Ontogenetic changes in insect herbivory in birch (*Betula pubsecens*): the importance of plant apparency

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Running headline: Plant apparency and insect herbivory

Summary

1. Several theories aim at predicting changes in the interactions between plants and herbivores over the lifetime of a plant. Hypotheses based on ontogenetic changes in resource allocation to plant defence and in plant apparency lead to partly opposing predictions regarding the differences in levels of herbivory between juvenile and mature plant individuals.

2. We tested these predictions by measuring background foliar losses to insects in saplings and mature trees of downy birch (*Betula pubescens*) in ten sites along a latitudinal gradient from 60 to 69ºN in boreal forests of Northern Europe.

3. The percentage of consumed leaf area increased, and the variation in the levels of herbivory among plant individuals decreased, for tree sizes ranging from small saplings (2−12 cm tall) to large saplings (13−80 cm tall) and then to mature trees (3−18 m tall).

4. Small saplings had higher foliar quality for insects, as indicated by better performance of leafmining larvae of *Eriocrania semipurpurella* and by greater specific leaf area, compared with large saplings and mature trees. The average percentage of leaf area consumed from a damaged leaf, which reflects the inducibility of local defence responses to insect damage, did not vary among the birch size classes.

5. The foliar losses to insects decreased nearly fivefold with an increase in latitude from 60 to 69ºN, and the relative differences in these losses among the birch size classes were independent of latitude.

6. Our findings fit well with the predictions based on a plant apparency hypothesis, but do not support predictions based on ontogenetic changes in resource allocation to plant antiherbivore defences. We conclude that the generally overlooked effects of apparency on plant damage by insects can explain, at least in some cases, the frequently observed lack of correspondence between the levels of plant defences and herbivory.

Key-words: background insect herbivory, dispersion of damage, foliar quality, latitudinal gradient, plant-herbivore interactions, plant size, plant ontogenetic stage.

Introduction

Mortality is especially high during the early stages of plant development, and insect herbivory is an important source of this mortality (Moles & Westoby 2004; Mooney & Linhart 2006; Eichhorn et al. 2010). However, the studies of herbivory experienced by juvenile plants have tended to concentrate on vertebrate grazers (Hanley 1998; Barton & Koricheva 2010), so the levels of background foliage losses due to insects, i.e. losses that occur when insect populations are at their 'normal' low densities (Kozlov et al. 2015b), have been estimated less frequently than for mature plants, especially in boreal forests (Barton & Koricheva 2010). This is particularly the case for seedlings and saplings of woody plants (629 measurements compared to 3542 measurements for mature plants in the unpublished database created by M.V.K.).

Vertebrate and invertebrate herbivores demonstrate different preferences with respect to plant ontogenetic stages (Boege & Marquis 2005; Barton & Koricheva 2010); therefore, the shortage of information on insect herbivory on juvenile plants creates a noticeable gap that hampers the development of ecological and evolutionary theories regarding the interactions between plants and herbivores over the lifetime of a plant. A recent meta-analysis (Barton & Koricheva 2010) found a general lack of correspondence between ontogenetic patterns in plant defence and herbivory, suggesting that factors other than resource allocation to defence compounds may underlie the observed variations in plant losses to insects. One of these other factors can be plant size, which, according to the plant apparency hypothesis (Feeny 1976), may influence the levels of herbivory both at interspecific and intraspecific levels. On the intraspecific level, the apparency of a plant individual increases both with its absolute size and with its height relative to neighbouring plants (Castagneyrol et al. 2013; Strauss et al. 2015).

Two main lines of theoretical argumentation are used to explain ontogenetic changes in plant losses to insects. One of these lines is based on ontogenetic changes in resource allocation to plant defence (Boege & Marquis 2005), while another focuses on ontogenetic changes in plant apparency (Feeny 1976). These two hypotheses, although not mutually exclusive, yield several opposing predictions concerning the levels of foliar losses in juvenile trees (seedlings and saplings) relative to conspecific mature individuals. Thus, a comparison of the observed patterns in herbivory with those predicted by ontogenetic changes in plant defence and in plant apparency can reveal the relative contributions of these two mechanisms to the changes in background losses of foliage to insects during plant ontogeny.

During the sapling stage, plant growth is accompanied by an increase in acquisition of resources that can be allocated to the resource-demanding plant constitutive resistance (Haukioja & Koricheva 2000); consequently, foliar quality and foliar damage by herbivores are expected to decrease as a plant matures (Boege & Marquis 2005). In contrast, the apparency hypothesis predicts that plant damage would increase with plant age, as the increase in plant size and, consequently, in plant apparency to herbivores, would increase the probability of herbivore encounters with a plant (Feeny 1976; Fowler 1984; Schlinkert et al. 2015).

The percentage of leaves developed on long shoots (which produce new leaves until mid-summer) within a plant individual decreases from 40-80% in small birch saplings to 5-10% in mature birch trees (V.Z. and M.V.K., personal observation). This decrease in the proportion of actively growing vs. differentiated leaf tissues as a plant ages suggests that juvenile plants should show, on average, a greater induction of plant defences in response to leaf damage when compared to mature plants (Boege & Marquis 2005), because this induction in angiosperms occurs primarily in tissues that are actively growing (Karban & Baldwin 1997). Furthermore, inducibility can decrease as a plant develops due to regulatory mechanisms associated with plant maturation (Diezel, Allmann & Baldwin 2011). These predictions concerning ontogenetic changes in the strength of induced defence were confirmed by the current meta-analysis (Barton & Koricheva 2010), which demonstrated that induced defence – a cost-saving strategy – was generally higher in juveniles than in mature plants.

The ontogenetic changes in inducibility of plant leaves can be detected by exploring the distribution of leaf damage within a plant (Edwards & Wratten 1983; Bergelson, Fowler & Hartley 1986; Lehtilä 1996), because many herbivores quickly abandon induced leaves and move on to non-induced parts of a plant (Paschold, Halitschke & Baldwin 2007; Perkins et al. 2013). Thus, the higher inducibility of juvenile plants could be expected to force insect herbivores to move to another leaf before they have inflicted as much leaf damage as they would in mature plants. The result would be a lower extent of damage, as reflected by the consumed biomass per damaged leaf, in juvenile plants than in mature plants. By contrast, the plant apparency hypothesis gives no reasons to expect that the consumed biomass per damaged leaf will differ between juvenile and mature plants.

Woody plants often survive for many years, even decades, as small juveniles, before light gaps allow them to grow and mature (Barton & Koricheva 2010). The resource allocation theory predicts that saplings undergoing this period of growth stagnation are likely to direct a large proportion of acquired resources to the synthesis of defence compounds to reduce herbivore attack on their limited leaf area (Boege, Barton & Dirzo 2011). However, the resource allocation strategy changes as light becomes available: under sufficient illumination, saplings could be expected to direct all their resources to growth, at the expense of defence (Herms & Mattson 1992). Thus, higher insect herbivory could be expected on fast-growing compared to slow-growing birch saplings, irrespectively of their size and age. The plant apparency hypothesis, on the other hand, would predict that larger (more apparent) birch saplings would suffer greater foliar damage than smaller saplings, irrespective of their growth rates.

The plant apparency hypothesis also predicts a higher among-individual variation in insect herbivory in smaller (juvenile) than in larger (mature) plants: many juveniles would avoid damage because they are hidden, but those discovered by herbivores would receive relatively high amounts of damage (Fowler 1984; Strauss et al. 2015). In contrast, the resource allocation theory would not predict that variation in foliar damage among individuals of the same size class would differ between juvenile and mature plants.

The differences in the background levels of insect herbivory between juvenile and mature plants may also arise from factors other than plant size or age; for example, the environment in which the plants grow, affects the probability of insect encounters with host plants (Strauss et al. 2015). In particular, plants are generally more apparent to insects in less vegetated areas: removal of the vegetation surrounding individual plants increases the rates of attack by herbivores (Grau et al. 2012; Heard & Sax 2013; Strauss & Cacho 2013). Based on this assumption, we suggest that a poleward simplification of plant community structure due to latitudinal decreases in diversity, biomass and average height of plants within the boreal forest zone (Hillebrand 2004; Moles et al. 2009; Gillman et al. 2015) will result in a poleward decrease in the differences in foliar losses to insects between small (less apparent) and large (more apparent) conspecific plant individuals. In contrast, the resource allocation theory does not allow an expectation of latitudinal changes in the differences in insect damage between juvenile and mature individuals. However, the change in plant chemical defences with latitude (Moles et al. 2011; Salazar & Marquis 2012) suggests that a similar latitudinal change may occur in terms of the relative importance of defensive chemistry and apparency in shaping the distribution of foliar losses between plant ontogenetic stages.

In this study, we document within- and among-site variation in foliar quality and background insect herbivory between juvenile (saplings) and mature trees of downy birch, *Betula pubescens* Ehrh. and ask which of the two suggested mechanisms—ontogenetic changes in resource allocation to plant antiherbivore defences or ontogenetic changes in plant apparency—provides a better explanation of this variation. The apparency hypothesis predicts that: (1) foliar losses to insects will be lower in juvenile (small) birches than in mature (large) trees; (2) the foliar losses in a juvenile birch due to insects will be better explained by sapling height than by sapling growth rate; (3) within-site variation in herbivory among birch saplings will be larger than among mature trees; and (4) the percentage of leaf area eaten away by insects from a damaged leaf will not differ between juvenile and mature birches. We conducted our study in multiple sites along a latitudinal gradient in the boreal forests of Northern Europe to test the prediction that (5) the difference in foliar losses to insects between juvenile and mature birches will decrease with an increase in latitude due to simplification of plant community structure. Data supporting these predictions would confirm the importance of ontogenetic changes in plant apparency, while contrary evidence would suggest the importance of ontogenetic changes in plant antiherbivore defences, as predicted by resource allocation theory.

Materials and methods

Study species

Downy birch is widely distributed in Eurasia and is common in most types of boreal forests. At the northern edge of its range, it forms stable climax forests, but in more southern regions, birches are colonists in primary and human-induced secondary successions. Downy birch is damaged by several hundreds of insect species (Sinadsky 1973; Fowler 1985; Atkinson 1992). Background foliar losses of mature birches to insects increase in Fennoscandia from 1–2% at 70°N to 5–7% at 60°N; this pattern is best explained by mean July temperatures (Kozlov 2008) and results from both a smaller number of herbivore species and their lower abundances at higher latitudes (Kozlov et al. 2013, 2015d).

Study area and sampling sites

Sampling was conducted at ten sites selected along the latitudinal gradient between St. Petersburg and Murmansk, Russia, which was ca. 1000 km in length (Fig. 1; Appendix S1 in Supporting Information). Boreal forests of this region are dominated by Scots pine (*Pinus sylvestris* L.) or Norway spruce (*Picea abies* (L.) Karst.), but also have significant numbers of downy and white birches (*B. pubescens* and *B. pendula* Roth, respectively).

Stand basal area was measured with a relascope, as described by West (2003). The Shannon diversity index was calculated from the numbers of trees of different species counted in the course of estimation of the stand basal area (five replicates per site). The cover of field layer vegetation was visually estimated, and the maximum height of field layer vegetation was measured in plots 1×1 m in size (ten replicates per site).

Assessment of herbivory

At each site, on August 17–22, 2014 and August 27–31, 2015, we measured foliar losses due to insects in small saplings (2−12 cm tall), large saplings (13–80 cm tall) and mature birches (3–18 m tall). Ten saplings of each size class (whenever possible) were collected on a ‘first found, first sampled’ basis. From each of five mature trees, we collected a haphazardly selected branch with approximately 80 leaves that could be reached from the ground (i.e. within 2 m height).

The height of a sapling was measured as the vertical distance from the base of the highest bud to the ground. The age was estimated by counting annual increments of the main stem, i.e. the number of annual shoots formed by the stem of a sapling during the lifetime. In problematic situations, the age was controlled by counting annual rings on stem cross-section. The average growth rate (more specifically, vertical increment) was quantified by dividing a sapling’s height by its age. Juvenile birches (saplings) classified as ‘small’ were, on average, 6 years old, 8 cm tall and had 6 leaves, whereas ‘large’ saplings were, on average, 11 years old, 35 cm tall and had 17 leaves.

In the laboratory, the leaves on each sapling/branch were counted, and each leaf was carefully examined for the presence of insect damage. Following a widely used methodology (Alliende 1989; Kozlov et al. 2015b), each leaf was assigned to one of the damage classes according to the percentage of the area of the leaf lamina that was consumed by insects: 0 (intact leaves), 0.01–1, 1–5, 5–25, 25–50, 50–75 and 75–100%. The plant-specific percentage of leaf area lost to insects was calculated as follows: the numbers of leaves in each damage class were multiplied by the respective median values of the damaged leaf area (i.e. 0 for intact leaves, 0.5% for the damage class 0.01–1%, 3% for the damage class 1–5%, etc.); the obtained values were summed for all damage classes and divided by the total number of leaves (including undamaged ones) in a sample. To explore the mechanisms behind the changes in plant-specific losses of foliage, we also calculated the percentage of damaged leaves and the percentage of leaf area consumed from a damaged leaf.

Assessment of foliar quality

As a direct measure of foliar quality for insects, we used the ratio between larval mass and frass mass accumulated during larval development within a mine of *Eriocrania semipurpurella* Stph. (Lepidoptera: Eriocraniidae); this ratio correlated positively with foliar nitrogen content and negatively with carbon:nitrogen ratio in leaf tissues (Koricheva & Haukioja 1995) and with foliar concentrations of heavy metals (Kozlov et al. 2000). Thus, the higher values of this index are indicative of better quality food, because a larger part of the ingested food is converted into insect body mass. The leaves with last instar larvae were collected from small saplings, large saplings and mature trees from two study sites located at 60 and 68°N (54 and 58 leaves, respectively). At the time of collection, the mines were close to their final size, and they were stored individually in plastic vials. After each larva had abandoned its mine for pupation, we measured leaf length, opened the mine and collected all frass accumulated in the mine.

Additionally, we measured the foliar quality for insects using the specific leaf area (SLA), which is the ratio of leaf area to leaf mass. The SLA reflects leaf mechanical properties that directly affect leaf palatability for chewing insects, which inflicted the largest fraction of the foliar losses measured in our study. The SLA also correlates positively with mass-based foliar nitrogen, which is one of the key determinants of the tissue quality for herbivores (Reich et al. 1999; Wright et al. 2004). The SLA measurements were obtained by excising leaf disks 8 mm in diameter with a cork borer from the intact part of each leaf. Larvae, frass and leaf disks were dried for 24 h at +80°C and weighed to the nearest 0.01 mg.

Data analysis

We compared two indices of foliar losses, plant-wide and per damaged leaf (transformed as $\ln[1 + \sqrt{x}]$), the percentage of damaged leaves (transformed as $\ln[1 + x]$) and two indices of foliar quality, leaf mass/frass mass ratio (transformed as $\sqrt{x}$) and SLA, among birch size classes (mature trees, large and small saplings) using a mixed model analysis of variance (ANOVA). In this analysis, the size class was a fixed factor, whereas study site, study year and the interaction between size class and study site were considered as random factors; estimated marginal means were compared by the Tukey-Kramer method, adjusted for multiple comparisons (procedure GLIMMIX; SAS Institute 2009). The effects of growth rate on herbivory were explored using a similarly structured analysis of covariance. The significance of the random factors in these analyses was evaluated by calculating the likelihood ratio and testing it against χ^2 distribution (as described in Littell et al. 2006).

The variation in foliar losses to insects among birch individuals was quantified by the log-transformed coefficient of variation for each size class × site × year combination and then compared among size classes by one-way ANOVA (procedure GLM; SAS Institute 2009). The latitudinal patterns in vegetation characteristics and in herbivory were explored by calculating Pearson correlation coefficients between respective site-specific values and the latitudes of study sites.

Results

Differences among birch size classes

Over 99% of the foliar damage recorded in the course of the present study was imposed by leaf-chewing insects, primarily by free-living defoliators. The percentage of leaf area lost to insects on a per plant basis showed significant increases from small saplings to large saplings and then to mature trees (Fig. 2a; $F_{2,\,452} = 22.7$, $P < 0.0001$) in both study years (between-year differences: Wald $\chi^2 = 0.07$, d.f. = 1, $P = 0.80$). The percentage of damaged leaves (Fig. 2b; $F_{2,\,452} = 21.9$, $P < 0.0001$) and the coefficient of variation in the levels of herbivory among plant individuals (Fig. 2c; $F_{2,\,56} = 29.9$, $P < 0.0001$) followed the same pattern.

The quality of birch leaves for insect herbivores demonstrated an opposing pattern: both the larval mass/frass mass ratio of *E. semipurpurella* and the SLA were higher in the small saplings than in large saplings and mature trees (Fig. 3; $F_{2,\,102} = 4.34$, $P = 0.016$ and $F_{2,\,41} = 7.69$, $P = 0.015$, respectively). These differences among birch ontogenetic stages were consistent between the study sites located at 60 and 68°N (interaction term: $\chi^2 = 0.23$, d.f. = 1, $P = 0.31$ and $\chi^2 = 0.01$, d.f. = 1, $P = 0.99$, respectively). The larval mass/frass mass ratio was higher at 60°N than at 68°N ($\chi^2 = 5.60$, d.f. = 1, $P = 0.02$), whereas the SLA did not differ between these localities ($\chi^2 = 2.40$, d.f. = 1, $P = 0.12$).

The percentage of leaf area consumed from a damaged leaf did not vary among the birch size classes (Fig. 2d; $F_{2,\,372} = 0.66$, $P = 0.52$). In both small and large saplings, the losses of foliage to insects did not depend on growth rate ($F_{1,\,152} = 0.06$, $P = 0.80$ and $F_{1,\,167} = 0.77$, $P = 0.38$, respectively), which varied from 0.3 to 11.6 cm year^{-1}.

Differences among study sites

The stand basal area decreased with increasing latitude of the study site ($r = -0.65$, $n = 10$ sites, $P = 0.04$), whereas the diversity of trees and the cover of the field layer vegetation were independent of latitude ($r = 0.51$, $n = 10$, $P = 0.13$ and $r = -0.22$, $n = 10$, $P = 0.53$, respectively). The maximum height of the field layer vegetation decreased from 36–44 cm in the southern part of the latitudinal gradient to 23–35 cm in the northern part (r = -0.68, n = 10, P = 0.03), thus confirming that apparency of birch saplings consistently increased poleward.

The among-site variation in the percentage of leaf area lost to insects was highly significant (Wald χ^2 = 28.6, d.f. = 1, P < 0.0001), and foliar losses of all size classes decreased with increasing latitude (Fig. 4). The relative differences among size classes with respect to foliar losses were similar for all study sites (interaction term: χ^2 = 0.01, d.f. = 1, P = 0.99).

Discussion

Ecological importance of minor foliar damage

The detected differences in insect herbivory between birch ontogenetic stages are relatively minor: on average, foliage loss to insects in the mature trees (2.83%) is 2% greater than in the small saplings (0.77%). On the other hand, this foliage loss to insects in mature trees is more than three times higher than in small saplings.

Steadily accumulating evidence (reviewed by Kozlov & Zvereva 2017) clearly shows that even minor changes in plant losses to insects may cause significant effects on plant growth and reproduction. In particular, a 2% increase in foliar losses of birch saplings during a seven-year period resulted in a 30% reduction in their vertical growth (Zvereva, Zverev & Kozlov 2012). Thus, the detected differences in foliar damage between birch ontogenetic stages are likely to have important consequences for the evolution of defensive strategies.

Ontogenetic changes in plant defences

Investigation of plant antiherbivore defences has been extremely popular during the past decades, and multiple theories have been suggested to explain variations in the expression of plant defensive traits and in herbivory at different scales (Price 1991; Karban & Baldwin 1997; Haukioja 2006; Kant et al. 2015). In particular, the optimal defence theory (Rhoades 1979; Bryant et al. 1992), in contrast to the resource allocation theory, predicts that plant parts with high fitness values, for example, leaves of juveniles, will be highly defended, and the level of defence will decrease as plants mature and become less susceptible to the fitness reductions of these attacks (Barton & Koricheva 2010). In agreement with this prediction, the background losses of birch foliage to insects increased from small saplings to mature trees. However, this pattern is unlikely to be attributable to strong constitutive defences specific to juvenile birches, because the SLA was lower and the performance of leafmining *Eriocrania* larvae was better on small saplings than on mature trees. The latter result may be explained by the greater nitrogen content in leaves of birch saplings than in leaves of mature trees (Weih & Karlsson 1999; Manninen et al. 2015).

Meta-analysis (Barton & Koricheva 2010) demonstrated that induced defence was generally stronger in juveniles than in mature plants. A high inducibility is frequently associated with less damage per affected leaf (Edwards & Wratten 1983; Bergelson et al. 1986; Lehtilä 1996); therefore, we expected that a smaller percentage of leaf area would be eaten away by insects for a damaged leaf from a juvenile birch than from a mature birch. However, this expectation was not met. We therefore suggest that factors other than ontogenetic changes in the expression of inducible defences are likely to underlie the observed differences in plant-specific damage between juveniles and mature birches.

We also found no correlation between vertical increment and herbivory in juvenile birches. If we assume that herbivory is inversely proportional to plant investments into defences, this result contradicts the basic idea of the growth-differentiation balance hypothesis

(Herms & Mattson 1992). It also conflicts with the empirical evidence on more intensive herbivory on fast-growing (i.e. more vigorous) plants that served as the basis of the plant vigour hypothesis (Price 1991). On the other hand, a recent study demonstrated that the expression of defence traits is generally costly, but the magnitude and importance of these costs are context-dependent (Cipollini, Walters & Voelckel 2014). Consistently, earlier studies of silver birch revealed controversial relationships between sapling growth rate and herbivory (Rousi et al. 1993; Mutikainen et al. 2000; Prittinen et al. 2003), indicating that environmental factors influence the variation in herbivory among juvenile birches. In particular, Prittinen et al. (2003) found a positive correlation between leaf damage and the height of birch saplings of the same age and explained this correlation by a trade-off between resource allocation to growth and defence. We found a similar correlation, but the explanation by Prittinen et al. (2003) cannot be applied to our data because the herbivory on naturally growing birch saplings did not correlate with their growth rate. Therefore, we attribute the observed variations in background herbivory to the effects of sapling height *per se*.

Finally, we found the plant age hypothesis difficult to use to explain our results, at least in the form in which this hypothesis was first applied to insects (Spiegel & Price 1996). These authors and their immediate predecessors (Kearsley & Whitham 1989) only claimed that individual plants undergo predictable changes in their susceptibility to herbivores as they mature, and that different herbivore species preferentially damage plants of different ages. Both these publications listed insects that are more common on young hosts, as well as those that are more common on older, mature hosts. In our opinion, this hypothesis does not allow any general prediction of the direction of ontogenetic changes in plant losses to insects, although these predictions have been occasionally made (e.g. by Barton & Koricheva 2010).

Top-down factors, sometimes considered as indirect defences, may also contribute to differences in herbivory between juvenile and mature trees. For example, herbivores feeding on small saplings are less exposed to birds but at the same time may suffer higher mortality from epigeic predators, particularly ants. Production of volatiles that act as cues for natural enemies may also alter as plant matures (Cole 1980). However, the shortage of information on the effects of plant age on herbivore mortality from natural enemies (reviewed by Boege & Marquis 2005) calls for further experimental studies addressing the role of top-down factors in shaping ontogenetic changes in plant losses to insects.

Ontogenetic changes in plant apparency

We found increases in the background losses of downy birch foliage to insects in natural environments as we moved from small saplings to large saplings and then to mature trees, while the among-plant variation in herbivory decreased. Both these patterns fit the predictions of the plant apparency hypothesis (Feeny 1976; Fowler 1984; Strauss et al. 2015). First, the larger (more apparent) individuals of downy birch suffered higher insect herbivory than was seen in the smaller (less apparent) individuals, presumably due to the higher probabilities of herbivore encounters with larger plants. Second, many of the small individuals, which exhibit higher quality of foliage for leaf-eating insects, simply escaped from herbivory. However, those that were found by herbivores received relatively high amounts of damage, which led to a more uneven distribution of damage among saplings, when compared to mature trees.

The resource concentration hypothesis (Root 1973) predicts that insect herbivores should be more abundant in large patches of host plants, because the insects are more likely to find these patches and to stay longer in them. This theory on patch size can be extended to the ontogenetic changes in size of woody plants, which can vary by several orders of magnitudes between juvenile and mature individuals. For example, insect herbivores were 19 times more abundant on the foliage of the mature trees of *Castilla elastica* in Panama than on the foliage of conspecific saplings (Barrios 2003). Similar, albeit smaller, differences in the communities of birch-feeding insects were reported in Northern England in early summer, when the abundance of insects feeding on mature birches was ca. 50% higher than on seedlings (Fowler 1985). A strong correlation between plant height and number of leaves did not allow us to decouple the contributions of these two variables to the observed variation in herbivory. Thus, we cannot definitively state which is more important—plant size *per se* or the amount of resources provided by the plant.

Apparency hypothesis also explains well the differences in herbivory between the two size classes of juvenile birches. These differences can be due to their height relative to the height of the field layer vegetation: large saplings are taller than the majority of the field layer plants and thus more exposed to herbivores, while small saplings are concealed beneath the field layer plants. The importance of field layer vegetation in the protection of tree seedlings was demonstrated in earlier studies: lower insect herbivory was observed for birch seedlings growing among bilberry (*Vaccinium myrtillus*) shrubs at a treeline when compared to seedlings where the surrounding bilberry shrubs had been removed (Grau et al. 2012). Similarly, higher insect herbivory was seen in oak (*Quercus rubra*) seedlings growing in fern-free areas than in areas sheltered by ferns (George & Bazzaz 1999).

Finally, we found that the damage inflicted by insects on a damaged leaf did not differ among birches of different age classes. This finding does not provide direct support for the apparency hypothesis, but it does not contradict the predictions of that hypothesis.

Latitudinal changes in herbivory

The existence of a latitudinal pattern in herbivory and the mechanisms behind this pattern have attracted an increasing attention from scientists during the past years. Despite some discrepancies, the current studies support the idea that herbivory within the boreal forest zone generally decreases with increasing latitude and/or decreasing mean annual temperature (Kozlov et al. 2015b; Lim, Fine & Mittelbach 2015; Zhang, Zhang & Ma 2016). Our findings are also in line with this general pattern.However, the recent studies addressing latitudinal changes in insect herbivory were either based exclusively on data collected from mature plant individuals (Kozlov 2008; Kozlov et al. 2013, 2015a-d) or on combined data collected from all ontogenetic stages of the plants (Moles et al. 2011; Turcotte et al. 2014; Lim et al. 2015). The use of data obtained from a combination of juvenile and mature plants is a risky approach for two reasons: the levels of herbivory might differ between plant ontogenetic stages (Swihart & Bryant 2001; Boege & Marquis 2005) and the preference of herbivores for a certain plant stage might also change with the biome (Barton & Koricheva 2010), with plant community characteristics (Strauss et al. 2015) or with resource availability (Wise & Abrahamson 2005).

The mechanisms behind the latitudinal patterns in insect herbivory remain poorly understood. The latitudinal gradient in insect herbivory may arise due to changes in a number of factors, including direct effects of climate on insect performance and diversity (Bale et al. 2002; Kozlov et al. 2013) and on the number of generations in some species (Yamamura & Kiritani 1998), the mechanical properties of the leaves (Onoda et al. 2011), plant defensive chemistry (Moles et al. 2011) and predator pressure (Björkman, Berggren & Bylund 2011). The multiplicity of factors affecting both latitudinal and ontogenetic changes in herbivory made predictions of their combined effects nearly impossible, and the existing empirical data did not allow exploration of these effects. This gives special importance to our first test for latitudinal gradients in ontogenetic trajectories in plant-herbivore interactions. Our finding that the relative differences in foliar losses among the birch size classes were independent of latitude suggests that latitudinal changes in the mechanisms controlling plant losses to insects do not change the distribution of herbivory among birch ontogenetic stages. More data of this kind, in particular those from longer latitudinal gradients, are needed to confirm whether herbivory on seedlings/saplings generally exhibits the same latitudinal pattern as is seen on mature plants.

Our results did not support the prediction that the difference in foliar losses to insects between small (less apparent) and large (more apparent) birch individuals will decrease with latitude due to changes in plant community structure. Several reasons could explain this result. First, we did not detect the expected poleward simplification of the environment (*sensu* Strauss et al. 2015): neither the diversity of top-canopy trees nor the cover of field layer vegetation decreased in our latitudinal gradient. Second, the poleward decrease in stand density caused similar changes in light conditions for the sampled (lower) branches of top-canopy trees and juvenile birches. Finally, the expected changes in distribution of herbivory among birch size classes may have been counterbalanced by changes in community structure of herbivores or predators.

Conclusions

The observed relationships between the levels of background insect herbivory and the size of downy birch individuals are consistent with ontogenetic changes in plant apparency, but do not fit predictions based on ontogenetic changes in resource allocation to plant antiherbivore defences, either constitutive or induced. Although escape from herbivory, in particular due to small size, is a well-known mechanism of resistance (Boege & Marquis 2005) and provides a first line of plant defence, the importance of this mechanism in shaping insect-plant interactions has only been appreciated in a few recent publications (Castagneyrol et al. 2013; Strauss et al. 2015). Generally overlooked effects of apparency on plant damage by insects can explain, at least in some cases, the lack of correspondence between the levels of plant defences and herbivory, that had been demonstrated by Barton and Koricheva (2010). We conclude that ontogenetic changes in the size of woody plants may considerably affect the losses of their foliage to insects (presumably through changes in the probabilities of herbivore encounters with plant individuals) and that in some situations, the observed patterns in insect herbivory are in a better agreement with predictions based on ontogenetic changes in apparency than with predictions based on ontogenetic changes in plant defences.

Authors' contribution

MVK conceived the ideas; VZ and ELZ designed methodology; VZ and MVK collected and analysed the data; ELZ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.p5c14 (Zverev, Zvereva & Kozlov 2017).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1 Characteristics of study sites.

Figures and figure legends

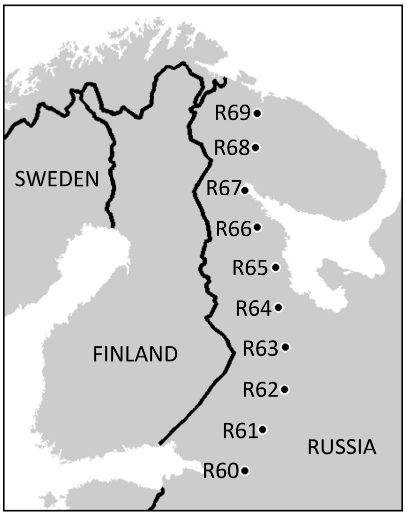

Fig. 1. Study area and study sites. The name of each site contains the approximate latitude (for the coordinates of study sites and vegetation characteristics see Appendix A1 in Supporting information).

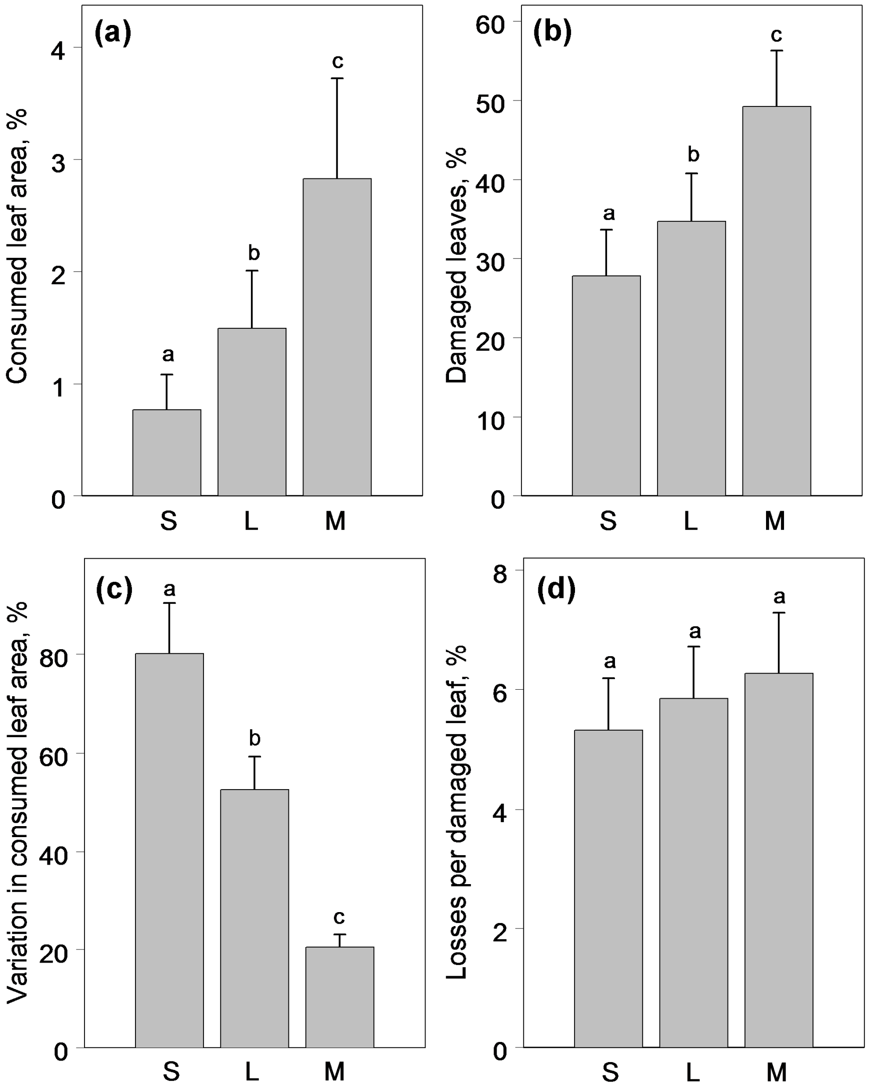

Fig. 2. Losses of downy birch (*Betula pubescens*) foliage to insects (back-transformed values; estimated marginal means + asymptotic S.E., each based on 10 site-specific values): (a) percent of consumed leaf area; (b) percent of damaged leaves; (c) coefficient of variation in percent of consumed leaf area among individuals within a site; (d) percent of leaf area consumed from a damaged leaf. Ontogenetic stages: S, small saplings (2−12 cm tall); L, large saplings (13−80 cm tall); M, mature trees (3−18 m tall). The values marked with different letters significantly differ from each other (Tukey test, experiment-wide $P = 0.05$).

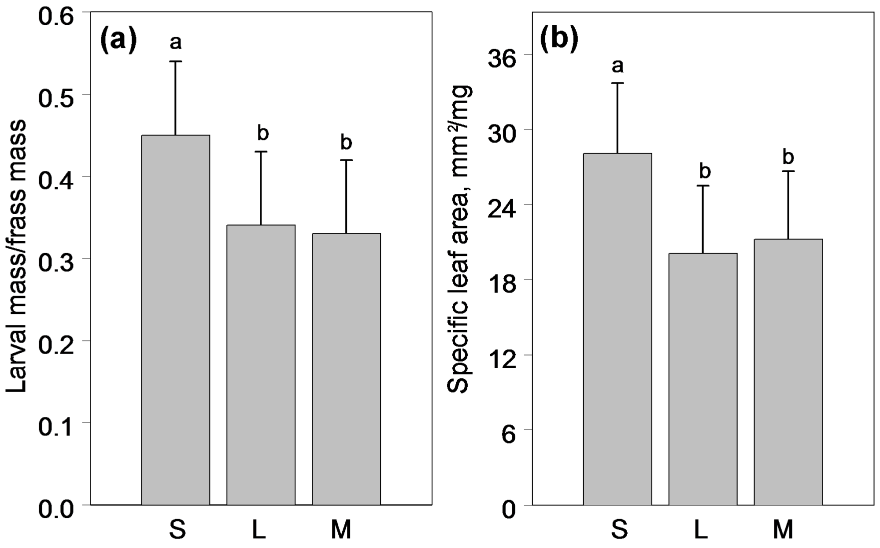

Fig. 3. Quality of downy birch (*Betula pubescens*) foliage for insects (estimated marginal means + asymptotic S.E.): (a) larval mass/frass mass ratio of a leafminer *Eriocrania semipurpurella* (n = 34–42); (b) specific leaf area (n = 11–20). Ontogenetic stages: S, small saplings (2–12 cm tall); L, large saplings (13–80 cm tall); M, mature trees (3–18 m tall). The values marked with different letters significantly differ from each other (Tukey test, experiment-wide $P = 0.05$).

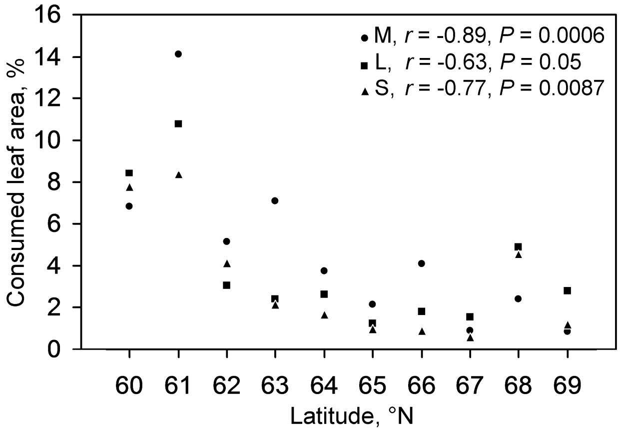

Fig. 4. Geographical variation (Pearson correlation coefficients with latitude) in average losses of downy birch (*Betula pubescens*) foliage to insects. Size classes: S, small saplings (2−12 cm tall); L, large saplings (13−80 cm tall); M, mature trees (3−18 m tall).