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Photosynthesis, Leaf longevity and Defense Characteristics in Trees of Betulaceae Planted in Northern Japan

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Abstract

Basic understanding of the relationship between photosynthetic capacity, leaf defenses and leaf longevity is important for the maintenance of forest vigor and health. We compared physical (leaf mass per area, leaf toughness, presence of trichomes) and chemical (total phenolics, condensed tannin) defense characteristics of leaves, net photosynthesis, and leaf carbon/nitrogen (C/N) ratio among trees and saplings of 18 Betulaceae species. These included five species (*Alnus glutinosa*, *A. incana*, *A. sibirica*, *Betula pendula*, and *B. verrucosa*) originating from the Eurasian continent. A weak negative correlation was found between leaf lifespan and LMA (leaf mass per area). No relation between the existence of trichome and leaf longevity was found. The amount of total phenolics showed a positive correlation with C/N ratio in leaves. Leaf longevity was negatively correlated with the amount of total phenolics. Except for *Betula platyphylla*, two species of *Carpinus* and *Corylus heterophylla*, there was a positive correlation between the amount of condensed tannin and leaf longevity. Successional traits of 18 Betulaceae species and leaf defense characteristics are discussed based on relations of the C/N ratio of leaves and adaptive radiation in the family. Early successional species had higher photosynthetic rate with short longevity of leaves and smaller content of defense chemicals and vice versa for late successional species.

Key words: Leaf defense, leaf longevity, trichome, Betulaceae, successional status

Introduction

Betulaceae trees are a common species in secondary forests in northern Japan (Koike 1988, 1990, 1995). Species in this family show a wide range of successional traits, i.e. early-, mid- (or gap phase), and late- successional characteristics (Koike 1988). Kikuzawa (1982) showed that the pattern of leaf survival and structure of winter buds correspond to the ecological characteristics of each species with phylogenetic constraints. In addition, there are several studies on the defense characteristics of Betulaceae (e.g., Rousi *et al.* 1996, Koike 1995, Matsuki and Koike 2006).

Some of alders and birches were imported from western Eurasian regions, especially from northern Europe, for breeding and silvicultural purposes (Somego 1985). Some of these species are resistant to insect herbivores while others are more susceptible to them. 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 (Koike *et al.* 2003, Matsuki and Koike 2006). This suggests a trade-off between defense characteristics and leaf longevity. In addition, for

species with long leaf life span, investment in defense characteristics may restrict investment in photosynthetic enzymes and result in lower photosynthetic capacity because most of defense chemicals in deciduous broad-leaved trees are originated from phenylalanine, a nitrogen compound (e.g. Kamata 1999). Basic understanding of leaf defense characteristics is important for maintaining growth and vitality of secondary forests dominated by Betulaceae species in northern Japan. However, it has not been well researched (Koike 1988, Matsuki and Koike 2004).

In general, contents of defense chemicals are not only affected by soil fertility, but also by the growth phase of the plant life cycle (Kamata 1999, Koricheva *et al.* 1998). The defense characteristics of plants change with season and in response to grazing by herbivores. In addition, these characteristics are strongly dependent on species-specific traits (Julkunen-Tiitto 1985, Matsuki *et al.* 2004, Matsuki and Koike 2006) as a result of evolution. Investment in leaf defense characteristics may vary widely, even among species in the same family. Based on field observations (Matsuki 2003), alder (Betulaceae) is a typical pioneer

and its leaves are grazed annually by leaf beetles (*Agelastica coerulea*). Leaf grazing, however, rarely kills these trees. In contrast, leaves of *Ostrya japonica* (gap phase species, also Betulaceae) 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 defense characteristics of Betulaceae trees and leaf longevity or photosynthetic capacity? In this study, we examined leaf defense characteristics of 18 Betulaceae species in relation to the trichome as physical defense, carbon/ nitrogen (C/N) content and leaf defense chemicals as chemical defense in leaves. We inferred differences among species in their defense characteristics in the context of adaptive mode in the Betulaceae family.

Materials and Methods

Plant materials:

Adult trees (ca. 25-years old as of 2002) of 18 Betulaceae species were used for the study, with the exception of *B. davurica*, which was 15 yrs old). All trees were grown at the Hitsujigaoka Experimental Forest of Forestry and Forest Products Research Institute (FFPRI) (Sapporo; 42°58'N, 141°23'E, 146 m a.s.l.). Sample leaves (leaf age = 60-80 days) were collected from the sun crown in late July to mid Aug, 2000. As shown in Table 1, 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. 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. Five species (*Alnus glutinosus*, *A. incana*, *A. sibirica*, *Betula pendula*, and *B. verrucosa* = *B. pubescens*) originated from the Eurasian continent (northern Europe). All *Alnus* species have symbiotic microorganisms (*Frankia* sp.) in their roots, which fix nitrogen directly from the air.

Measurement of light-saturated photosynthetic rate

We measured light-saturated photosynthetic rate of early and late leaves using a portable gas-exchange system (LI-6400, Li-Cor, NE, USA). Light intensity, leaf temperature and CO₂ concentration during the measurement were 1500 μmol m⁻² s⁻¹, 25°C and 350 ppmV, respectively.

Measurement of defensive characteristics of leaves

As indicators of physical defense characteristics of leaves, we measured leaf mass per area (LMA) and leaf toughness and observed the presence of trichomes. We sampled two leaf discs with a leaf punch (diameter 5mm; Kokuyo LtD. Tokyo). One was used for analysis of C/N and the other was for defense chemicals. After dryness of the leaf disk in oven for 48 hrs, we determined leaf dry mass. We measured the toughness of fresh leaves using a push-pull gauge (CPU gauge, AIKOH, Nagoya, Japan) (Matsuki and Koike 2006).

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). We also determined the content of defense chemicals of leaves. After the photosynthesis measurement, the leaves were immediately freeze-dried (FLEXI-DRY, FTS Systems, USA) and mill-ground (TM10, Tescom, Tokyo, Japan) down to powder. The concentration of total phenolics in 20mg leaf powder was determined by the Folin-Ciocalteu method (Julkunen-Tiitto 1985). The concentration of condensed tannin was determined using the proanthocyanidin method (Bate-Smith 1977). The remaining sample leaves were used for the measurement of carbon and nitrogen content by a N/C analyzer (NC-900, Shimadzu, Kyoto, Japan) after being dried at 60°C for 48 hours.

Results

Photosynthetic characteristics

Except in several alders, there was a strong positive correlation between net photosynthetic rate per area (Pn) and nitrogen content per area (Fig. 1). Alders (*Alnus japonica*) had low Pn despite high nitrogen content. There was a weak negative correlation between leaf lifespan and net photosynthetic rate ($r = -0.68$, $P = 0.0523$; data not shown).

Defense characteristics

A clear positive correlation was found between leaf mass per area (LMA) and leaf toughness (Fig. 2). There was no clear tendency among species in the relationship between LMA and leaf toughness. Tree species originating from high mountain areas (e.g., *Alnus maximowiczii*, *Betula ermanii*) or high latitude regions (e.g., *A. sibirica*, *B. verrucosa*) had a high value of LMA and toughness.

No relation between growth traits and the existence of trichome was found. Most species (e.g. alder, birch, *Carpinus* sp., *Ostrya*) 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 (see obtained from nursery). *B. maximowicziana* seedlings and young trees had thick trichome in both adaxial and abaxial sides in early and late leaves, but in older trees and adult trees there is almost no trichome (data not shown). Epicuticle wax was found in abaxial side of *A. hirsuta* leaves.

We found no relationship between C/N ratio and the concentration of condensed tannin ($r = -0.08$, $P = 0.01$). *B. platyphylla* had an extremely high value of condensed tannin (33.7 mg g⁻¹) despite intermediate value of C/N ratio (19.8). Excluding 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 was positively correlated with C/N ratio of leaves, whereas it was negatively correlated negatively with LMA (Fig 5). Excluding four species (*B. platyphylla*, *C. cordata*, *C. laxiflora* and *Corylus*

heterophylla), leaf longevity was positively correlated with the amount of condensed tannin (Fig. 6). By

contrast, leaf longevity was negatively correlated with the amount of total phenolics.

Table 1. Latin name and ecological characteristics of material species.

Latin name (abbreviation)	Japanese common name	Successional status and habitat
<i>Alnus hirsta</i> (Ah)	Keyama hannoki	Light demanding, early-mid successional trait
<i>Alnus inokumae</i> (= <i>A. hirsuta</i> var. <i>microphylla</i>) (Ai)	Kobano yamahannoki or Tanigawahannoki	Light demanding, early-mid successional trait, Leaf size is smaller than <i>A. hirsta</i>
<i>Alnus japonica</i> (Aj)	Hannoki	Light demanding, early-mid successional trait, High flooding tolerance
<i>Alnus maximowiczii</i> (Am)	Miyama Hannoki	Light demanding, early-mid successional trait, They grow at cool habitat, shrub
<i>Betula davurica</i> (Bd)	Yaegawa kanba	Light demanding, early-mid successional trait, They grow at high mountain site and cool habitat
<i>Betula ermanii</i> (Be)	Dake kanba	Light demanding, early-mid successional trait, They grow at high mountain site and cool habitat
<i>Betula obovata</i> (= <i>B. tatewakiana</i>) (Bo)	Yachi kanba	Light demanding and flood tolerant shrub species. Similar to <i>Betula nana</i> in north Europe
<i>Betula platyphylla</i> var. <i>japonica</i> (Bp)	Shira kanba	Typical early successional species and grows well on dry and wet conditions at lowland.
<i>Betula maximowicziana</i> (Bm)	Udai kanba	Early-mid successional species. They become a member of mixed conifer and broadleaf forests because their long lifespan upto 250 years old
<i>Corylus heterophylla</i> var. <i>thunbergii</i> (Ch)	Hashibami	Gap phase species but light demanding shrub
<i>Ostrya japonica</i> (Oj)	Asada	Gap phase species and they become a member of mixed conifer and broadleaf forests
<i>Carpinus cordata</i> (Cc)	Sawashiba	Late successional species with high shade tolerant capacity
<i>Carpinus laxiflora</i> (Cl)	Akashide	Mid-Late successional species with intermediate shade tolerant capacity
From Europe		
<i>Alnus glutinosa</i> (Ag)	Guruchinoza hannoki	Light demanding, flooding tolerant
<i>Alnus incana</i> (Ai)	Inkana hannoki	Light demanding, flooding tolerant
<i>Alnus sibirica</i> (As)	Shiberia hannoki	Light demanding, flooding tolerant
<i>Betula pendula</i> (Bpa)	Penjyura kanba	Early successional species and they grow open site after disturbance
<i>B. verrucosa</i> (= <i>B. pubescens</i>) (Bv)	Berukoza kanba	Early successional species and they grow open site st cool climate

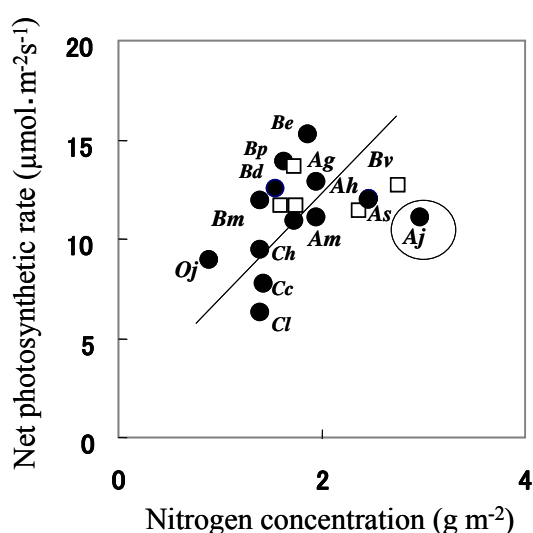


Fig. 1. Light saturated photosynthetic rate in relation to leaf nitrogen content of 18 Betulaceae species. Circle and square denote species of Japanese and Eurasian origin, respectively. *Alnus japonica* (circled) had low photosynthetic rate despite high leaf nitrogen concentration. Abbreviation of species names are referred to Table 1.

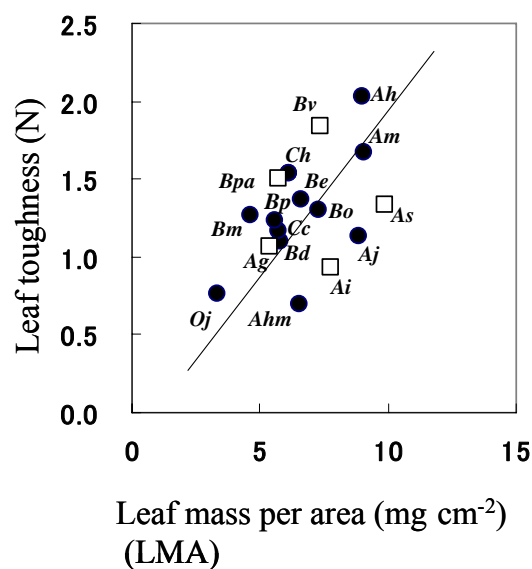


Fig. 2. Leaf mass per area (LMA) in relation to leaf toughness for 18 Betulaceae species. Symbols are the same as in Figure 1.

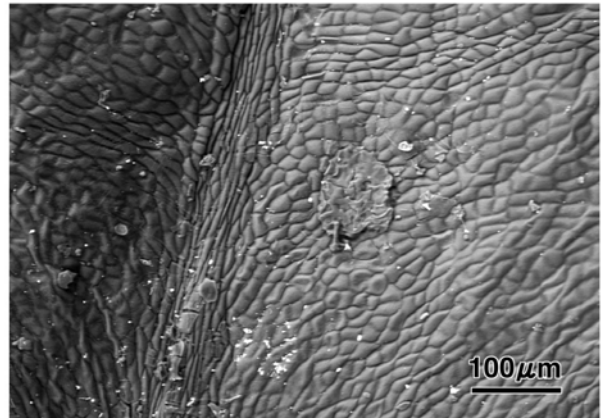
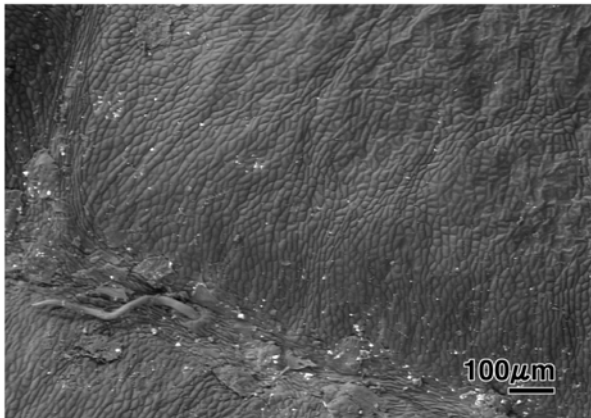
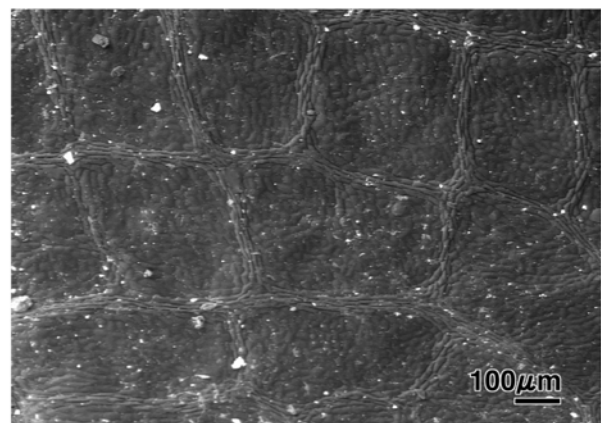
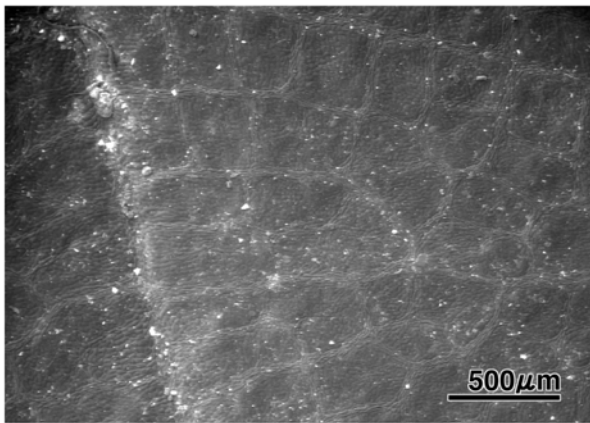
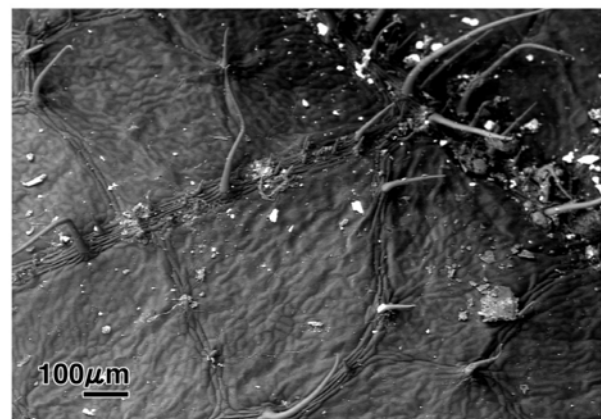
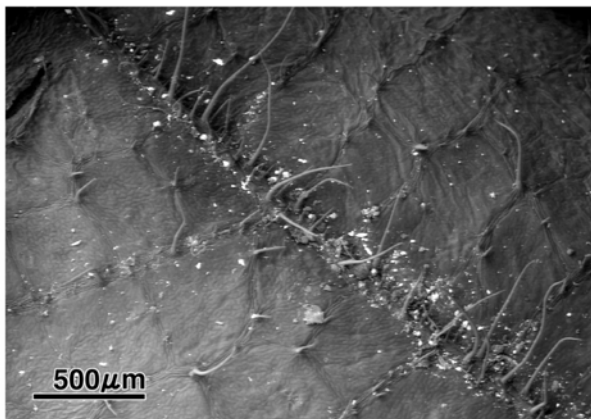
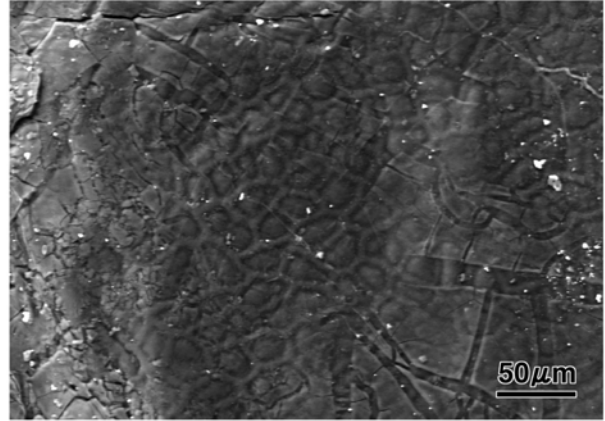
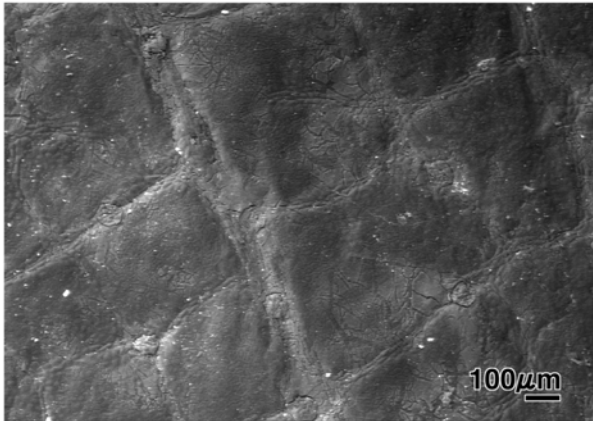
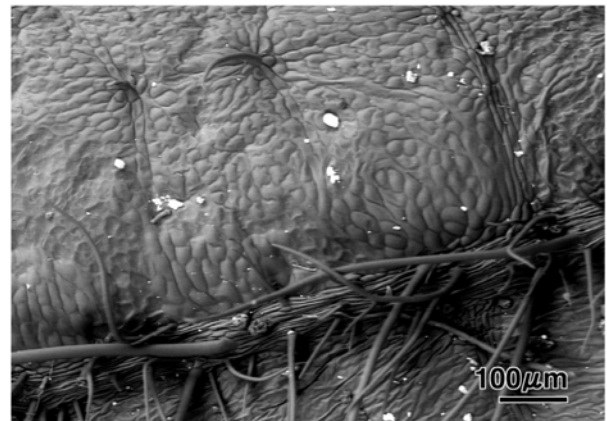
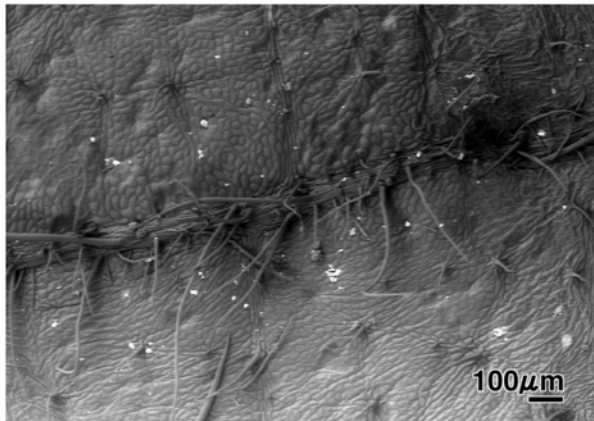
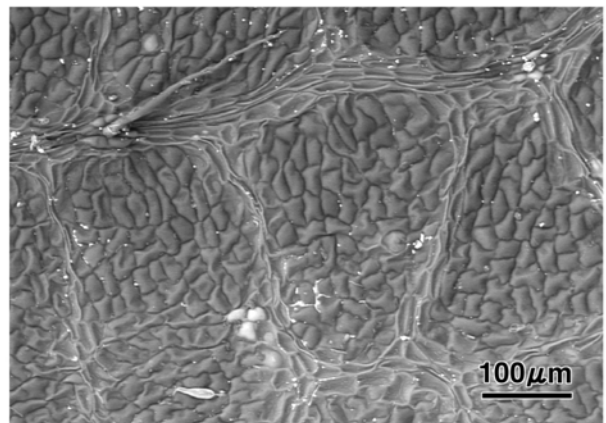
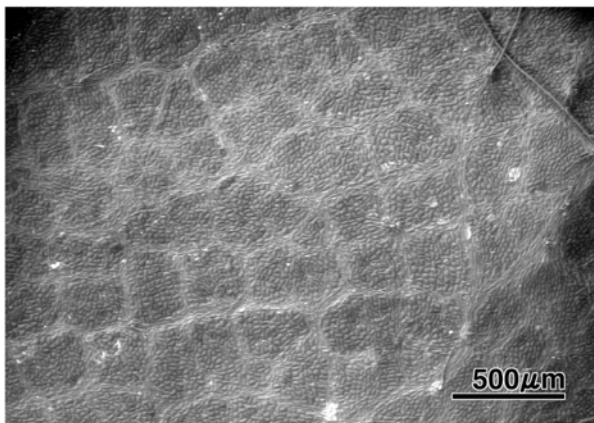
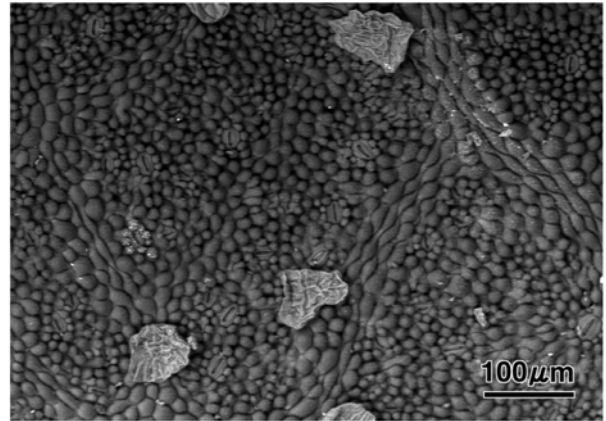
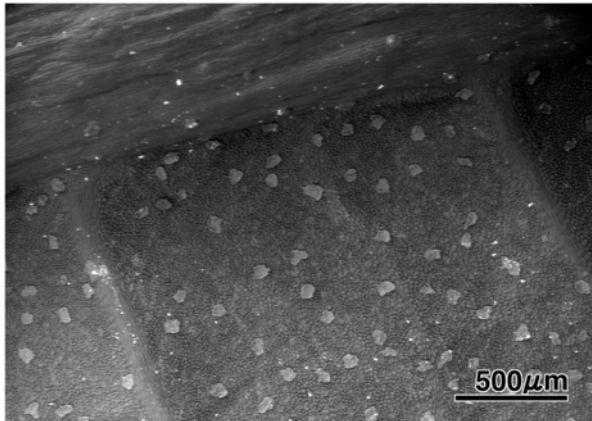
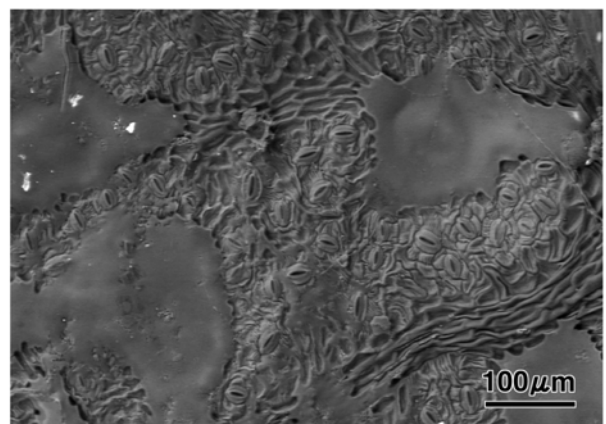
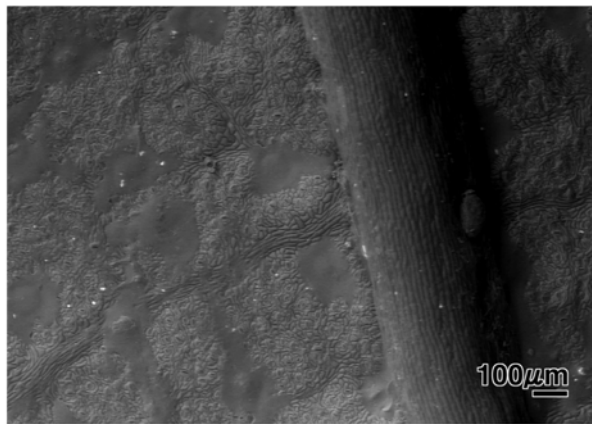
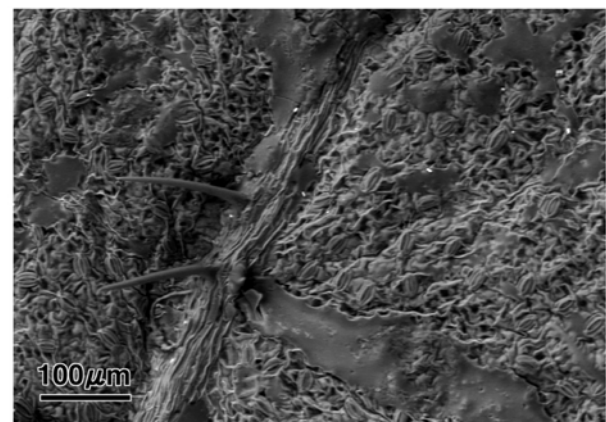
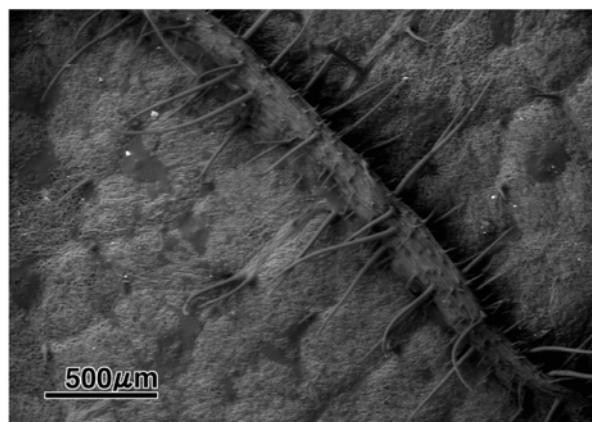
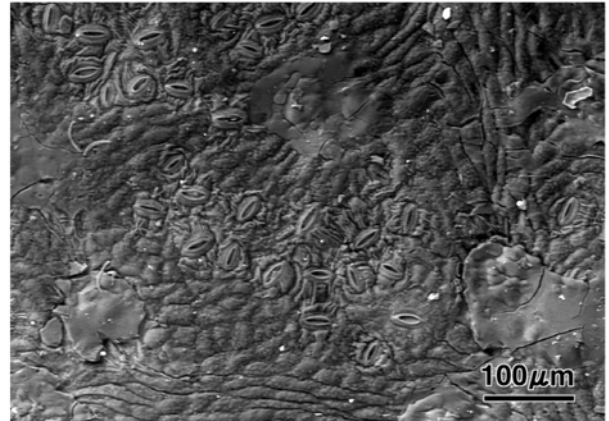
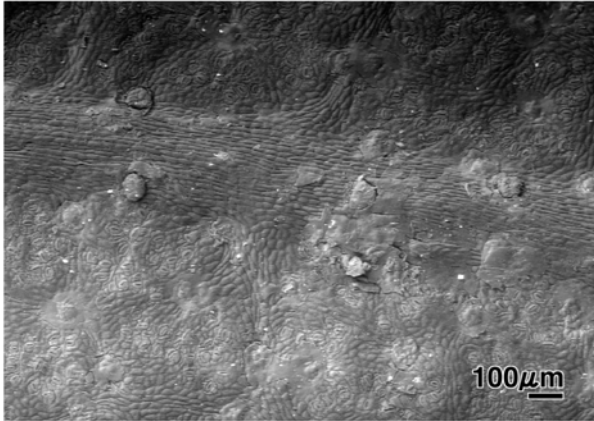
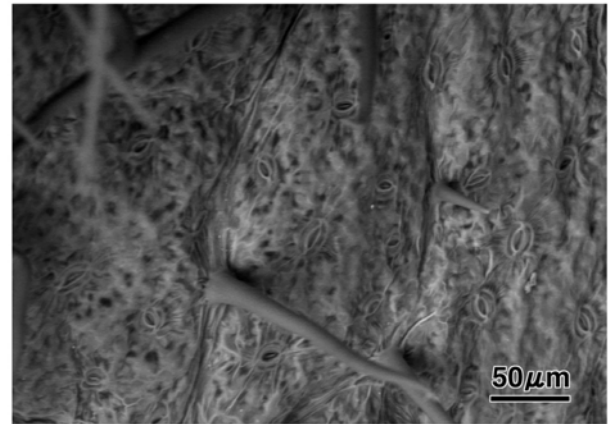
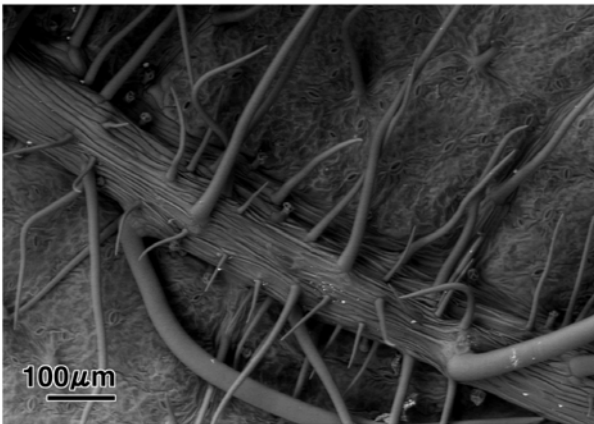
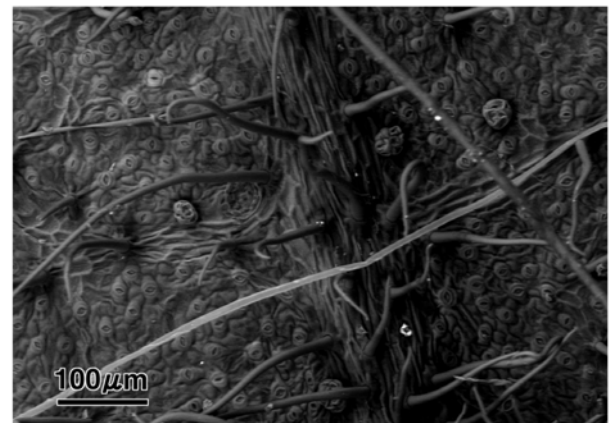
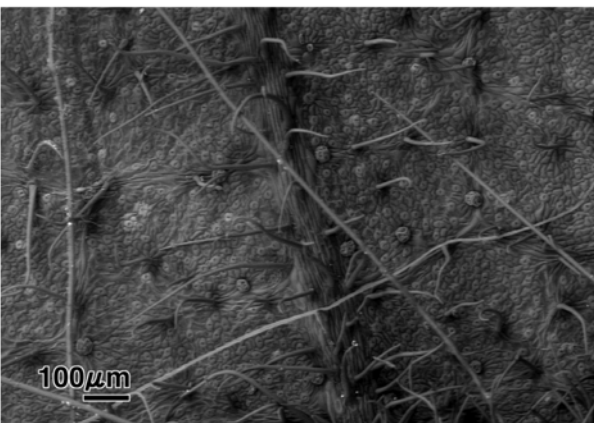
Alnus hirsuta*Betula maximowicz**Betula maximowicz* (nursery)

Fig. 3. Examples of trichomes along leaf veins on the abaxial sides of leaves of Betulaceae species.

Betula platyphylla*Ostrya japonica**Carpinus cordata*

Alnus hirsuta*Betula maximowicz**Betula maximowicz* (nursery)

Betula platyphylla*Ostrya japonica**Carpinus cordata*

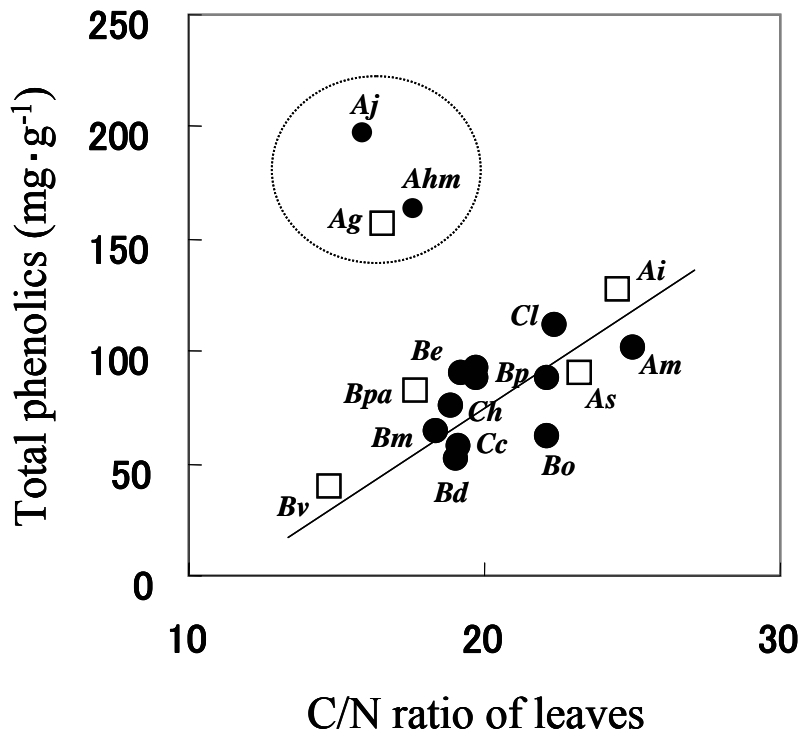


Fig. 4. Relationship between C/N ratio and the amount of total phenolics. Symbols are referred to Table 1.

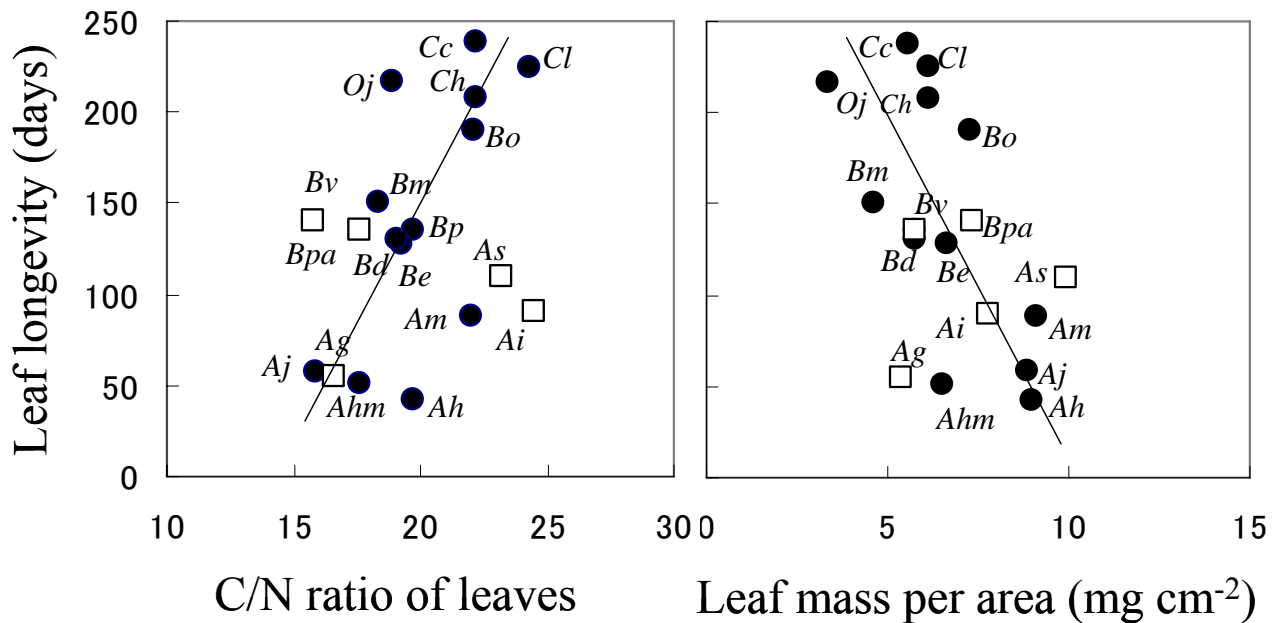


Fig. 5. Leaf longevity in relation to C/N ratio and leaf mass per area (LMA). Explanation of the same as in Figure 4.

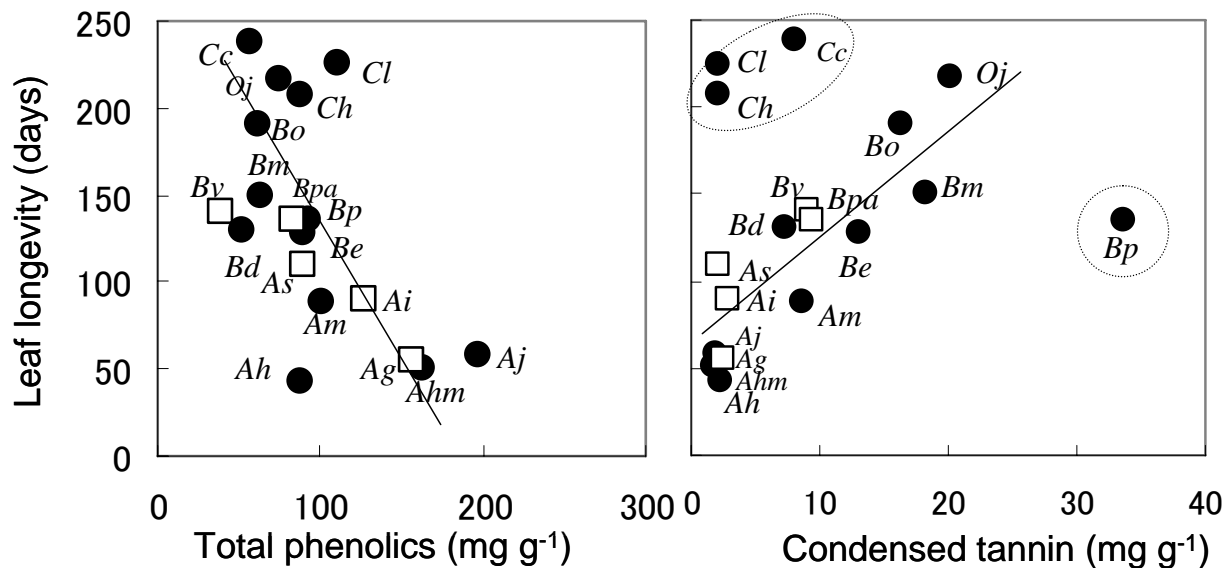


Fig. 6. Leaf longevity in relation to total phenolics and condensed tannin. Symbols are referred to Table 1 and Figure 4.

Discussion

In deciduous broad-leaved trees, species with long leaf longevity have a relatively lower photosynthetic rate per area (Koike 1988, Matsuki and Koike 2006). Species of deciduous leaf habit with low leaf turnover rate usually have a high defense capacity (Mooney and Gulmon 1982, Koike 1988, Reich *et al.* 1999). This suggests that greater allocation to defense capacity may come at the cost of reduced photosynthetic capacity but could prolong leaf longevity. Leaf mass per area (LMA) represents a physical defense, such as mechanical protection and leaf toughness (Koike 1988). We found, however, a weak negative correlation existed between LMA and leaf lifespan (Fig. 5). Throughout the six biomes, species with a high photosynthetic rate usually have a high LMA (Koike 1988, Reich *et al.* 1999) because the amount of photosynthetic organs per area is large, while their leaf longevity is relatively short.

High photosynthetic capacity is usually associated with high leaf turnover to maximize carbon gain (Kikuzawa 1991). Therefore, the negative correlation between LMA and leaf longevity (Fig. 5) may be determined by carbon gain rather than defense characteristics. Trichome of leaves acts as a physical defense in some birch species (Valkama *et al.* 2003, Matsuki *et al.* 2004). Most alders have a high density of trichome on the abaxial side, especially along leaf veins (Fig. 3). Trichomes may be an alternative strategy for physical defense of leaves that requires less biomass investment than increasing leaf thickness. Epicuticle of *A. hirsuta* may have some improvement of extra-transpiration of leaf surface (Kitahashi *et al.* 2005).

The amount of chemical defense should correlate positively with leaf longevity. Excluding *B. platyphylla*, there was a weak positive correlation between the amount of condensed tannin and leaf lifespan (Fig 6). 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 *Co. heterophylla* had lower amount of condensed tannin but longer leaf lifespan. Excluding the alders, the amount of total phenolics in leaves increased with increasing C/N ratio (Koike *et al.* 2006). Alders have 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 sheath in leaves may maximize their mechanical protection (Koike 1996). The inner structure of a leaf should be analyzed from the viewpoints of function and structure (Mooney and Gulmon 1982, Koike 1988). Moreover, alders may compensate for their grazed leaves by continuously producing new leaves. In this experiment, we matched the age of sample leaves ca. 60-80 days. For some species, such as alders, with short leaf lifespan, we may have missed the peak value of photosynthetic rate (Koike 1990). Typically, early successional species have high photosynthetic rate and short leaf lifespan with lower defense traits. *B. platyphylla* was an exception to this trend. By contrast, two species (*Carpinus* sp. and *Ostrya*) had lower photosynthetic rates and longer leaf lifespan with higher defense traits.

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 (Kamata 1999, Koricheva *et al.* 1998). These hypotheses predict that the characteristics and quantity of leaves herbivores use as food are strongly affected by environmental changes and species growth characteristics. The defense traits of plants change with season progress and in response to grazing by herbivores (Matsuki *et al.* 2004,

Matsuki and Koike 2005) and with elevated CO₂ (Koike *et al.* 2006). However, these characteristics are strongly dependent on species-specific traits (Koricheva *et al.* 1998, Koike 1990). Based on the present results, C/N ratio in leaves is a valuable criterion for explaining the relations of leaf longevity and defense traits. 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.

Maintaining growth and vitality of secondary forests consisting of Betulaceae species in northern Japan requires basic knowledge of the ecological role of defense characteristics of the representative tree species and the interactions among defense characteristics, photosynthetic capacity and longevity of leaves. For this, further analysis of plant protection is needed.

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