Original article

Season and light affect constitutive defenses of understory shrub species against folivorous insects

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ABSTRACT

Understory shrubs contribute to overall species diversity, providing habitat and forage for animals, influence soil chemistry and forest microclimate. However, very little is known about the chemical defense of various shrub species against folivorous insects. Using six shrub species, we tested how seasonal changes and light conditions affect their constitutive defense to insect damage. We monitored leaf perforation, concentrations of total phenols, condensed tannins, nitrogen (N), and total nonstructural carbohydrates (TNC). Leaf damage caused by insects was low in *Sambucus nigra*, *Cornus sanguinea*, and *Frangula alnus*, intermediate in *Corylus avellana* and *Prunus serotina*, and high in *Prunus padus*. Leaves of all the species, when growing in high light conditions, had high concentrations of defense metabolites. Except for *C. avellana*, leaves of the other shrub species growing in full sun were less injured than those in shade. This may be due to higher concentrations of defense metabolites and lower concentrations of nitrogen. Similar patterns of the effects of light on metabolites studied and N were observed for leaves with varying location within the crown of individual shrubs (from the top of the south direction to the bottom of the north), as for leaves from shrubs growing in full sun and shrubs in the shade of canopy trees. A probable cause of the greater damage of more sunlit leaves of *C. avellana* was the fact that they were herbivorized mostly by *Altica brevicollis*, a specialist insect that prefers plant tissues with a high TNC level and is not very sensitive to a high level of phenolic compounds.

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1. Introduction

Understory shrubs play an important role in forests, particularly in forest monocultures (i.e. planted stands of one tree species). Shrubs are important for biodiversity and ecosystem function (Szymański, 1986). First of all, the understory protects the soil against erosion. Secondly, it lowers evaporation from the soil surface, and improves the microclimate of the forest interior, by limiting the penetration of wind. Thirdly, it ensures the formation of structurally and chemically diverse forest litter, contributing to soil biodiversity. Finally, it mobilizes mineral nutrients (Tuszyński and Gorzelak, 1998), and thus prevents soil degradation, improving soil structure and chemical composition (Siles et al., 2010; Sardans and Peñuelas, 2013). That is why shrubs are more often taken into account in afforestation schemes of recent years.

Shrubs of some species are severely defoliated by folivorous insects. The species of understory shrubs vary considerably in the degree of leaf perforation (Karolewski et al., 2007; Żmuda et al., 2008). Frequently, shrubs of some species (e.g. *Prunus padus*) may be nearly completely defoliated by insects (Leather and Lehti, 1982; Leather, 1986). Defoliation is strongly influenced by light conditions, which depend on forest type and location in the forest interior or at forest edges, e.g. along roads (Roberts and Paul, 2006). Hence, it is important to understand the mechanisms by which light affects their resistance to insect herbivores (Karolewski et al., 2010). Moreover, herbivory influences litter chemistry, and thus the cycling of nutrients (Chapman et al., 2003; Ayres et al., 2004; Kurokawa and Nakashizuka, 2008; Whitham et al., 2008).

Light conditions significantly modify the structure, water content and concentration of metabolites in leaves. Most studies show that leaves of woody plants growing in full sun are more resistant to insect damage than those growing in shade (Roberts and Paul, 2006, and literature cited therein). The lower leaf damage in plants growing in high light conditions than in shade, as well as the

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less frequent perforation of sunlit leaves than shaded leaves of the same plant, may be due to chemical defenses. This may be resistance, which consists of the accumulation of large amounts of defense metabolites in leaves, e.g. simple phenolic acids, tannins, flavonoids, or phenolic glycosides (Hwang and Lindroth, 1997; Oleksyn et al., 1998; Hemming and Lindroth, 1999; Henriksson et al., 2003). However, leaf damage by insects may be less dependent on the concentration of defense compounds and more dependent on nutritional value (Fortin and Mauffette, 2002; Hódar et al., 2002) or other potential defensive plant traits, for example physical resistance (Carmona et al., 2011). Studies vary regarding the effects of light availability on the nutritional value of leaves and on their consumption. It has been suggested that sunlit leaves have a low nutritional value, i.e. low nitrogen and water content, but high concentrations of defense compounds per unit of leaf mass (Henriksson et al., 2003; Yamasaki and Kikuzawa, 2003; Koricheva et al., 1998). They are therefore less attractive to insects and less damaged, while the survival and performance of the insects that feed on sunlit leaves are poorer: lower final mass, longer duration of larval development and pupation, etc. (Lindroth et al., 1993; Jansen and Stamp, 1997). Only rarely have cases of greater leaf damage in plants growing in high light conditions been reported (Roberts and Paul, 2006; Żmuda et al., 2008). Alternatively, it is supposed that leaves of plants growing in full sun have a higher nutritional value (higher content of protein and carbohydrates per unit leaf mass), so folivores do not need to consume large amounts of such nutritious food and they may even reach higher mass and larger dimensions, with faster development (Niesenbaum and Kluger, 2006; Oishi et al., 2006; Osier and Jennings, 2007; Mooney et al., 2009). Despite the relatively large number of studies allowing a comparison of the effects of light conditions on leaf chemistry, the causes of greater leaf perforation in well-lit than in shaded plants have not been sufficiently explained.

We hypothesized that the level of defensive compounds in the leaves depends on the light conditions, regardless of plant species. At the same time, we test the hypothesis that, for some shrub species (e.g. Corylus avellana) growing under full sunlight, high levels of defense compounds in the leaves do not guarantee an effective defense against insect grazing. Instead we hypothesized that the rate of herbivory is determined most by the direct influence of external conditions on insects (sunlight and temperature) than by the content of defensive substances in the leaves.

The aims of this study were: (1) to assess the effects of leaf chemistry on leaf damage (perforation) by insects among six species of understory shrubs during the whole growing season, and (2) to assess the effects of light growing conditions on constitutive resistance against folivores. Furthermore, seasonal changes in defense compounds and nutrients may explain and/or predict the responses of shrub species to other biotic factors, for example pathogenic fungi. Our studies also allow a comparison of the impact of light conditions between the native European P. padus and the highly invasive North American Prunus serotina, with respect to leaf chemistry and damage caused by herbivorous insects. This aspect is further discussed.

2. Materials and methods

2.1. Study site and plant material

Our study was conducted from April to October 2007 in the experimental forest of the Poznań University of Life Sciences. The forest is located near Bolechowo, Łopuchówko Forest Division, in western central Poland (52°33′N, 17°01′E; altitude 70 m). Observations were carried out on six forest understory shrub species: European elder (Sambucus nigra L.), black cherry (P. serotina Ehrb.),

European bird cherry (P. padus L.), common dogwood (Cornus sanguinea L.), glossy buckthorn (Frangula alnus Mill.) and common hazel (C. avellana L.). Six individuals were selected: three shrubs growing in high light conditions (along wide forest roads or in forest gaps) and three growing in shaded conditions (under a dense canopy) for each species. We used 3—5 m high shrubs growing under a canopy of Pinus sylvestris L. with admixture of Quercus robur L., Fagus sylvatica L., Carpinus betulus L. and Ulmus laevis Pall. In order to avoid the influence of different habitats — ecotone effect (Leather and MacKenzie, 1994) we did not sample shrubs from forest edges.

We examined shrub species that differ greatly in terms of light requirements (Ellenberg et al., 1992). These authors, using a ten-point scale (10 — most light-demanding species) assigned a value of 7 for S. nigra and C. sanguinea, 6 for F. alnus, P. serotina and C. avellana, and 5 for P. padus.

2.2. Experimental design

The first experiment examined the influence of sampling date in shrubs growing in full sun and in shade (Figs. 1—7). The second experiment, performed on one sampling date, examined the effect of light differences in exposure of leaves of the same individual, depending on their location in the shrub crown (Figs. 8 and 9). For this purpose, we collected leaves from 3 shrubs of each species in a gradient from the south-facing top of the crown (most sunlit) to the north-facing crown base (most shaded). With each shrub we collected some shoots, and all the leaves of the shoots were a single sample. These samples were collected on 1st August, when leaves of all the species were fully developed. Light conditions of leaf growth were assessed on the basis of species-specific values of SLA (projection leaf area per unit dry leaf mass, $cm^2 \ g^{-1}$). The average value of the SLA of all leaves on a single shoot was used as an indicator of light condition of individual plants. The number of samplings depended on crown size — if it was larger, then the leaves were collected from a larger number of locations within the crown. Number of samples for each species corresponds to the number of points in Figs. 8 and 9. SLA value for a single sample was calculated based on weighted mean taking into account the mass participation of individual leaves.

2.3. Assessment of herbivory

Leaf damage caused by folivorous insects was assessed as the percentage of leaf perforation (leaf area loss), estimated on the day of their collection. Leaves with sporadic damages (e.g. mining, skelotonizing, partially eaten by mammals etc.) were not taken for the analyses. Because of the relatively large size of shrubs and the variation in insect damage of leaves (perforation) among branches of the same shrub, the percentage of leaf perforation was estimated visually (to the nearest 5%). This was always done by the same person, after earlier multiple comparisons of the percentage of leaf perforation estimated visually with results of scanning of the same leaves by means of WinFOLIA software (version 2003b, Regent Instruments Inc., Quebec, Canada; http://www.regentinstruments.com/). Also, a large number of defects at the edges of leaves did not allow the use of instrumental methods. However, the program WinFOLIA was used to fully measure the surface area of leaves collected for chemical analyses and SLA assessment (see below).

2.4. Sample collection for chemical analyses and SLA assessment

For constitutive defenses, undamaged leaves were sampled from 3 shoots per shrub. At the beginning and end of the
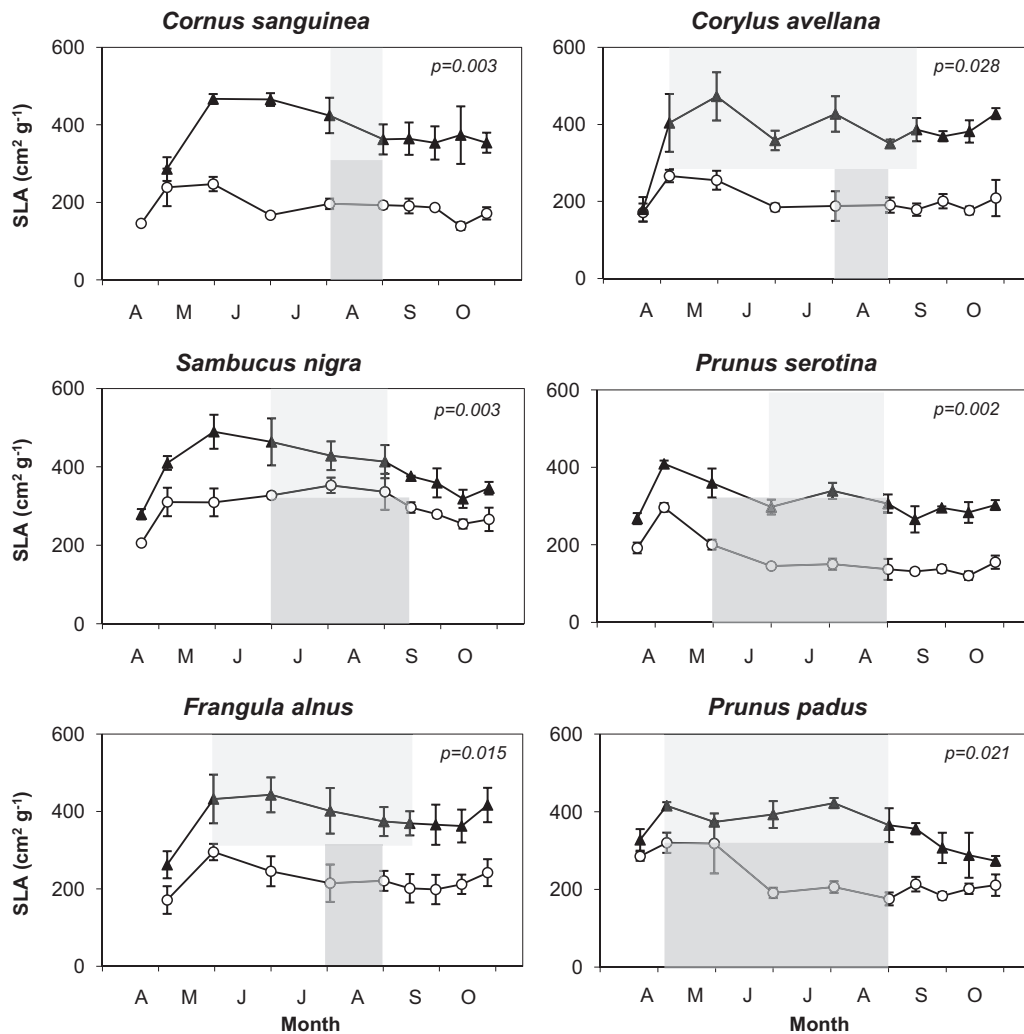
Fig. 1. Specific leaf area (SLA, mean ± SE) of six shrub species in relation to long-term light conditions (○ − full sun, ▲ − shade), for uninjured leaves. Gray shading indicates the periods between the earliest and maximum leaf perforation (see Fig. 2) for shrubs growing in full sun (light gray) and in shade (dark gray). Repeated measures ANOVA analysis was used to assess the influence of light conditions on the level of significance (p) separately for each species.

growing season of each species, leaves were collected every two weeks, while at the middle of the season they were collected every four weeks. The leaves were dried at 40 °C, and tannins were determined. Then leaves were dried at 65 °C. Simultaneously, leaves were also collected for the assessment of specific leaf area (SLA). Each leaf was scanned, dried (65 °C) and weighed. Each sample for SLA analyses was composed of 10−20 leaves per shoot (depending on species), 3 shoots per shrub.

2.5. Chemical analyses

Defense compounds (tannins and total soluble phenolics) as well as nutrients (N and non-structural carbohydrates) were measured in dried (40 °C only for tannins, and for others in 65 °C) tissue powdered in a Mikro-Feinmühle-Culatti mill (IKA Labortechnik Staufen, Germany).

Total soluble phenolic (TPh) content was measured according to Johnson and Schaal (1957), as modified by Singleton and Rossi (1965). Thus it was determined using Folin Ciocalteu's Phenol Reagent (Sigma F − 9252) at $\lambda = 660$ nm, and results were expressed as μM chlorogenic acid g^{-1} dry matter (d.m.).

Condensed tannins, after extraction with absolute methanol, were determined by a colorimetric method, using a color reaction with vanillin in an acid medium (Price et al., 1978). Readings of absorption were taken at $\lambda = 500$ nm, while results were expressed as μM catechin g^{-1} d.m.

Total non-structural carbohydrates (TNC, i.e. soluble carbohydrates and starch) were determined as described by Haissig and Dickson (1979) and Hansen and Møller (1975). Soluble carbohydrates were assayed in methanol-chloroform-water extracts. The precipitate remaining after extraction was used to determine starch content. Starch analysis consisted of its transformation into glucose, with amyloglucosidase, and oxidation using the peroxidase−glucose oxidase complex. Concentrations of soluble carbohydrates were measured at $\lambda = 625$ nm, following a color reaction with anthrone, while concentrations of starch were measured at $\lambda = 450$ nm, following the reaction with dianisidine. The total amount of soluble carbohydrates and starch (with glucose as a standard) was expressed as percentage of dry mass.

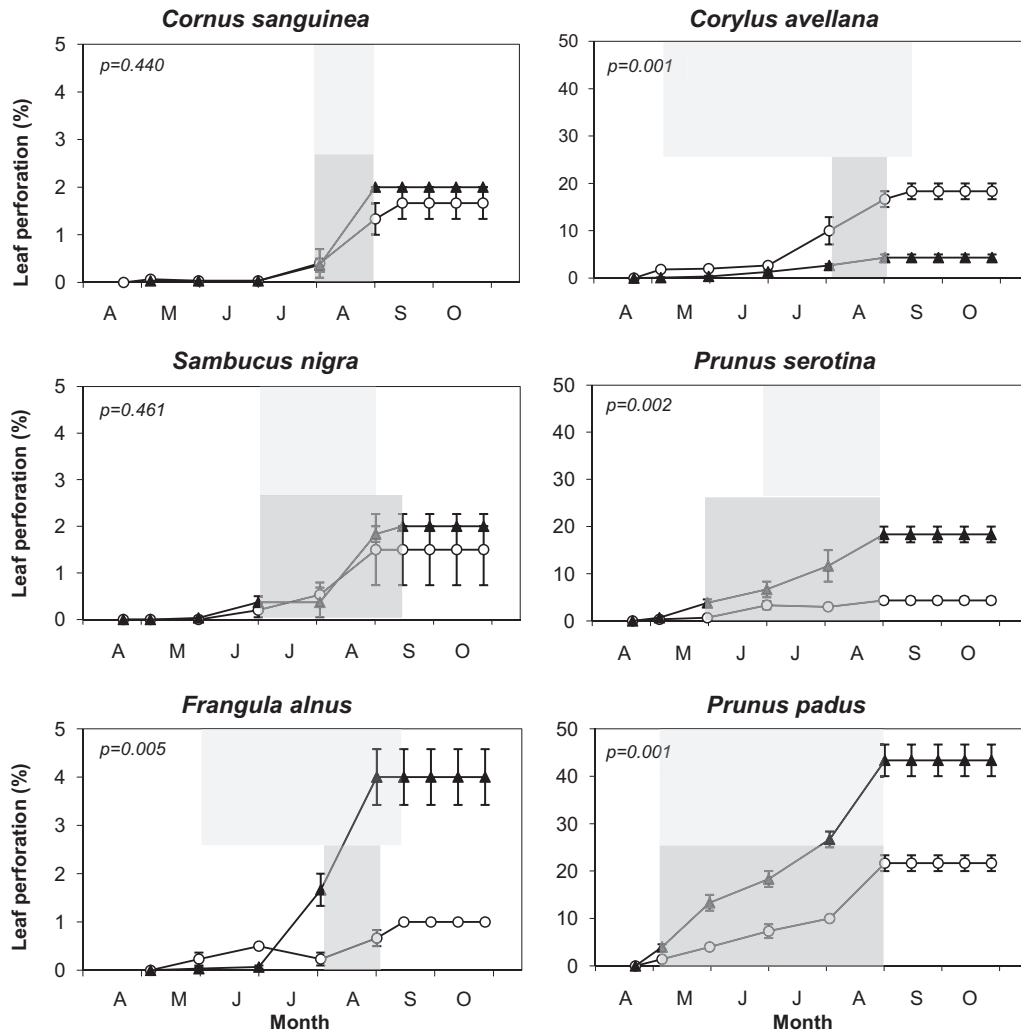Nitrogen content (% d.m.) was determined using an Elemental Combustion System CHNS-O 4010 analyzer (Costech Instruments, Italy/USA; http://www.costechanalytical.com).

Fig. 2. Leaf perforation (mean ± SE) of six shrub species in relation to long-term light conditions (○ − full sun, ▲ − shade). Gray shading and level of significance (p) as in Fig. 1.

2.6. Statistical analyses

Statistical analyses were conducted using JMP 8.0 software (SAS Institute Inc., Cary, NC, USA; http://www.jmp.com). Values presented on graphs are means of 3 replicates (shrubs) for each variant. Vertical segments on graphs mark the values of standard error (SE) of means. Repeated measures ANOVA was used to assess the influence of light conditions and sampling date on the traits investigated for each species separately (see Figs. 1–7). Repeated measures analysis was also used to estimate the influence of species, light, and sampling date on the traits studied (see Table 1). For traits expressed as percentages, the Bliss formula for leaf perforation (Snedecor and Cochran, 1976), and logarithms of the values for water, nitrogen and non-structural carbohydrates, were used. For interactions of time with other effects the Pillai's Trace test was used.

The Tukey test ($p = 0.05$) was applied when grouping the species in terms of leaf injury caused by insect feeding and the level of all the other parameters. We used the results of repeated measures ANOVA analysis for interpreting results of the experiment on the relationships between percentage of leaf perforation and concentrations of metabolites and N during the growing season.

To assess the effect of light conditions of leaves on metabolites and N, resulting from their location in the shrub crown (reflected in SLA), we applied regression equations (see Figs. 8 and 9). The shape of the regression equations (linear or quadratic) describing the relationships between SLA and concentrations of metabolites and N was selected so as to maximize the value of the correlation coefficient (r^2) and its significance (p).

3. Results

Including all sampling dates during the growing season and both light conditions of plant growth, shrub species differed in the percentage loss of leaf area (Table 1 and Fig. 2). ANOVA and the Tukey test allowed a division of the species studied into three groups with respect to leaf perforation. The mean leaf area loss at both light conditions was the lowest in *S. nigra* (1.0%), *C. sanguinea* (1.1%), and *F. alnus* (1.5%), (group a, see Table 1). In contrast, *C. avellana* (7.4%) and *P. serotina* (8.0%) exhibited intermediate vulnerability to leaf grazing insects (group b), while *P. padus* (22.8%) was the most affected among the species studied (group c).

Light conditions caused large differences in SLA. Well-lit shrubs had low SLA and shaded shrubs had high SLA (Fig. 1). We found wide ranges of SLA of leaves located in various parts of the crown (Figs. 8 and 9). The leaf perforation depended strongly on light conditions (Table 1). In four species (*C. sanguinea*, *F. alnus*,
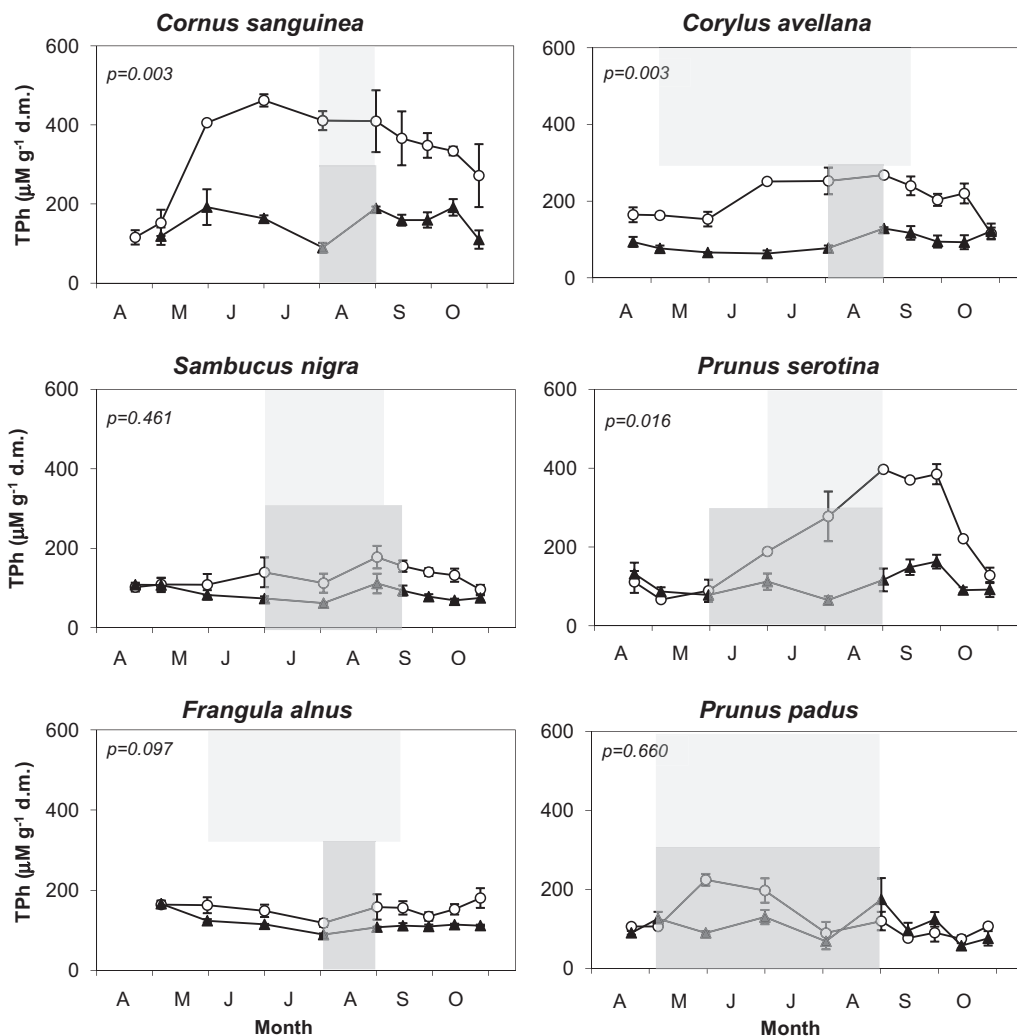
Fig. 3. Concentration of total soluble phenolics (TPh, mean ± SE, expressed as chlorogenic acid) in relation to long-term light conditions (○ − full sun, ▲ − shade) in uninjured leaves. Gray shading and level of significance (*p*) as in Fig. 1.

P. serotina, and *P. padus*), the leaves of the shrubs growing in full sun were significantly less injured than those from shaded conditions (Fig. 2). In *S. nigra* herbivore damage was low (0.8–2.3%) and the differences were not significant. In contrast, the opposite relationship between leaf perforation and light conditions was found in *C. avellana*; leaves in full sun were more injured than leaves in the shade.

The time of initiation and end of leaf perforation varied among shrub species (Fig. 2). Later in the season, leaf perforation remained at a stable level, but concentrations of the metabolites and nitrogen changed in uninjured leaves. We focused on constitutive defenses. Thus, all the data on SLA, concentrations of the metabolites, and N (presented in Table 1 and in Figs. 1 and 3–9) apply to uninjured leaves. However, we additionally marked these diagrams by shading the intervals between initiation of leaf perforation and time of maximum perforation (followed by a lack of changes in leaf area), based on the data presented in Fig. 2.

3.1. Defence compounds

TPh and condensed tannins played a significant role in the constitutive defense of the shrub leaves studied (Table 1). TPh content of leaves was highest in *C. sanguinea*, which was most

resistant to leaf perforation by insects (mean for both light conditions). Also leaves of *P. serotina* contained more TPh and tannins than leaves of *P. padus*, but only on the shrubs growing in full sun. In such light conditions, the differences in leaf perforation between the two *Prunus* species were higher (4.6-fold, average for the whole season) than in shade (2.4-fold). In *C. sanguinea*, *C. avellana*, *P. serotina*, *P. padus* and *F. alnus*, leaves of plants growing in high light conditions showed significantly higher concentrations of TPh (Figs. 3 and 8), while higher condensed tannins occurred with higher light in *P. serotina*, and similar trends occurred in *C. sanguinea*, *P. padus*, and *C. avellana* (Fig. 4). We found the largest effect of light on TPh in *C. sanguinea*. The difference in TPh level between the most and the least sunlit leaves in the crown of this species was 7-fold and reached 320 μM g^{-1} d.m. Relatively large differences in TPh between the most and least sunlit leaves were also recorded in *C. avellana* (6-fold) and *P. padus* (5-fold), where they reached about 200 μM g^{-1} d.m. In contrast to *P. padus*, in the closely related *P. serotina*, the difference reached only 135 μM g^{-1} d.m., and the correlation between TPh and SLA was only marginally significant (*p* = 0.061). Among the species studied, only *P. serotina* had significant negative correlations between SLA and tannin content of leaves growing in various parts of the crown (data not shown). Leaves of *S. nigra* and *F. alnus* exhibited relatively low
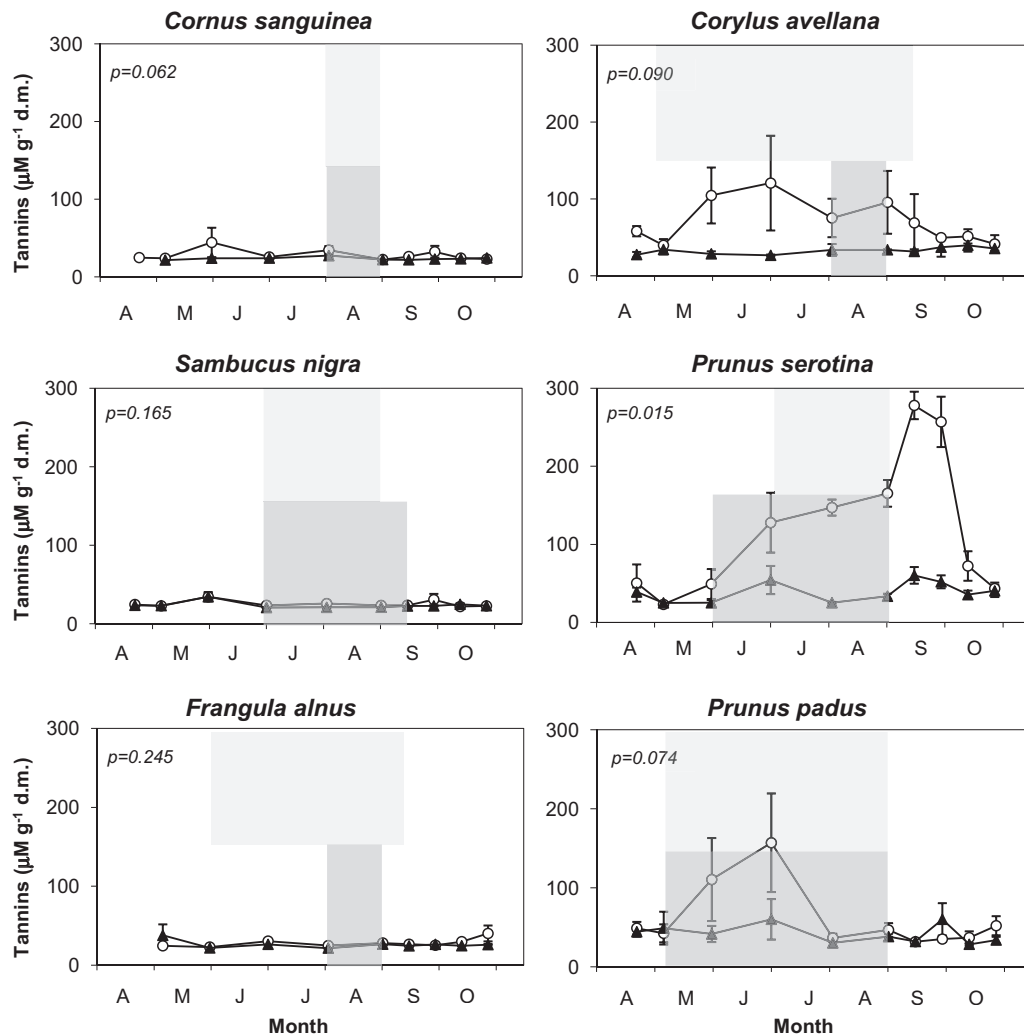
Fig. 4. Concentration of tannins (mean ± SE, expressed as catechin) in relation to long-term light conditions (○ − full sun, ▲ − shade) in uninjured leaves. Gray shading and level of significance (*p*) as in Fig. 1.

concentrations of tannins in both light conditions (Fig. 3). Also, there was no significant relationship between SLA and tannin content in leaves from well-lit and shaded parts of the same shrub (data not shown). In all species except *C. avellana*, leaves growing in full sunlight were less susceptible to insect damage than those from shaded conditions.

3.2. Water, nitrogen and nonstructural carbohydrates

As expected, leaves of shaded shrubs of all species had higher water content than leaves of shrubs growing in full sun (Fig. 5). Differences in water content between the two types of leaves were the largest in *C. avellana*, *C. sanguinea*, *P. padus*, and *P. serotina* (mean for the whole season: 12−14%), moderate in *F. alnus* (6%), and smallest (3%) in the light-demanding species, *S. nigra*.

For all species analyzed jointly, light conditions did not affect the nitrogen content of leaves (Table 1). However, a significantly higher N content was detected in leaves of *F. alnus* and marginally for *P. serotina* growing in strong light (Fig. 6). Similarly, using SLA as a proxy for light, we found no effect of light on nitrogen content (data not shown). Only in *P. serotina* did leaves growing in more sunlit parts of the crown (lower SLA) have a significantly higher nitrogen

content, very much like the case of shrubs growing in full sun (Fig. 6).

Leaves in full sun had a higher TNC content than leaves of shrubs growing in shade (Table 1). The effect of light on TNC content depended mostly on soluble carbohydrates. The differences between the light conditions of leaves on their starch level were not statistically significant (data not shown). The largest effect of light was recorded in *C. avellana*, as TNC concentration in leaves of well sunlit shrubs was 87% higher and the level of soluble carbohydrates was 108% higher than in leaves of shaded shrubs. Light conditions did not significantly affect the TNC content of leaves of *S. nigra* and *P. padus* (Fig. 7). In the other species, the effect of light was significant but not as strong as in *C. avellana*.

We noted similar effects of light on the TNC content of leaves when using SLA as a proxy for light. In *C. sanguinea*, *C. avellana*, *P. serotina*, and *F. alnus*, TNC content of leaves was significantly higher in shrubs growing in full light than in shade (Fig. 7), and a similar relationship was observed within shrubs, when leaves located in well-lit parts of the crown (lower SLA) were compared with those growing in shade (Fig. 9). In *C. avellana*, TNC content of leaves was much more variable than in the other species. For the SLA range of 100−500 cm^2 g^{-1}, TNC content varied from nearly 9% to 1% d.m., respectively (Fig. 9).
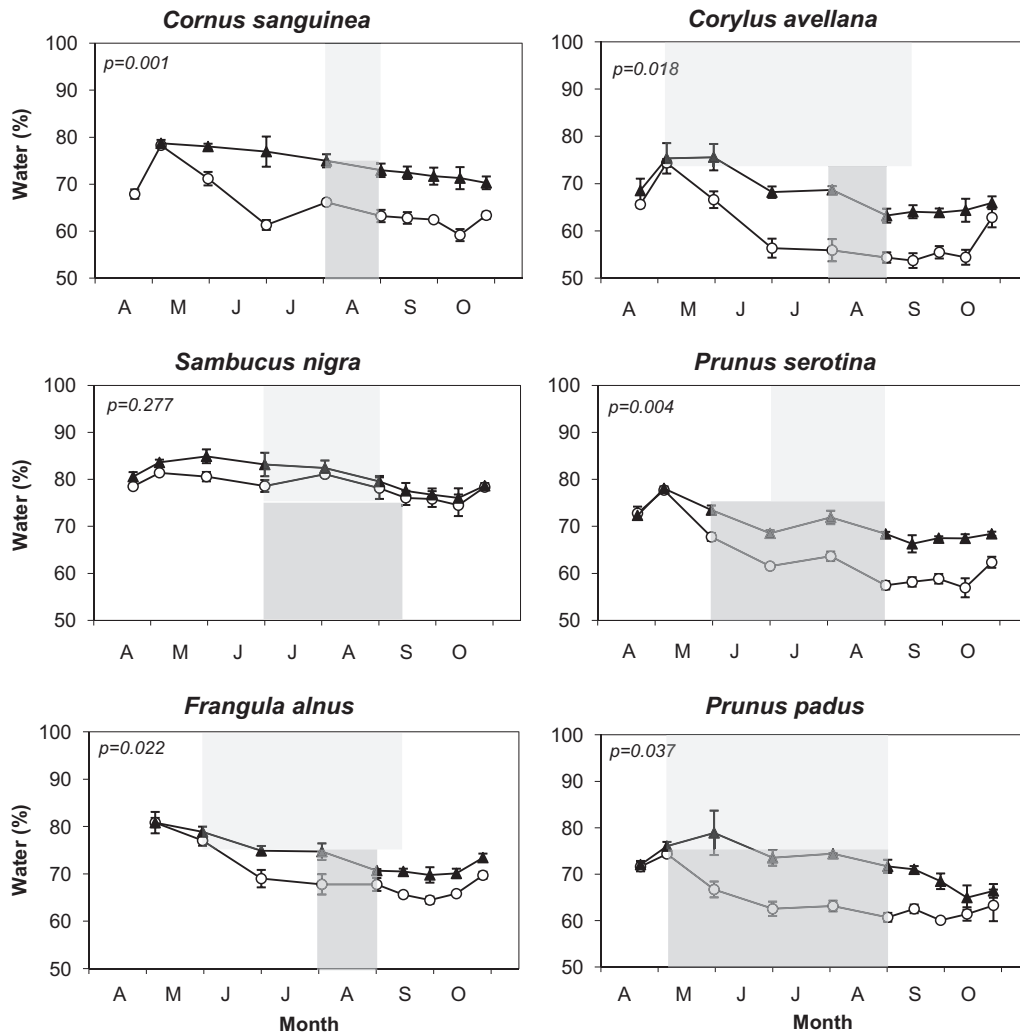
Fig. 5. Leaf water content (mean ± SE) in relation to long-term light conditions (○ − full sun, ▲ − shade) in uninjured leaves. Gray shading and level of significance (*p*) as in Fig. 1.

4. Discussion

4.1. Defense compounds

Our results indicate that the differences in leaf perforation of understory shrubs are determined mostly by concentrations of their defense compounds (soluble phenolics, condensed tannins), and − to a lesser extent − water content and concentrations of substances that are favorable for folivores (nitrogen compounds, TNC). The high level of phenolic compounds is the most common constitutive defenses of plants against folivores (Bennett and Wallsgrove, 1994; Henriksson et al., 2003; Barton and Koricheva, 2010). Results of this study suggest that a high level of TPh effectively protects leaves against folivores in *C. sanguinea*. Soluble phenolics, mostly the so-called phenolic acids, are a frequently mentioned group of phenolic compounds believed to prevent leaf damage by herbivores (Jansen and Stamp, 1997; Covelo and Gallardo, 2001; Ikonen et al., 2001; Coviella et al., 2002). However, there are few published reports on resistance of *Cornus* species to leaf damage by insects. For example, *Cornus florida* was classified by Dudt and Shure (1994) as a species very resistant to insect herbivory. Those authors believed that chemical defense against insect damage was due to high concentrations of both TPh and condensed tannins. They reported that light conditions in this

species determined to a large extent the level of TPh and − to a small extent − also the level of tannins, i.e. very much like *C. sanguinea* in the present study. Exceptionally high resistance of *C. sanguinea* to leaf damage by polyphagous insects was observed by Heskova (1973), who fed larvae of *Lymantria dispar* (Lymantriidae, Lymantrinae) leaves of 10 woody species. In comparison to many other shrub species, *C. sanguinea* is infested by a very small number of herbivorous insects, and their abundance is low (Duffey et al., 1974).

Beside *C. sanguinea*, another species showing little leaf damage was *S. nigra*, regardless of light conditions. Leaves of this species were characterized by a relatively low level of both soluble phenolics and condensed tannins. As mentioned earlier, *S. nigra* is a strongly light-demanding species (Ellenberg et al., 1992). In plants of this group, carbon is invested mostly in growth rather than in defense metabolites (Imaji and Seiwa, 2010). The causes of low leaf damage in *S. nigra* are poorly understood. However, research conducted by other authors confirms our earlier observations that *S. nigra* is highly resistant to leaf damage by insects (Varga, 1969). This species is one of the highest in leaf N content (Ellenberg et al., 1992). Possibly its defense strategy is based on defense compounds containing nitrogen (Craine et al., 2003).

We found that leaves of *C. avellana*, *P. serotina*, and *P. padus* contain not only high concentrations of soluble phenolics, but
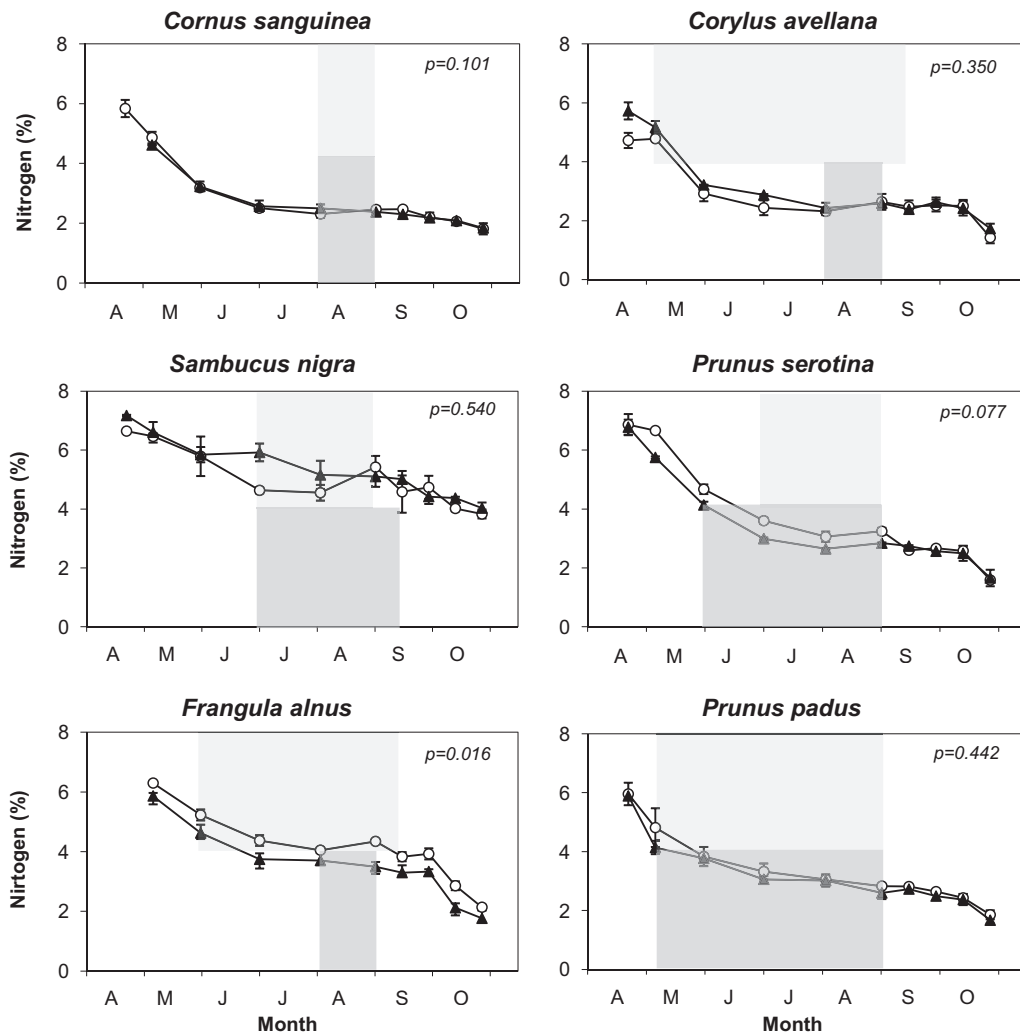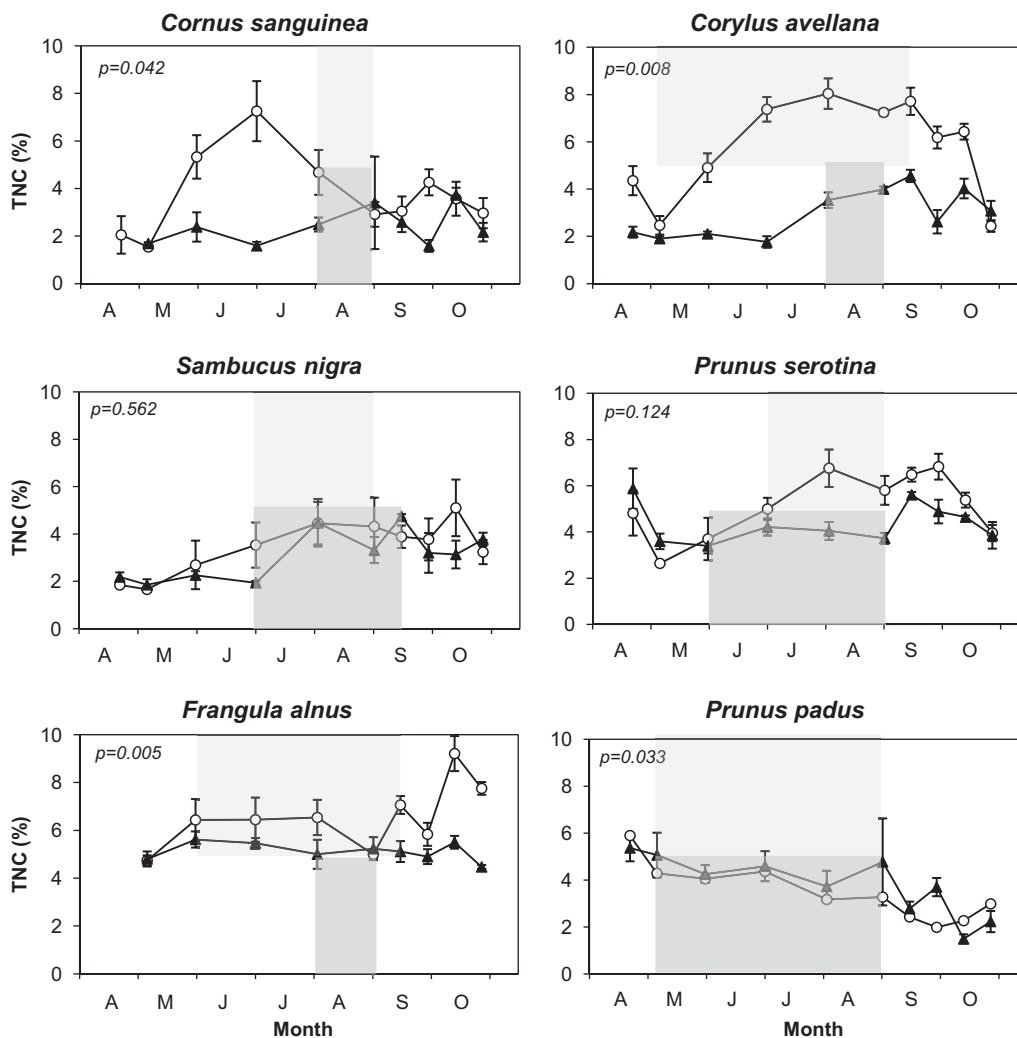
Fig. 6. Leaf nitrogen concentration (N, mean ± SE) in relation to long-term light conditions (○ − full sun, ▲ − shade) in uninjured leaves. Gray shading and level of significance (*p*) as in Fig. 1.

also relatively high concentrations of condensed tannins. Among phenolic compounds, tannins (condensed tannins in particular) are believed to play an important role in plant resistance to damage by folivores (Forkner et al., 2004; Dixon et al., 2005; Salminen and Karonen, 2011). Despite this, however, leaves of these three species are strongly damaged by insects (Fig. 2). Others report similar results for *P. padus* (Leather, 1986; Uusitalo, 2004), *P. serotina* (Kulman, 1965; Rieske, 2004; Osier and Jennings, 2007), and *C. avellana* (Heskova, 1973; Gantner and Jaśkiewicz, 2002). Thus, in these three species, the high concentrations of phenolic compounds in leaves do not protect them sufficiently against leaf damage by folivores − the oligophagous insect *Gonioctena quinque-punctata* on *P. padus* and *P. serotina*, and the monophagous *Altica brevicollis* on *C. avellana*.

The important role of phenolic compounds in plant defense is best reflected in the interaction between their concentration, light and leaf damage. SLA is a better indicator of the effects of light conditions than periodic measurements of light intensity, as it reflects light conditions throughout the entire growth and development of a given plant (Elemans, 2004). In our study, this is most conspicuous in both *Prunus* species, where lower leaf perforation (Fig. 2) and higher levels of TPh (Fig. 3) and tannins

(Fig. 4) were recorded in well-lit shrubs than in shaded ones. The lower defoliation of *P. serotina* than of *P. padus* (mean for both light conditions) probably results from the fact that the former species is introduced into Europe, and thus is less infested by folivores. This is also consistent with the enemy release hypothesis (Adams et al., 2008; Cincotta et al., 2008; Jogesh et al., 2008). Besides, the lower defoliation of *P. serotina* may be due to its initiation of leaf development up to 2 weeks later than in *P. padus*. This indicates that phenological differences between congeneric alien and native species may contribute to the success of insect pests. Similar effects of phenological differences on biotic factors (insects, fungal pathogens) have been reported many times (Aide, 1993; Martel et al., 2001; Dixon, 2003, and literature cited therein). The high level of phenolic compounds at an early stage of leaf development in *P. padus* (Figs. 3 and 4) is probably a defense strategy adapted to its phenology. This species is particularly severely infested by folivores (Leather, 1986; Uusitalo, 2004). The level of phenolics is also more sensitive to light in *P. padus* than in *P. serotina*. The increase in TPh associated with increasing sunlight is greater in this species (Fig. 8). This indicates that the defense strategy of this species against insect damage includes the use of phenolics. Such constitutive defense of sunlit leaves in *P. padus* is stronger than in *P. serotina*, whose leaves in such conditions are

Fig. 7. Total nonstructural carbohydrates in leaves (TNC, mean ± SE) in relation to long-term light conditions (○ − full sun, ▲ − shade) in uninjured leaves. Gray shading and level of significance (*p*) as in Fig. 1.

more rigid and leathery (Abrams et al., 1992), so they do not need to invest so much in chemical defense.

Apart from this study and our earlier research (Giertych et al., 2008), most other authors have also reported that increased access to light is related to higher levels of phenolic compounds in leaves: both of TPh (Dudt and Shure, 1994; Jansen and Stamp, 1997; Graglia et al., 2001) and of tannins (Dudt and Shure, 1994; Graglia et al., 2001; Yamasaki and Kikuzawa, 2003). Except for *C. avellana*, we also found that the lower leaf perforation in shrubs growing in full sun than in shade corresponds to their higher content of soluble phenolics (Figs. 3 and 8) and/or condensed tannins (Fig. 4). Thus, this may be explained by higher concentration of defense metabolites, TPh, and partially by tannins. Reduced herbivory in plants growing in full sun could be explained through the partial convergence between the responses of plants to UV radiation and herbivory. Under high UV radiation plants tend to accumulate phenolic compounds in the epidermis that may protect DNA from radiation, reducing its palatability at the same time (Roberts and Paul, 2006). Also, other authors have shown that the conditions of high solar radiation and the related high levels of UV-B radiation, reduce insect herbivory (Mazza et al., 2013). An important role is attributed here to phenolic compounds (Ballaré et al., 2012).

### 4.2. Water, nitrogen and nonstructural carbohydrates

In contrast to defense compounds, the variation in levels of substances that are favorable for folivores (nitrogen compounds, TNC, water) only slightly affected leaf perforation of the shrub species included in this study. The higher concentration of nitrogen and carbohydrates in leaves of shrubs growing in high light conditions did not reveal any important role of these substances in food preferences of insects in most of the species studied (Figs. 6 and 7). The higher concentration of these substances should make them more attractive and cause higher leaf damage. By contrast, in all species except *C. avellana*, leaves from high-light branches were less injured. One of the causes of lower leaf damage in high light conditions may be their greater thickness and rigidity, as indicated by low SLA values (Fig. 1) and low water content (Fig. 5). Muth et al. (2008) suggested that the most significant cause of greater damage of shaded leaves of the deciduous shrub *Lindera benzoin* was their lower mechanical strength and higher water content and nitrogen content, as compared to well-lit leaves. However, those authors, in contrast to our study, did not detect any significant differences in concentrations of defense compounds (TPh and tannins) among leaves of plants of the same species in dissimilar light conditions.
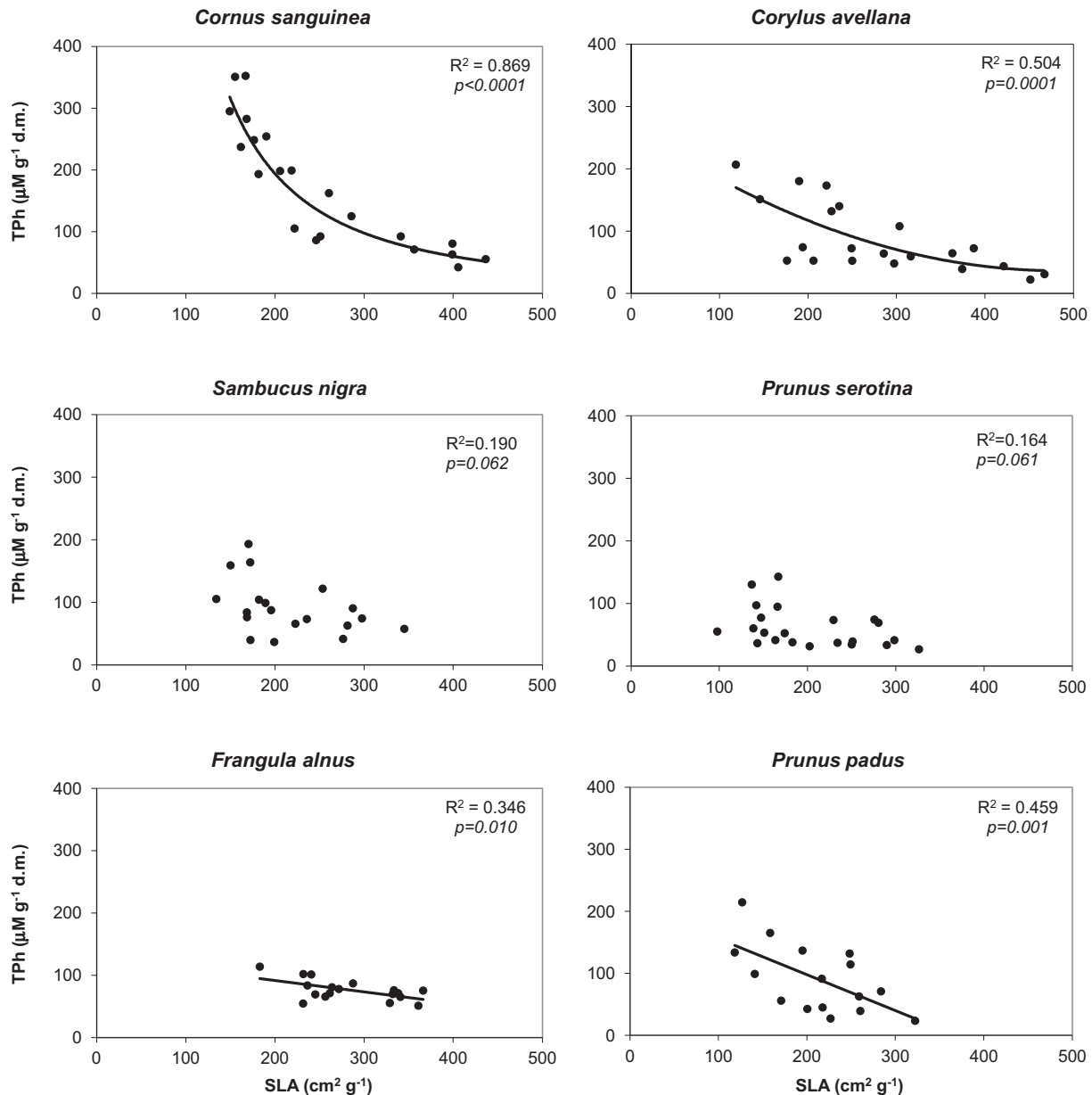
Fig. 8. Influence of light conditions, expressed as specific leaf area (SLA), on the concentration of total soluble phenolics (TPh) in uninjured leaves differently located in the crown of six shrub species.

We found that the differences in TNC content of leaves of shrubs growing in contrasting light conditions corresponded to differences in their content between sunlit and shaded leaves of the same shrub (Figs. 7 and 9). Carbohydrates are important as nutrients but they are also known as phagostimulants (i.e. feeding stimulants), which determine plant palatability to insects (Harborne, 1993). When feeding on leaves with higher levels of these substances, insects grow faster, reach a higher weight, and/or their larval development is shorter (Hwang and Lindroth, 1997; Kainulainen et al., 1998). However, in our study, the insect preference for leaves with a higher TNC level (more sunlit) in spite of the high level of defense compounds was noticed only in *C. avellana*. In this species, the much higher (about 4-fold, on average for the whole season) leaf perforation of sun-lit than shaded shrubs (Fig. 2) may be explained by their nearly 2-fold higher TNC content (Fig. 7),

including chiefly soluble carbohydrates (Table 1). In high light conditions, concentrations of both soluble carbohydrates and starch are higher (Hemming and Lindroth, 1999; Henriksson et al., 2003). Our observations (Karolewski et al., unpubl. data) and other reports (Gantner and Jaśkiewicz, 2002; D'Alessandro, 2004) indicate that the leaf-eating beetle *A. brevicollis* Foudras, 1860 (syn. *A. brevicollis* ssp. *coryletorum* Kral, 1964; Coleoptera, Chrysomelidae) is very common on *C. avellana*. Perhaps this beetle — usually regarded as oligophagous (Gantner and Jaśkiewicz, 2002) — has adapted to higher concentrations of phenolic compounds. Some researchers suggest that insect specialists are more resistant to elevated levels of defensive compounds than generalists (Van Zandt and Agrawal, 2004; Mathur et al., 2011). In addition, for example, Mooney et al. (2009) found that larvae of an insect specialist (*Epimecis hortaria*) consumed a greater mass of leaves
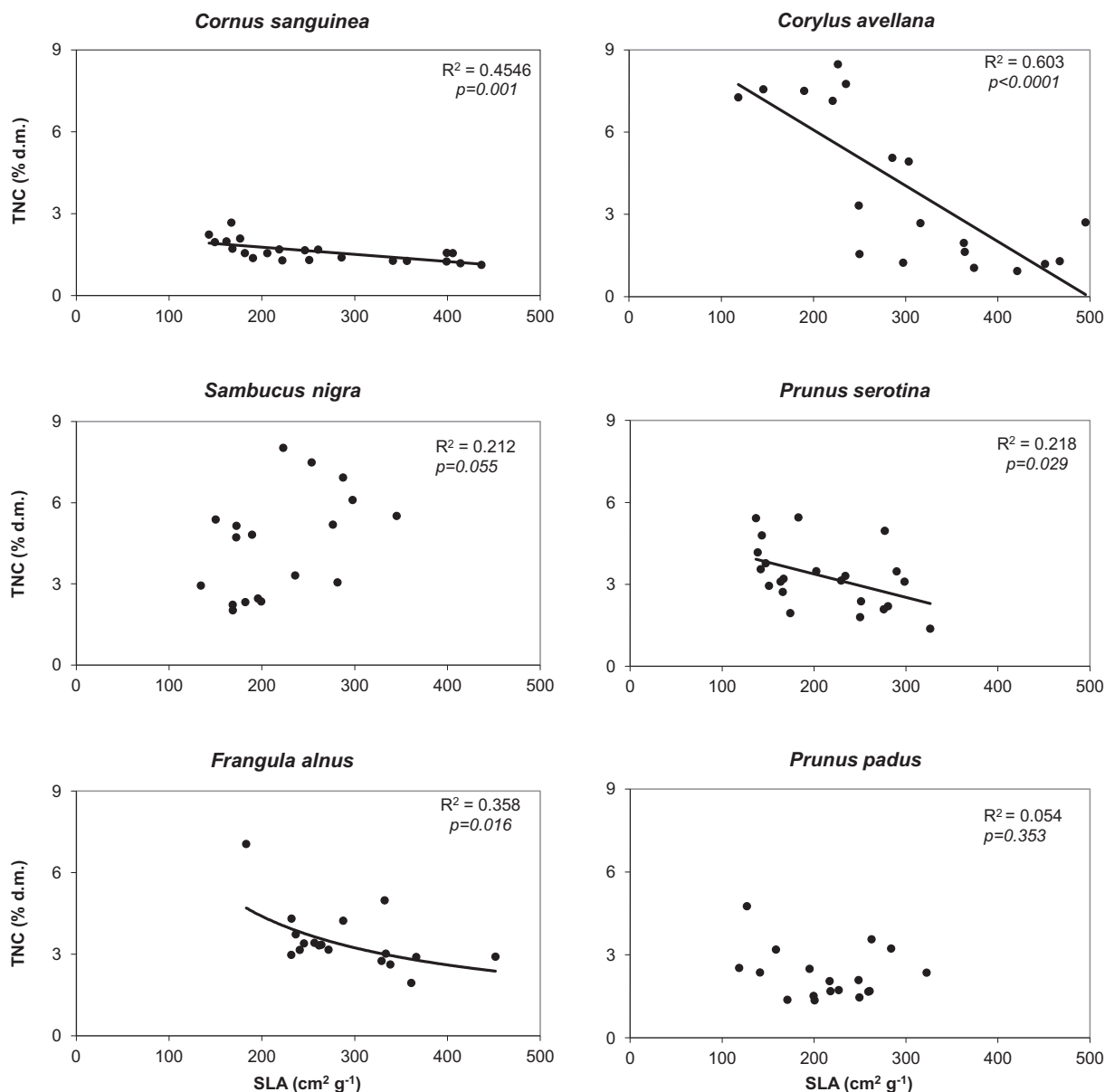
Fig. 9. Influence of light conditions, expressed as specific leaf area (SLA), on the concentration of total nonstructural carbohydrates (TNC) in uninjured leaves differently located in the crown of six shrub species.

(*Lindea benzoin*) and achieved greater final mass in the sun than in shade. We found that larvae and beetles of *A. brevicollis* feed more frequently on more sun-lit leaves, i.e. those containing more phenolics, but simultaneously with an exceptionally high level of carbohydrates. Similar relationships were also reported in other studies (Fortin and Mauffette, 2002; Leather and MacKenzie, 1994; Kainulainen et al., 1998). Our observations made throughout the growing season indicated that *A. brevicollis* was more abundant on well-lit shrubs than on shaded ones (Karolewski et al., unpubl. data). Adult *A. brevicollis* is a beetle which, in case of danger, jumps over long distances (Král, 1864). Perhaps, both a highly calorific diet (rich in sugars), and higher temperatures, are the reasons why the insect prefers to feed on sunlit shrubs and sunlit leaves. This hypothesis is supported by the observation in another study where we found that the beetles held for 3 h at 20 °C on average jumped 1.5 times the distance of those held at 10 °C (paper in preparation).

Differences in nitrogen concentration between leaves of shrubs growing on sunlit and shaded sites (Fig. 6) did not correspond to N concentration in sunlit and shaded leaves from various parts of the same shrub (data not shown). This probably results from a much lower effect of light conditions on the level of N than on TNC (Hemming and Lindroth, 1999). Nitrogen compounds are also important components of insect diet (Giertych et al., 2005, 2007; Chen et al., 2008), but according to McNeil and Southwood (1978) their concentration in plants is relatively stable and affects insect feeding only if nitrogen availability to plants is very low.

4.3. Additional observations and conclusions

In this study, the low leaf perforation in *S. nigra* probably resulted from the fact that in comparison to other shrub species, this species is injured by a very small number of phytophagous insect species

Table 1

Influence of shrub species (S), light conditions (L) and time in growing season (T) on leaf perforation (% leaf area), and specific leaf area − SLA (cm^2 g^{-1} d.m.), content of total phenols (TPh, expressed as μM of chlorogenic acid g^{-1} d.m.), condensed tannins (expressed as μM of catechin g^{-1} d.m.), water (% f.m.), nitrogen (% d.m.), total non-structural carbohydrates (TNC, % d.m.), soluble carbohydrates (SC, % d.m.) and starch (% d.m.), in non-herbivorized leaves of six shrub species growing in sun and shade conditions, at all terms in the season. Mean values ±SE are shown. Different letters indicate significant differences in leaf traits between species on average in both light conditions ($p < 0.05$, Tukey test). Analysis of repeated measures ANOVA with Phllai's Trace test for interactions of $T \times S$ and $T \times S \times L$ was used to assess the influence of species, time and light conditions on measured traits. In bold are p values <0.05.

Species		Perforation		SLA		Water		TPh		Tannins		Nitrogen		TNC		SC		Starch	
		Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade
Cornus sanguinea		1.0	1.2c	192	383bc	65.3	74.1bc	350	153a	28.4	23.5c	2.7	2.6d	4.0	2.4d	3.4	1.9d	0.54	0.47a
		±0.2	±0.2	±8	±16	±1.1	±0.8	±22	±9	±2.4	±0.4	±0.2	±0.2	±0.4	±0.3	±0.4	±0.3	±0.02	±0.01
Sambucus nigra		0.9	1.2c	304	405a	78.3	80.3a	128	83d	25.3	23.6c	5.0	5.2a	3.6	3.1cd	3.2	2.7cd	0.45	0.44b
		±0.2	±0.2	±10	±15	±0.6	±0.8	±8	±4	±1.2	±1.0	±0.2	±0.2	±0.3	±0.2	±0.3	±0.2	±0.01	±0.01
Frangula alnus		0.6	2.4c	222	381ab	69.8	73.8b	153	117bc	28.0	26.1c	4.1	3.6b	6.5	5.1a	6.1	4.7a	0.43	0.44b
		±0.1	±0.4	±12	±16	±1.1	±0.8	±6	±4	±1.4	±1.6	±0.2	±0.2	±0.3	±0.1	±0.3	±0.1	±0.00	±0.01
Corylus avellana		11.8	2.9b	205	399b	59.2	67.7e	205	96bc	73.6	33.5ab	2.7	2.8cd	5.8	3.1bc	5.4	2.6bc	0.43	0.44b
		±1.5	±0.4	±9	±14	±1.4	±1.1	±12	±6	±11.1	±1.9	±0.2	±0.2	±0.4	±0.2	±0.4	±0.2	±0.00	±0.01
Prunus serotina		3.2	12.7b	164	318c	62.7	70.0de	230	106b	105.0	38.9a	3.5	3.1c	5.2	4.2ab	4.7	3.8ab	0.43	0.44b
		±0.3	±1.4	±11	±10	±1.3	±0.8	±25	±8	±19.4	±3.4	±0.3	±0.2	±0.3	±0.2	±0.3	±0.2	±0.01	±0.01
Prunus padus		14.6	31.0a	225	354b	63.8	71.4cd	123	106cd	56.5	40.4b	3.1	2.9cd	3.2	3.6cd	2.8	3.2cd	0.43	0.45b
		±1.7	±3.0	±14	±14	±0.9	±1.0	±12	±10	±9.8	±4.1	±0.2	±0.1	±0.2	±0.3	±0.2	±0.3	±0.00	±0.01
Mean		5.4	8.6	219	373	66.6	72.9	198	111	51.2	30.9	3.5	3.4	4.7	3.6	4.3	3.2	0.45	0.45
ANOVA	d.f.	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Species (S)	5	278.33	<0.0001	10.54	<0.0001	87.04	<0.0001	19.04	<0.0001	49.46	<0.0001	52.98	<0.0001	22.76	<0.0001	23.66	<0.0001	47.47	<0.0001
Light (L)	1	38.72	<0.0001	221.23	<0.0001	170.65	<0.0001	95.26	<0.0001	74.45	<0.0001	2.82	0.1092	31.52	<0.0001	25.42	<0.0001	0.28	0.5993
Time (T)	8	142.61	<0.0001	25.25	<0.0001	49.88	**0.0007**	10.09	**0.0417**	5.91	**0.0057**	146.28	<0.0001	15.20	<0.0001	17.26	<0.0001	2.54	0.0606
S × L	5	52.81	<0.0001	2.57	0.0656	6.59	**0.0010**	4.91	**0.0157**	13.05	<0.0001	2.40	0.0761	5.92	**0.0016**	5.14	**0.0034**	18.28	<0.0001
S × T	40	1.54	**0.0417**	1.93	**0.0081**	1.73	**0.0191**	2.18	**0.0104**	3.27	<0.0001	4.46	<0.0001	3.58	<0.0001	3.15	<0.0001	1.72	**0.0173**
L × T	8	3.14	**0.0226**	6.53	**0.0039**	9.05	**0.0005**	1.31	0.4534	4.73	**0.0126**	3.27	**0.0318**	6.81	**0.0013**	8.88	**0.0004**	1.61	0.2096
S × L × T	40	1.11	0.3276	1.37	0.1241	1.40	0.1035	1.65	0.0683	2.23	**0.0016**	0.83	0.7356	2.15	**0.0016**	1.99	**0.0041**	1.23	0.2094
Error d.f.		24		17		19		10		17		19		20		20		21	

(Atkinson and Atkinson, 2002), and in addition only a small number of individual insects of each species were observed. Additionally, on *S. nigra*, predatory insects and parasites of insect folivores are relatively abundant (Győrfi, 1954; Defrance et al., 1987).

Concentrations of defense metabolites and nitrogen did not explain the higher insect damage of well-lit leaves than of shaded leaves of *C. avellana*. This difference is also not due to variation in the number of hairs (trichomes) on leaves, as on sunlit leaves their number is 2-fold higher (Karolewski et al., in preparation). This is consistent with earlier reports on the dependence of leaf "hairiness" on light conditions (Molina-Montenegro et al., 2006). No published information is available on higher leaf damage of more sunlit *Corylus* shrubs or on any factors that could explain such a relationship.

On shrubs of all the other species, regardless of light conditions, except monophagous *Yponomeuta evonymellus* on bird cherry (Leather, 1986), all the insects recorded by us in this study were generalists. The list of insect species that grazed on the shrub species studied is an element of a separate publication in preparation. In addition, we did not find in the available literature any data on specialist herbivorous insects grazing on these shrub species. Generalist herbivorous insects are more sensitive to defense compounds than specialist insects (Niemela, 1983; Mooney et al., 2009). The polyphagous beetle *G. quinquepunctata* Fabr. (Coleoptera, Chrysomelidae) was one of the most frequent species, found by us on all the six shrub species (more than 5% of the total number of folivores feeding on shrubs of the given species). This insect was more common on shrubs growing in shade than in full light. This may explain why in the other species (in contrast to *C. avellana*), leaf perforation was greater in shrubs growing in shade, with low concentrations of defense compounds.

In summary, we found that interspecific differences in leaf herbivory among the six species of shrubs studied were not systematically related to concentrations of defense metabolites (TPh and tannins). Contrary to our expectations, concentrations of these metabolites were higher in leaves of the species vulnerable to insect damage and lower in the species resistant to it. This indicates that phenolic compounds participate in the defense of the shrub species that are often infested by generalist herbivorous insects. Results of this study are consistent with our initial hypothesis that the level of defensive compounds in the leaves depends on the light conditions, regardless of the plant species. In five of the six species, leaves that developed in high light conditions were also less susceptible to insect grazing. The only exception was *C. avellana*. We suppose that in this species, higher leaf perforation in shrubs growing in full sun and in sunlit leaves was caused by grazing of a specialist herbivorous insect (*A. brevicollis*), adapted to high concentrations of phenolic compounds (TPh and condensed tannins). In the other five species, the lower leaf perforation of more sunlit shrubs and leaves is a typical relationship, frequently reported in the literature, and there are also no reports that these species are infested by specialist insects.

Acknowledgments

References

Abrams, M.D., Kloeppel, B.D., Kubiske, M.E., 1992. Ecophysiological and morphological responses to shade and drought in two contrasting ecotypes of *Prunus serotina*. Tree Physiol. 10, 343–355.

Adams, J.M., Fang, W., Callaway, R.M., Cipollini, D., 2008. A cross-continental test of the Enemy Release Hypothesis leaf herbivory on *Acer platanoides* (L.) is three times lower in North America than in its native Europe. Biol. Invasions 11, 1005–1016.

Aide, T.M., 1993. Patterns of leaf development and herbivory in a tropical understory community. Ecology 74, 455–466.

Atkinson, M.D., Atkinson, E., 2002. Sambucus nigra. J. Ecol. 90, 895–923.

Ayres, E., Heath, J., Possell, M., Black, H.I.J., Kerstiens, G., Bardgett, R.D., 2004. Tree physiological responses to above-ground herbivory directly modify belowground processes of soil carbon and nitrogen cycling. Ecol. Lett. 7, 469–479.

Ballaré, C.L., Mazza, C.A., Austin, A.T., Pierik, R., 2012. Canopy light and plant health. Plant Physiol. 160, 145–155.

Barton, K.E., Koricheva, J., 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. Am. Nat. 175, 481–493.

Bennett, R.N., Wallsgrove, R.M., 1994. Secondary metabolites in plant defence mechanisms. New Phytol. 127, 617–633.

Carmona, D., Lajeunesse, M.J., Johnson, M.T.J., 2011. Plant traits that predict resistance to herbivores. Funct. Ecol. 25, 358–367.

Chapman, S.K., Hart, S.C., Cobb, N.S., Whitham, T.G., Koch, G.W., 2003. Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. Ecology 84, 2867–2876.

Chen, Y., Ruberson, J.R., Olson, D.M., 2008. Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. Entomol. Exp. Appl. 126, 244–255.

Cincotta, C.L., Adams, J.M., Holzapfel, C., 2008. Testing the enemy release hypothesis: a comparison of foliar insect herbivory of the exotic Norway maple (*Acer platanoides* L.) and the native sugar maple (*A. saccharum* L.). Biol. Invasions 11, 379–388.

Covelo, F., Gallardo, A., 2001. Temporal variation in total leaf phenolics concentration of *Quercus robur* in forested and harvested stands in northwestern Spain. Can. J. Bot. 79, 1262–1269.

Coviella, C.E., Stipanovic, R.D., Trumble, J.T., 2002. Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. J. Exp. Bot. 53, 323–331.

Craine, J., Bond, W., Lee, W.G., Reich, P.B., Ollinger, S., 2003. The resource economics of chemical and structural defenses across nitrogen supply gradients. Oecologia 137, 547–556.

Defrance, H., Marboutie, G., Atger, P., 1987. Experimentation d'une haie brise-vent composite. Rev. Hort. 280, 25–29.

Dixon, A.F.G., 2003. Climate change and phenological asynchrony. Ecol. Entomol. 28, 380–381.

Dixon, R.A., Xie, D.-Y., Sharma, S.B., 2005. Proanthocyanidins – a final frontier in flavonoid research? New Physiol. 165, 9–28.

Dudt, J.F., Shure, D.J., 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. Ecology 75, 86–98.

Duffey, E., Morris, M.G., Sheail, J., Ward, L.K., Wells, D.A., Wells, T.C.E., 1974. Grassland Ecology and Wildlife Management. Chapman & Hall, London, UK.

D'Alessandro, P., 2004. Coleoptera, Chrysomelidae. In: Cerretti, P., Hardersen, S., Mason, F., Nardi, G., Tisato, M., Zapparoli, M. (Eds.), Invertebrati di una foresta della Pianura Padana, Bosco della Fontana, Secondo contributo, Conservazione Habitat Invertebrati 3, pp. 261–262.

Elemans, M., 2004. Light, nutrients and the growth of herbaceous forest species. Acta Oecol. 26, 197–202.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D., 1992. Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobot. 18, 3–258.

Forkner, R.E., Marquis, R.J., Lill, J.T., 2004. Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. Ecol. Entomol. 29, 174–187.

Fortin, M., Mauffette, Y., 2002. The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple. Can. J. For. Res. 32, 379–389.

Gantner, M., Jaśkiewicz, B., 2002. Beetles (Coleoptera) occurring on hazel (*Corylus* L.) in different habitat conditions. Acta Sci. Pol. Hort. Cult. 1, 55–66.

Giertych, M.J., Bąkowski, M., Karolewski, P., Żytkowiak, R., Grzebyta, J., 2005. Influence of mineral fertilization on feed quality of oak leaves and utilization efficiency of feed components by the gypsy moth. Entomol. Exp. Appl. 117, 59–69.

Giertych, M.J., Karolewski, P., Grzebyta, J., Oleksyn, J., 2007. Feeding behavior and performance of *Neodiprion sertifer* larvae reared on *Pinus sylvestris* needles. For. Ecol. Manag. 242, 700–707.

Giertych, M.J., Karolewski, P., Oleksyn, J., 2008. Early-season defoliation of *Sorbus aucuparia* (L.) and *Acer platanoides* (L.) can induces defense mechanisms against the spider mite (*Tetranychus urticae* (Koch)). Pol. J. Ecol. 56, 443–452.

Graglia, E., Julkunen-Titto, R., Shaker, G.R., Schmidt, I.K., Jonasson, S., Michelsen, A., 2001. Environmental control and intersite variations of phenolics in *Betula nana* in tundra ecosystems. New Phytol. 151, 227–236.

Győrfi, J., 1954. A cserebogár-kérdés jelenlegi helyzete. Erdő 3, 24–33.

Haissig, B.E., Dickson, R., 1979. Starch measurement in plant tissue using enzymatic hydrolysis. Physiol. Plant 47, 151–157.

Hansen, J., Møller, I., 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Anal. Biochem. 68, 87–94.

Harborne, J.B., 1993. Introduction to Biochemical Ecology, fourth ed. Academic Press, London.

Hemming, J.D.C., Lindroth, R.L., 1999. Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. J. Chem. Ecol. 25, 1687–1714.

Henriksson, J., Haukioja, E., Ossipov, V., Osipova, S., Sillinpää, S., Kaspari, L., Pihlaja, K., 2003. Effects of host shading on consumption and growth of the geometrid *Epirrita autumnata*: interactive roles of water, primary and secondary compounds. Oikos 103, 3–16.

Heskova, A., 1973. Effect of a change of food on the development and health of *Lymantria dispar* larvae. Acta Inst. For. Zvol. 4, 269–311.

Hódar, J.A., Zamora, R., Castro, J., 2002. Host utilisation by moth and larval survival of pine processionary caterpillar *Thaumetopoea pityocampa* in relation to food quality in three *Pinus* species. Ecol. Entomol. 27, 292–301.

Hwang, S.-Y., Lindroth, R.L., 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moth and forest tent caterpillars. Oecologia 111, 99–108.

Ikonen, A., Tahvanainen, J., Roininen, H., 2001. Chlorogenic acid as an antiherbivore defence of willows against leaf beetles. Entomol. Exp. Appl. 99, 47–54.

Imaji, A., Seiwa, K., 2010. Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species. Oecologia 16, 273–281.

Jansen, M.P.T., Stamp, N.E., 1997. Effects of light availability on host plant chemistry and the consequences for behavior and growth of an insect herbivore. Entomol. Exp. Appl. 82, 319–333.

Jogesh, T., Carpenter, D., Cappuccino, N., 2008. Herbivory on invasive exotic plants and their non-invasive relatives. Biol. Invasions 10, 797–804.

Johnson, G., Schaal, L.A., 1957. Accumulation of phenolic substances and ascorbic acids in potato tuber tissue upon injury and their possible role in disease and resistance. Am. Potato J. 34, 200–209.

Kainulainen, P., Ruohomäki, K., Ossipov, V., Haukioja, E., Pihlaja, K., 1998. Delayed induced changes in the biochemical composition of host plant leaves during an insect outbreak. Oecologia 116, 182–190.

Karolewski, P., Żmuda, M., Giertych, M.J., Oleksyn, J., 2007. Chemical leaf composition affects insect grazing on shrubs growing under different light conditions. Acta Physiol. Plant 29 (Suppl. 1), 71.

Karolewski, P., Zadworny, M., Mucha, J., Napierała-Filipiak, A., Oleksyn, J., 2010. Link between defoliation and root vitality in five understory shrubs with different resistance to insect herbivores. Tree Physiol. 30, 969–978.

Koricheva, J., Larsson, S., Haukioja, E., 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. Annu. Rev. Entomol. 43, 195–216.

Král, J., 1864. Zur Kenntnis der palaearktischen Altica-Arten HI (Col. Phytophaga, Alticidae). IX. Beitrag zur Kenntnis der Alticiden. Ent. Bl., Krefeld 60, 126–133.

Kulman, H.M., 1965. Natural control of the eastern tent caterpillar and notes on its status as a forest pest. J. Econ. Entomol. 58, 66–70.

Kurokawa, H., Nakashizuka, T., 2008. Leaf herbivory and decomposability in a Malaysian tropical rain forest. Ecology 89, 2645–2656.

Leather, S.R., 1986. Insects on bird cherry 1: the bird cherry ermine moth, *Yponomeuta evonymellus* (L.) (Lepidoptera: Yponomeutidae). Entomol. Gaz. 37, 209–215.

Leather, S.R., Lehti, J.P., 1982. Abundance and distribution of *Yponomeuta evonymellus* (Lepidoptera, Yponomeutidae) in Finland during 1981. Not. Entomol. 62, 93–96.

Leather, S.R., MacKenzie, G.A., 1994. Factors affecting the population development of the bird cherry ermine moth, *Yponomeuta evonymella* (L.). Entomol. 113, 86–105.

Lindroth, R.L., Kinney, K.L., Platz, C.L., 1993. Response of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry, and insect performance. Ecology 74, 763–777.

Martel, J., Hanhimäki, S., Kause, A., Haukioja, E., 2001. Diversity of birch sawfly responses to seasonally atypical diets. Entomol. Exp. Appl. 100, 301–309.

Mathur, V., Ganta, S., Raaijmakers, C.E., Reddy, A.S., Vet, L.E.M., van Dam, N.M., 2011. Temporal dynamics of herbivore-induced responses in *Brassica juncea* and their effect on generalist and specialist herbivores. Ent. Exp. Appl. 139, 215–225.

Mazza, C.A., Giménez, P.I., Kantolic, A.G., Ballaré, C.L., 2013. Beneficial effects of solar UV-B radiation on soybean yield mediated by reduced insect herbivory under field conditions. Physiol. Plant 147, 307–315.

McNeil, S., Southwood, T.R.E., 1978. The role of nitrogen in the development of insect–plant relationships. In: Harborne, J.B. (Ed.), Biochemical Aspects of Plant and Animal Coevolution. Academic Press, London, pp. 77–99.

Molina-Montenegro, M.A., Ávila, P., Hurtado, R., Valdivia, A.I., Gianoli, E., 2006. Leaf trichome density may explain herbivory patterns of *Actinote* sp. (Lepidoptera: Acraeidae) on *Liabum mandonii* (Asteraceae) in a montane humid forest (Nor Yungas, Bolivia). Acta Oecol. 30, 147–150.

Mooney, E.H., Tiedeken, E.J., Muth, N.Z., Niesenbaum, R.A., 2009. Differential induced response to generalist and specialist herbivores by *Lindera benzoin* (Lauraceae) in sun and shade. Oikos 118, 1181–1189.

Muth, N.Z., Kluger, E.C., Levy, J.H., Edwards, M.J., 2008. Increased per capita herbivory in the shade: necessity, feedback, or luxury consumption? EcoScience 15, 182–188.

Niemela, P., 1983. Seasonal patterns in the incidence of specialism: macrolepidopteran larvae on Finnish deciduous trees. Ann. Zool. Fenn. 20, 199–202.

Niesenbaum, R.A., Kluger, E.C., 2006. When studying the effects of light on herbivory, should one consider temperature? The case of *Epimecis hortaria* F. (Lepidoptera: Geometridae) feeding on *Lindera benzoin* L. (Lauraceae). Environ. Entomol. 35, 606.

Oishi, M., Yokota, T., Teramoto, N., Sato, H., 2006. Japanese oak silkmoth feeding preference for and performance on upper-crown and lower-crown leaves. Entomol. Sci. 9, 161–169.

Oleksyn, J., Karolewski, P., Giertych, M.J., Żytkowiak, R., Reich, P.B., Tjoelker, M.G., 1998. Primary and secondary host plants differ in photosynthetic response to herbivory: evidence from *Alnus* and *Betula* grazed by the alder beetle, *Agelastica alni*. New Phytol. 140, 239–249.

Osier, T.L., Jennings, S.M., 2007. Variability in host-plant quality for the larvae of a polyphagous insect folivore in midseason: the impact of light on three deciduous sapling species. Entomol. Exp. Appl. 123, 159–166.

Price, M.L., van Scoyoc, S., Butler, L.G., 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26, 1214–1218.

Rieske, L.K., 2004. Age-specific host utilization in the eastern tent caterpillar (Lepidoptera: Lasiocampidae). J. Entomol. Sci. 39, 94–100.

Roberts, M.R., Paul, N.D., 2006. Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. New Phytol. 170, 677–699.

Salminen, J.-P., Karonen, M., 2011. Chemical ecology of tannins and other phenolics: we need a change in approach. Funct. Ecol. 25, 325–338.

Sardans, J., Peñuelas, J., 2013. Plant–soil interactions in Mediterranean forest and shrublands: impacts of climatic change. Plant Soil 365, 1–33.

Siles, G., Rey, P.J., Alcántara, J.M., Bastida, J.M., Herreros, J.L., 2010. Effects of soil enrichment, watering and seedling age one establishment of Mediterranean woody species. Acta Oecol. 36, 357–364.

Singleton, V.I., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. Am. J. Enol. Viticult. 16, 144–158.

Snedecor, G.W., Cochran, W.G., 1976. Statistical Methods, sixth ed. The Iowa State University Press, Ames, Iowa, USA.

Szymański, S., 1986. Ecological Bases of Silviculture. PWRiL, Warszawa (in Polish).

Tuszyński, M., Gorzelak, A., 1998. An attempt of evaluating the impact of understory on soil, microclimate, and vegetation in pine stands on poor sandy forest soil. Sylwan 142, 15–26 (in Polish).

Uusitalo, M., 2004. European Bird Cherry (*Prunus Padus* L.) – a Biodiverse Wild Plant for Horticulture. Agrifood Res. Rep. 61. MTT Agrifood Res. Finl., Jokioinen Finland.

Van Zandt, P.A., Agrawal, A.A., 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*. Oikos 104, 401–409.

Varga, F., 1969. Adatok a gyapjaspille (*Lymantria dispar* L.) táplálkozásbiológiájához és ennek összefüggése a tömegszaporodással. EFE Tudományos Közleményei 1, 71–82.

Whitham, T.G., DiFazio, S.P., Schweitzer, J.A., Shuster, S.M., Allan, G.J., Bailey, J.K., Woolbright, S.A., 2008. Extending genomics to natural communities and ecosystems. Science 320, 492–495.

Yamasaki, M., Kikuzawa, K., 2003. Temporal and spatial variations in leaf herbivory within a canopy of *Fagus crenata*. Oecologia 137, 226–232.

Żmuda, M., Karolewski, P., Giertych, M.J., Żytkowiak, R., Bąkowski, M., Grzebyta, J., Oleksyn, J., 2008. The effect of light conditions on leaf injury in underbrush shrubs caused by leaf-eating insects. Acta Sci. Pol., Silv. Colendar. Rat. Ind. Lignar. 7, 47–57.