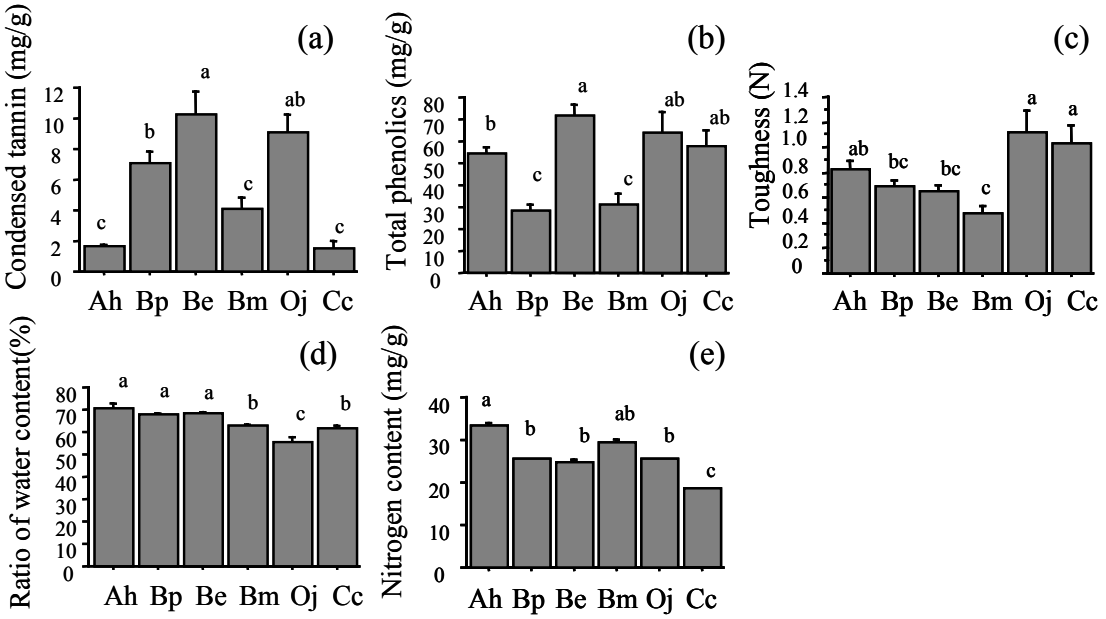

Fig. 2 Each parameter measured in the leaves of six species

B. Chemical and physical analysis

Although there was a significant difference in the concentration of total phenolics (Fig. 2b) and condensed tannin (Fig. 2a) across species, no correlation was found between these concentrations and the successional stage of trees. The leaves of Oj and Cc were significantly tougher than the others (Fig. 2c). Ratios of water content were higher in fast growing species (Fig. 2d). The nitrogen content also tended to be higher in fast growing species, although it was greater in Bm than in other *Betula* species (Fig. 2e).

C. Correlation between larva growth and leaf quality

No significant correlation was found between pupa mass and any measured parameter (total phenolics, condensed tannin, toughness, ratio of water content, nitrogen content) (Table 1). For example, pupal mass was not diminished (Fig. 1) by higher concentrations of total phenolics (Fig. 2b) or condensed tannin (Fig. 2a) in Be. Also, pupal mass (Fig. 1) was unrelated to low concentrations of total phenolics (Fig. 2b) or condensed tannin (Fig. 2a), low leaf toughness (Fig. 2c) or high nitrogen content (Fig. 2e) in Bm.

Table 1 Correlation between larva growth and each parameter

	phenolics	tannin	toughness	water	nitrogen
P	0.770	0.915	0.979	0.180	0.985
F	-0.167	0.061	-0.015	0.650	0.009

D. Hairs on the leaf surface

Short and dense hairs were observed on the upper (adaxial) leaf surface in Ah and Bp (Fig. 3). Long and dense hairs were observed in Be, Bm and Oj, though the hairs in Be were thin (Fig. 3). Long thick hairs were observed in Cc (Fig. 3). The growth of larva on species having long and thick hairs was relatively small (Fig. 1).

IV. Discussion

Although the gypsy moth could feed on all Betulaceae species used in this study and enter the pupal stage, there was a difference in the growth of larvae among the species studied. Ref. [9] also reported large differences in the acceptability of species by gypsy moth larvae. The growth of larvae grown on leaves of fast growing species (Ah, Bp and Be) was greater than on slow growing species (Bm, Oj and Cc). These results support the growth / differentiation hypothesis [4] and the cost-benefit hypothesis [5]. However, the parameters measured in this study do not explain the difference in the mass of larvae. Ref. [9] also failed to explain the acceptability to the gypsy moth of several species according to nitrogen content, water content and toughness. We expect low concentrations of defensive chemical compounds in the leaves of fast growing species. Nevertheless, leaves of Be had greatest concentration of phenolic compounds and relatively large pupal masses. Characteristics that were not measured in this study would be important in the gypsy moth's choice of host. One possibility is the effect of hairs on the leaf surface. Hairs (non-glandular trichomes) are effective in preventing feeding [10, 11 and 12]. However, as well as density, structure (length and thickness) also seems to be important. Our observations suggest that long and thick hairs might prevent

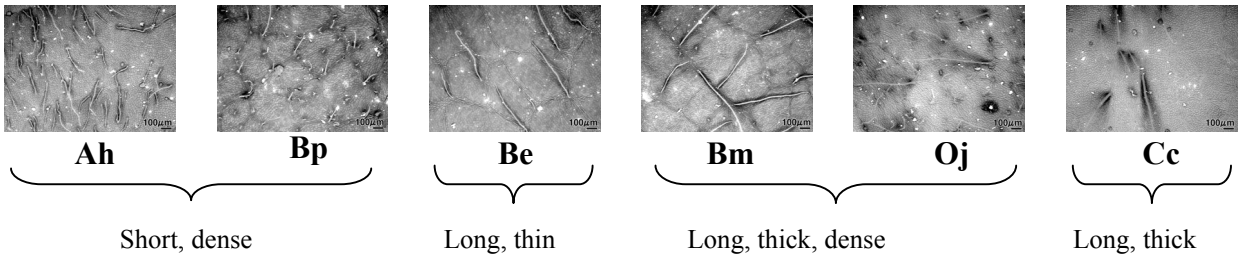

Fig. 3 Photograph of hairs on the leaf adaxial surface

feeding by the gypsy moth. In order to clarify these issues, further study is needed.

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References

[1] Y. Higashiura, "Pest control in natural and man-made forests in northern Japan," *Forest Ecology and Management*, Vol. 39, pp. 55-64, 1991.
[2] A. Liebhold, Y. Higashiura and A. Unno, "Forest type affects predation on gypsy moth (Lepidoptera: Lymantriidae) pupae in Japan," *Population Ecology*, Vol. 27, pp. 858-862, 1998.
[3] H. Hara, "Major insects injurious to the trees of *Betula* spp. and *Fraxinus mandschurica* var. *japonica* in Hokkaido and their management," *Bulletin of the Hokkaido Forestry Research Institute*, Vol. 37, pp. 67-74, 2001 (In Japanese).
[4] H. A. Mooney and S. L. Gulmon, "Constraints on leaf structure and function in reference to herbivory," *BioScience*, Vol. 32, pp. 198-206, 1982.
[5] K. Kikuzawa, "Cost-Benefit analysis of leaf habit and leaf longevity of trees and their geographical pattern," *American Naturalist*, Vol. 138, pp. 1250-1263, 1991
[6] O. Folin and V. Ciocalteu, "On tyrosine in proteins as determination in proteins," *Journal of Biological Chemistry*, Vol. 27, pp. 627-650,1927.
[7] 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.
[8] E. C. Bate-Smith, "Astringent tannins of *Acer* species," *Phytochemistry*, Vol. 16, pp. 1421-1426, 1977.
[9] A. F. Hunter and M. J. Lechowicz, "Foliage quality change during canopy development of some northern hardwood trees," *Oecologia*, Vol. 89, pp. 316-323, 1992.
[10] M. B. Traw and T. E. Dawson, "Differential induction of trichomes by three herbivores of black mustard" *Oecologia*, Vol. 131, pp. 526-532, 2002
[11] 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.
[12] 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*, in press.