INTRODUCTION

The three birch species _Betula ermanii_ Cham, _B. maximowicziana_ Regel and _B. platyphylla_ var. _japonica_ Hara are pioneers within the deciduous broadleaf habit of northern Japan, although _B. maximowicziana_ can, like late-successional species, act as a dominant species in mixed forests (Tabata, 1966). These three species prefer sunny locations and have an indeterminate growth pattern (Tabata, 1964, 1966; Tatewaki, 1964). They have heterophyllous leaves—both early and late (Clausen and Kozlowski, 1965). The early leaves flush immediately after bud breaking, and the late leaves appear after the early leaves are mature and continue to appear until early September (Kikuzawa, 1982; Koike and Sakagami, 1985; Koike, 1995). Kozlowski and Clausen (1966) found in heterophyllous _B. papyrifera_ that the contribution of early leaves to subsequent shoot growth was greater than that of late leaves. This indicates that early leaves are crucial to the production of new shoots.

It is suggested that defence systems in early leaves are important, since the period of 3–4 weeks from bud-breaking in spring is the crucial time for assimilation to produce new shoots and also the time of greatest pressure from herbivores (e.g. Varley, 1967; Feeny, 1970; Kamata, 2000). When the physiological trade-off within a plant is considered in isolation, the growth–differentiation balance (GDB) hypothesis considers that both growth and defence require photosynthate, and are negatively correlated (Mooney and Gulmon, 1982; Lerdau et al., 1994). This hypothesis asserts that allocation to carbon-rich secondary metabolites of early leaves during high relative rates of growth will be regulated, since leaf expansion and active shoot growth require large quantities of photosynthates (Riipi et al., 2002). However, when the selective pressure of herbivory and competition are taken into account, optimal defence (OD) theory predicts that strong defence will be allocated to tissue which has both ‘value’ and ‘vulnerability’ (McKey, 1974; Ohnemeiss and Baldwin, 2000; Stamp, 2003).

In woody plants, carbon-based chemical compounds such as phenolics and terpenes comprise the main chemical defence (Waterman and Mole, 1994). These compounds inhibit the digestion of protein in various herbivores (Waterman and Mole, 1994; Nomura and Itioka, 2002). Physical defence can also be effective against herbivores. Leaf toughness is a major deterrent to numerous herbivores (Feeny, 1976), and surface hairs (trichomes) on leaves are also effective. Ehleringer and Clark (1988) suggested that trichomes are for regulation of leaf spectral properties, temperature and water loss, but defence against herbivores may also be important (Gregory et al., 1986; Agrawal, 1999; Kostina et al., 2001; Traw and Dawson 2002). High molecular weight compounds such as phenolics, that are
immobile, cannot be recycled and are held until the leaves fall (Mooney et al., 1983). Physical constructs are also unlikely to be reusable for other functions. It is therefore expected that old leaves should have stronger defences than young leaves. It has often been reported that new leaves are of higher quality (more nitrogen, fewer phenolics, reduced toughness and other characteristics) than old leaves, so that they are preferred by herbivores (Feeny, 1970; Hunter and Lechowicz, 1992). Birch species have an indeterminate growth pattern, meaning that development of leaves continues throughout the growing season. Is there, then, any difference in defence between leaves at similar ages that develop at different times of year (i.e. early and late leaves)? Studies of the defence system in heterophyllous leaves are scarce (but see Lechowicz and St-Jacques, 2000). The hypothesis is that the ‘value’ and ‘vulnerability’ of heterophyllous leaves—early and late leaves in birch species—will be different, resulting in different strengths of defence between early and late leaves.

The present study focuses on three species of birch. Previous studies indicate a difference in the importance of early and late leaves in these species. Shoot development in B. platyphylla depends more on the activity of early leaves than in B. ermanii (Koike, 1995). Koike and Sakagami (1985) reported that the photosynthetic rate of early leaves was greater in B. platyphylla than in B. maximowicziana, and the early leaves in B. platyphylla lived 15 d longer than those of B. maximowicziana. These observations suggest that growth of B. platyphylla is more dependent on photosynthesize assimilated by prevailing early leaves than in the latter two species. Betula ermanii has a short growth period during which most of its leaves develop, and it shows a peak in its photosynthetic rate early in the growing season (Koike et al., 1988), suggesting that it invests strongly in defence early in the growing season. Betula maximowicziana, in contrast, shows a peak photosynthetic rate later in the growing season (Koike et al., 1988). Also, the trunk : root ratio is lower in B. ermanii and B. maximowicziana than in B. platyphylla (Fujimura and Sakagami, 1984). The high biomass of the root system relative to the above-ground biomass suggests a high storage carry-over from previous years, and less dependence on current assimilation for subsequent growth.

From these observations, it is predicted that assimilation of early leaves is more important in B. platyphylla than in the other two species. It is proposed that B. platyphylla invests in strong defence for early leaves, since damage to these leaves is liable to reduce subsequent growth. In contrast, B. maximowicziana needs to defend its late leaves, since it is less dependent on the early leaves and has its photosynthetic peak later in the growing season. Betula ermanii might need to invest in continuing high defence because of the shortness of its growing season (Koike 1995).

Comparisons were made across these three birch species of the amount of total phenolics and condensed tannin, the leaf toughness, and the density of trichomes on the leaf surface between early and late leaves harvested at different times during the growing season. The effect of defoliation of early leaves (in May) on subsequent growth was examined. A comparison between the photosynthetic rate in control and defoliated seedlings was also made, since it is possible that this might change after defoliation. The aims of the study were: (a) to determine differences in the seasonal pattern of defence between the three birch species, and (b) to investigate whether the defence pattern depends on the relative importance of early leaves.

**MATERIALS AND METHODS**

**Plant material**

Two-year-old seedlings of the three birch species (numbers of seedlings: B. platyphylla, 137; B. ermanii, 120; B. maximowicziana, 57) were obtained from Kuriyama town near Sapporo and were planted directly in the field at the experimental nursery of Hokkaido University Forests (43°44′N, 141°20′E) in full sun in April 2001. Seedlings of each species were planted randomly over the field. The soil was well fertilized and was watered regularly. Herbivores found on the seedlings were removed by hand to protect the seedlings from damage. Between 1 and 2 weeks after planting, two leaves of each bud on the seedlings started to flush; these are classified as ‘early leaves’. Betula platyphylla and B. ermanii began foliation about 5 d earlier than B. maximowicziana. Betula platyphylla and B. ermanii seedlings started to develop ‘late leaves’ about 4 weeks after the flush of the early leaves, and continued to flush until mid-September 2001. Betula maximowicziana seedlings started to develop ‘late leaves’ about 3 weeks after the flush of the early leaves. In contrast to previous studies (e.g. Fujimura and Sakagami, 1984), almost no after-effects of transplantation were observed in these three birch species.

**Timing of harvest and defoliation**

Parameters that define growth and defensive characteristics for the leaves were measured three times in the growing season of 2001. The first measurements were carried out in late May, 3 weeks after bud-break began. Only the early leaves were developed completely by this stage. Immediately after these first measurements, a half-length of the lamina in all early leaves of some randomly selected seedlings was clipped by scissors. In B. platyphylla, 59 seedlings were clipped and 77 remained as controls. In B. ermanii, 38 seedlings were clipped and 81 remained as controls. In B. maximowicziana, 20 seedlings were clipped and 37 remained as controls. The second set of measurements was made in late July and the third set in early October. All leaves used for measurement were about 3 weeks of age from bud-break and the leaves were essentially fully developed.

**Measurement of dry mass of seedlings**

For each seedling, the above-ground height and the diameter of the bottom of the stem was measured. The total dry mass of each seedling was then estimated from these values using a formula appropriate to each species. Parameters in the formula were found from previous measurements of the total dry mass ($M$), the height ($H$)
and the diameter ($D$) of a number of seedlings ($B. ermanii$, $n = 20$, $B. maximowicziana$, $n = 10$, $B. platyphylla$, $n = 19$).

The harvested seedlings were dried in an oven at 60 °C for at least 2 d.

The dry mass of the seedlings was estimated using the following equation:

$$M = a D^2 + b$$

where $a$ and $b$ are parameters related to growth.

The relative growth rate (RGR) was calculated using the following equations (Hunt, 1982):

$$RGR_{1 \rightarrow 2} = \frac{1}{M_1} \times (M_2 - M_1)/\left(t_2 - t_1\right)$$

$$RGR_{2 \rightarrow 3} = \frac{1}{M_2} \times (M_3 - M_2)/\left(t_3 - t_2\right),$$

where $RGR_{1 \rightarrow 2}$ denotes the RGR from first to second harvest, $RGR_{2 \rightarrow 3}$ denotes the RGR from second to third harvest, $M_1$ is the total dry mass at the first harvest, $M_2$ is the total dry mass at the second harvest, $M_3$ is the total dry mass at third harvest, and $t_1$, $t_2$ and $t_3$ are the respective dates of these harvests.

Measurement of defensive characteristics of early and late leaves

After measurement of the parameters defining growth, five seedlings per treatment were selected at random and harvested; this was done three times during the growing season. Measurements of parameters defining defence were made on the early leaves (about five leaves per seedling) of seedlings harvested in late May, the late leaves (one or two leaves per seedling) of seedlings harvested in late July, and the late leaves (one or two leaves per seedling) of seedlings harvested in early October. All leaves studied at each harvest were about 3 weeks old. The leaves were immediately freeze-dried (FLEXI-DRY; FTS Systems, New York, USA) and mill-ground (TM10; Tescom, Osaka, Japan) down to powder. The concentration of total phenolics in 20 mg leaf powder was determined by the Folin–Ciocalteu method (Folin and Ciocalteu, 1927), as modified by Julkunen-Tiitto (1985). The concentration of condensed tannin was determined using the proanthocyanidin method (Bate-Smith, 1977). The toughness of fresh leaves not used for chemical analysis was also measured, using a push–pull gauge (CPU gauge; AIKOH, Nagoya, Japan). One leaf was used from each seedling. Toughness was measured at three points on each leaf and the mean value determined. Trichomes on the upper surfaces of the leaves (adaxial side) were observed by microscope and were counted using the Sump method that is Suzuki’s universal micro-printing method (Koike et al., 1998) using a single leaf per seedling.

Measurement of light-saturated photosynthetic rate

The light-saturated photosynthetic rate ($P_{sat}$) of early and late leaves for defoliated and control seedlings was determined in mid-August 2001. A portable system dedicated to the measurement of photosynthetic rates (LI-6400; Li-Cor, Nebraska, USA) was used at a light saturation of 2000 μmol m$^{-2}$s$^{-1}$ and a leaf temperature of 25 °C, with a CO$_2$ concentration of 360 ppm as chosen previously by Koike (1995). Three seedlings were selected at random from each treatment for these measurements.

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
<th>Phenolics (mg g$^{-1}$)</th>
<th>Tanin (mg g$^{-1}$)</th>
<th>Toughness (N)</th>
<th>Trichomes (cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (S)</td>
<td>2</td>
<td>&lt;0.001 (11.58)</td>
<td>&lt;0.001 (25.12)</td>
<td>0.459 (0.80)</td>
<td>&lt;0.001 (18.65)</td>
</tr>
<tr>
<td>Harvest time (H)</td>
<td>2</td>
<td>0.101 (2.46)</td>
<td>0.012 (5.07)</td>
<td>0.024 (4.22)</td>
<td>&lt;0.001 (34.22)</td>
</tr>
<tr>
<td>$S \times H$</td>
<td>4</td>
<td>0.071 (2.379)</td>
<td>0.176 (1.66)</td>
<td>0.984 (2.16)</td>
<td>&lt;0.001 (18.29)</td>
</tr>
</tbody>
</table>

ANOVA probability values are shown, with $F$-values in parentheses.

Data are shown for species ($Betula platyphylla$, $B. ermanii$ and $B. maximowicziana$), harvest time (early leaves harvested in May, late leaves harvested in July, and late leaves harvested in October) and their interaction.
Statistics

Two-factor factorial ANOVA was used to compare total phenolics, condensed tannin, toughness and density of trichomes between the different stages of harvest (early leaves, late leaves in July and late leaves in October) in the three birch species. Leaf parameters at the different times of harvest were then compared across species by one-way ANOVA. Interspecific differences in leaf parameters at the same stage of harvest were also compared with one-way ANOVA. RGR values between defoliated and control in each species in each period (May–July, July–October) were compared with one-way ANOVA. Species or treatment differences in $P_{sat}$ of early and late leaves were compared with one-way ANOVA. All statistical analyses used Stat View statistical software (version 5.0.1; SAS Institute, North Carolina, USA).

RESULTS

Comparison of defence between early and late leaves and between species

Parameters relating to chemical and physical defence were compared across leaves harvested in May (early leaves), July (late leaves) and October (late leaves). There was a significant difference across species in the total phenolics but no significant differences with time of harvest (Table 1). For *Betula platyphylla*, the concentration of total phenolics in early leaves was about 40 mg g$^{-1}$ higher than in late leaves (Fig. 1A). The concentration of total phenolics in early leaves also tended to be higher than in late leaves in *B. ermanii* (Fig. 1A), although the difference was not statistically significant. For *B. maximowicziana*, in contrast, the concentration of total phenolics in late leaves was about 15 mg g$^{-1}$ higher than in early leaves (Fig. 1A, d.f. 2, $F = 12.6$, $P < 0.01$). In late leaves in July, total phenolics in *B. ermanii* were significantly higher than in the other species (Fig. 1A, d.f. 2, $F = 8.3$, $P = 0.01$), although there was no significant difference in total phenolics between the species in the late leaves in October. There was a significant difference in condensed tannin between species and at differing harvest times (Table 1). The concentration of condensed tannin tended to be higher in the early leaves than in late leaves (Fig. 1B). Across species, condensed tannin was significantly higher in *B. ermanii* than in the other species in the early leaves in May (Fig. 1B, d.f. 2, $F = 12.6$, $P < 0.01$). In late leaves in July, condensed tannin was significantly higher in *B. ermanii* than in *B. maximowicziana*. 

**FIG. 2.** (A) Leaf toughness (mean ± SE, $n = 5$) and (B) trichome density (mean ± SE, $n = 5$) of early leaves (black), late leaves in July (shaded) and late leaves in October (white) in *Betula platyphylla* (Bp), *B. ermanii* (Be) and *B. maximowicziana* (Bm). Different letters denote significant differences ($P < 0.05$) between leaves harvested at different times.

**FIG. 3.** Photographs of leaf upper surfaces (adaxial side) observed by microscope in *Betula platyphylla* (Bp), *B. ermanii* (Be) and *B. maximowicziana* (Bm). Early leaves were harvested in May and late leaves were harvested in July. Magnification ×40.
Leaf toughness differed significantly with time of harvest, but not between species (Table 1). Late leaves were significantly tougher than early leaves in *Betula maximowicziana* (Fig. 2A, d.f. 2, $F = 7.6, P = 0.01$); the difference was not significant in *B. platyphylla* and *B. ermanii*. In *B. platyphylla* and *B. ermanii*, the trichome density was significantly higher in early leaves than in late leaves (Figs 2B and 3; *B. platyphylla*, d.f. 2, $F = 50.8, P < 0.01$; *B. ermanii*, d.f. 2, $F = 27.5, P < 0.01$). In *B. maximowicziana* the trichome density tended to be higher in late leaves than in early leaves (Figs 2B and 3), although without statistical significance. Across species, the trichome density was significantly higher in *B. platyphylla* than in the other species in the early leaves (Figs 2B and 3), although without statistical significance. Across species, the trichome density was significantly higher in *B. platyphylla* than in the other species in the early leaves in May (Fig. 2B, d.f. 2, $F = 9.5, P < 0.01$). In contrast, the trichome density was significantly higher in *B. maximowicziana* than in the other species in late leaves (Fig. 2B, July, d.f. 2, $F = 39.6, P < 0.01$; October, d.f. 2, $F = 51.5, P < 0.01$).

### DISCUSSION

**Comparison of defence between early and late leaves**

Regardless of actual leaf age, clear differences exist in strength of defence between early and late leaves in these three birch species. Chemical defence (total phenolics and condensed tannin) in early leaves tended to be greater than for late leaves in *B. platyphylla* and *B. ermanii* (Fig. 1A and B). Lechowicz and St-Jacques (2000) also found higher total phenolics and condensed tannin in young early leaves (5–20 d from bud-breaking) than in young late leaves in *Betula papyrifera*. In contrast, total phenolics in *B. maximowicziana* tended to be greater in late leaves than in early leaves (Fig. 1A), although there was no significant difference in condensed tannin (Fig. 1B). In *Eucalyptus* leaves, Macauley and Fox (1980) also found no seasonal trend in total phenolics or condensed tannin. However, significant differences in physical defences between early and late leaves were found. Late leaves of *B. maximowicziana* were significantly tougher than early leaves (Fig. 2A). The trichome density of *B. platyphylla* and *B. ermanii* was significantly greater on early leaves than on late leaves (Fig. 2B), although the trichomes on *B. platyphylla* were much shorter than the others (Fig. 3). Stronger defence of early than late leaves is contrary to the growth/differentiation balance hypothesis (GDBH); the RGR of the early season (from May to July) was higher than in the late season (from July to October) in *B. platyphylla* and *B. ermanii* (Fig. 4A and B). There is still a possibility of a trade-off between growth and defence because the plants with high rates of growth and defence would have had even higher rates of growth in the absence of defence. Nonetheless, several previous studies have also suggested the rejection of...
the lifespan of early leaves of *B. maximowicziana* and *B. platyphylla* was higher in *Sakagami* (1985). Also, a shorter lifespan for early leaves of *B. platyphylla* was observed. In addition, bud-breaking of late leaves is shorter in *B. platyphylla* or *B. papyrifera*. Differences were observed in *P. lena* et al. (Weltzin et al., 1998). The RGR of defoliated *B. platyphylla* was affected only at the early stage of growth (Fig. 4A). No differences were observed in *F* sat values of early and late leaves between defoliated and control seedlings in August. These findings imply that damage to early leaves directly influenced the growth at the early stage, but did not affect the photosynthetic function of remaining leaves and of late leaves that expanded after the defoliation. *Koike* and *Sakagami* (1985) reported that *P* sat for early leaves was higher in *B. platyphylla* than in *B. maximowicziana*. Also, the lifespan of early leaves of *B. maximowicziana* was 15 d shorter than in *B. platyphylla*, whereas the longevity of late leaves of both species was almost the same (*Koike* and *Sakagami*, 1985). Also, a shorter lifespan for early leaves of *B. maximowicziana* than *B. platyphylla* or *B. ermanii* was observed. In addition, bud-breaking of late leaves began about 1 week earlier in *B. maximowicziana* than in *B. platyphylla* or *B. ermanii*; this implies that the interval during which early leaves were developing alone, without late leaves, is shorter in *B. maximowicziana* than in *B. platyphylla* or *B. ermanii*. These observations suggest that the importance of early leaves to subsequent growth is greater in *B. platyphylla* and *B. ermanii* than in *B. maximowicziana*, even though the three species belong to the same genus and have the same type of heterophyllous leaves. On the other hand, the reduction in the photosynthetic rate of late leaves started later in *B. maximowicziana* (in mid-October) than in *B. ermanii* (late September) (*Koike* et al., 1988). This suggests that late leaves are more important than early leaves in *B. maximowicziana*. Based on the observed differences in defence of early and late leaves and the contribution of early leaves, it is proposed that early leaves which play an essential role in subsequent growth have stronger defences, as seen in *B. platyphylla* and *B. ermanii*. In contrast, those early leaves that contribute less to subsequent growth do not have strong defences, and defence is then stronger in late leaves, as in *B. maximowicziana*. *Betula maximowicziana* produces fewer late leaves than *B. platyphylla* (although larger in area) (*Koike* and *Sakagami*, 1985), so that damage to late leaves is more likely to have a serious effect on growth. In fact, it has been reported that canopy trees of *B. maximowicziana* suffer significant mortality in outbreaks of Lepidoptera (*Operophtera brumata*) larvae (*Hara*, 2000).

**Interspecific differences in strategy of defence**

Although the chemical defences of *B. maximowicziana* are weaker than those of the other birches throughout the growing season, the enormous numbers of long thick trichomes on its leaf surfaces could compensate for its weak chemical defences. The gypsy moth (*Lymantria dispar*) is a notorious pest that feeds on birches and other deciduous trees, and outbreaks occur cyclically across large regions of Japan (*Higashiura*, 1991; *Liebhold* et al., 1998). It has been found that gypsy moths prefer leaves of *B. platyphylla* and *B. ermanii* to leaves of *B. maximowicziana* (*S. Matsuki* et al., unpub. res.). Trichomes of *B. maximowicziana* appear to be effective at deterring herbivores regardless of the reduced concentration of phenolics. The defensive roles of trichomes in birch species deserve fuller investigation. To confirm the effects of the various defensive strategies against herbivores, more detailed feeding tests by herbivores should be undertaken (*Kause* et al., 1999; *Underwood* et al., 2002).

**CONCLUSIONS**

It is suggested that *B. platyphylla* and *B. ermanii* invest highly in defence in their early leaves, which make a major contribution to subsequent growth. In contrast, *B. maximowicziana* invests its defence in late leaves, since its early leaves contribute less to subsequent growth and its late leaves appear to be functionally important. These results support the optimal defence theory, which predicts that organisms evolve and allocate defences in a way that maximizes individual inclusive fitness (*McKey*, 1974; *Ohnemeiss* and *Baldwin*, 2000; *Stamp* 2003). The defence strategy of *B. maximowicziana* is different from the other two species, and is similar to defensive trends in late-successional species (*Feeny*, 1970; *Riipi* et al., 2002). Although the three species studied all show strong preference for sunny locations and are categorized as ‘pioneer-species’ (*Tabata*, 1964, 1966; *Tatewaki*, 1964), *B. maximowicziana* tends to be a component of mixed forests (*Tabata*, 1964; *Koike*, 1995). The differing defence strategies may provide a plausible explanation for these differences across species in forest regeneration.

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