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Light as a regulator of structural and chemical leaf defenses against insects in two *Prunus* species

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ABSTRACT

Light is a key factor influencing competition between species, and the mechanisms by which trees overcome insect outbreaks can be associated with alternation of the leaves structure, which then prevent or promotes their susceptibility to herbivores. It was predicted that leaf tissue anatomy would likely be different in sun and shade leaves, with a gradual decline of leaves resistance coupled with reduction of accessible light. We quantified anatomical patterns and the distribution of defence compounds (phenols, total tannins, catechol tannins) within heavily grazed leaves of *Prunus padus*, native in Europe and *Prunus serotina*, an invasive to Central Europe. Both species were strongly attacked by folivorous insects when shrubs grew in the shade. In the sun, however only *P. padus* leaves were grazed, but *P. serotina* leaves were almost unaffected. We identified that anatomical characteristics are not linked to different *P. padus* and *P. serotina* leaf vulnerability to insects. Furthermore, the staining of defence compounds of *P. serotina* leaves grown in full sun revealed that the palisade mesophyll cells had a higher content of phenolic compounds and catechol tannins. Thus, our results indicate that a specific distribution of defence compounds, but not the anatomical relationships between palisade and spongy mesophyll, may be beneficial for *P. serotina* growth outside its natural range. The identified pattern of defence compounds distribution is linked to a lower susceptibility of *P. serotina* leaves to herbivores, and is associated with its invasiveness. This likely reflects that *P. serotina* is a stronger competitor than *P. padus*, especially at high sunlit sites i.e. gaps in the forest.

1. Introduction

Two *Prunus* species, the native *Prunus padus* L. and the introduced *P. serotina* Ehrb., contribute significantly to the composition of the forest understory in Europe (Zerbe and Wirth, 2006; Pairon et al., 2010). *P. serotina* was one of the first North American tree species introduced into Europe as an ornamental for parks and gardens (Starfinger et al., 2003), and was later planted to improve soil productivity and prevent erosion (Starfinger, 1997). Currently, *P. serotina* occurs throughout Europe and is recognized as a highly invasive shrub that excludes native species and diminishes the diversity present in plant communities (Starfinger, 1997). The successful colonization by *P. serotina* is the result of its greater ability, relative to the native *P. padus,* to tolerate relatively dry and nutrient poor soils (Starfinger, 1991), as well as its higher tolerance to spring frosts (Seneta and Dolatowski, 2011). Deckers et al. (2005) reported that *P. serotina* is highly adapted to changing light conditions; however, the general adaptability of both *Prunus* species to changing light conditions may be associated with resistance to herbivory (Łukowski et al., 2016).

As wide-spread shrubs growing under canopy conditions, *P. padus* and *P. serotina* are important sources of food for herbivores. The main herbivore in central and eastern Europe that utilises both cherry species is a polyphagous beetle, *Gonioctena quinquepunctata* Fabricius (Coleoptera: Chrysomelidae; Leather and MacKenzie, 1994; Uusitalo, 2004). The level of leaf perforations produced by beetle feeding differs in the two *Prunus* species, and is largely dependent on light conditions. The herbivory of shaded leaves is extensive in both species; however, insect feeding on sunny leaves is significantly greater in *P. padus* than it is in *P. serotina* (Karolewski et al., 2013, 2014; Łukowski et al., 2016). Although levels of leaf perforations may be a consequence of plant adaptation to growth in shaded conditions, as both species of *Prunus* can adjust leaf morphology and anatomy in response to various light intensities, the extent to which these shifts impact the intensity of herbivory

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has not been fully determined. Most research indicates that the food preferences of phytophagous insects are based on leaf chemistry (Awmack and Leather, 2002; Henriksson et al., 2003). There are reports, however, that leaf digestibility and palatability also depend on leaf anatomy (Dominy et al., 2003; Koricheva et al., 2004). Furthermore, leaf anatomy mirrors light availability (Lambers et al., 1998).

Plant species growing in high light conditions are characterized by thick leaves (Wilson et al., 1999, 2000; Rodriguez-Calcerrada et al., 2008) and palisade parenchyma that are composed of long cells with thick cell walls (Uemura et al., 2000; Yamashita et al., 2002). The structure of the palisade cells reflects the adaptation of sun-exposed leaves to maximise photosynthesis (Niinemets et al., 1998) and, hence, the production of higher amounts of carbohydrates than shade leaves (Fortin and Mauffette, 2002; Karolewski et al., 2013). High photosynthetic rates, however, also lead to a higher allocation of nitrogen (N) to light-intercepting proteins (Niinemets, 1997). Both features can have positive and negative consequences. The higher levels of photosynthesis result in greater carbohydrate production, providing energy for the generation of chemical defense compounds. Conversely, leaves growing under high light conditions have a higher nutrient content and hence are more attractive as a food source for herbivores (Hemming and Lindroth, 1999; Niesenbaum and Kluger, 2006).

The strength and extent of leaf defenses against herbivores are typically evaluated on the basis of the general concentration of defense compounds in the leaves (Grime et al., 1996). Increased tissue construction cost is associated with increased concentrations of defense compounds in tissues (McCall and Fordyce, 2010). Similarly to many other reports, our previous studies only considered the average content of defense compounds within entire leaves (leaf mass per unit). The localization of defense compounds within different leaf tissues, however, may optimize plant defense, especially since leaves may be attacked by herbivores with different feeding habits. Additionally, the vertical localization of defense compounds within a leaf may also reflect the presence of different chemical structures (Nuringtyas et al., 2012). The distribution of defense-related compounds in different tissues may therefore impact the intensity of leaf perforation. Clarifying the relationship between leaf structure and the localization of defense compounds will improve our understanding of leaf susceptibility to herbivory in leaves growing under diverse light conditions. Thus, the following question was posed in the present study: Do variations in leaf structure cause differences in the intensity of herbivory observed in the two *Prunus* species growing under different light conditions? Additionally, does the localization of defense compounds in specific leaf tissues make *P. serotina* less susceptible to insect herbivory by reducing the palatability of leaves grown under sun conditions? The aim of the study was to determine whether or not the thickness of the photosynthetic mesophyll layer in leaves could explain the variation in leaf damage observed between shaded and sun-exposed leaves. More specifically, the study aimed to determine if the distribution of defense compounds in palisade and spongy mesophyll cells would explain the pronounced differences in the levels of herbivory observed between sun and shade leaves in *P. padus* and *P. serotina*, as a result of their adaptation to a diverse light environment.

In the present study, the effects of light availability on leaf structure, the production of defense compounds, and leaf herbivory were determined in native *P. padus* and non-native *P. serotina*. The concentration of defense compounds in leaves grown in full sun or shade conditions was determined, as well as based on the level of insect-related perforations present on the leaves. The main objectives of this study were: (i) to determine whether or not the level of insect-related leaf perforations was associated with the ratio of palisade to spongy mesophyll cells in the two *Prunus* species, grown in shade and high light; and (ii) to test whether the localization of defense compounds within palisade and spongy mesophyll cells was associated with the level of leaf perforations.

2. Materials and methods

2.1. Plant material

Experiments were conducted in (i) an outdoor greenhouse with shade cloth located at the Institute of Dendrology in Kórnik, Poland (52°14′N; 17°05′E; 75 m altitude) and (ii) within a forest plot located in the Palędzie Forest, Konstantynowo Forest District, Poland (52°23′N, 16°40′E). Seeds of *P. padus* and *P. serotina* used in the greenhouse experiment were obtained from the Palędzie Forest and stratified at the Laboratory of Seed Biology, Institute of Dendrology, Polish Academy of Sciences. The seeds of the two *Prunus* species were sown in 1.5 dm^3 pots in the spring of 2007. The planting soil was composed of forest soil (collected in a mature oak/pine forest) mixed with neutralized peat (1:1), and received an application of the slow-release N-P-K fertilizer Osmocote (2 kg m^{-3}). The seedlings were randomly assigned to one of two light conditions (15% or 100% full sun), which were measured using a FF-01 photometer (Sonopan, Poland). Each treatment (species × light) was composed of 20 seedlings, and leaves were harvested for analysis from six-year-old trees. Details of the experimental design have been described previously (Karolewski et al., 2010).

Site and growth conditions of the approximately ten-year-old *Prunus* shrubs used in the forest plot have been previously described in Mąderek et al. (2015). *P. padus* and *P. serotina* trees used in the present study were all 3–5 m tall, growing under a canopy of *Pinus sylvestris* L., with a mixture of mature *Quercus robur* L., *Fagus sylvatica* L., *Carpinus betulus* L., and *Ulmus laevis* Pall. trees. Leaves from three shrubs (n = 3) within each species (*P. padus* and *P. serotina*)/light condition (sun and shade) were analyzed. Three fully expanded leaves from three different shoots per shrub were harvested in order to obtain representative plant material. The same number (108) of leaves was separately used for the anatomical and histochemical analyses. This pattern (2 species × 2 light treatments × 3 shrubs × 3 shoots × 3 leaves) was replicated to encompass perforated and non-perforated (control) leaves. The levels of insect-related perforations in the leaves relative to the light conditions were those established by Karolewski et al. (2013) – namely, 18% leaf perforation (the average percent of leaf area loss due to feeding) for shade leaves of *P. serotina*, 40% leaf perforation for shade leaves of *P. padus*, 4% leaf perforation for sun leaves of *P. serotina*, and 20% leaf perforation for sun leaves of *P. padus*. Leaf anatomical traits (thickness of the palisade layer, spongy mesophyll, and epidermis) were measured, as was the localization of defense compounds within the leaves of the two *Prunus* species grown under both controlled and field conditions.

2.2. Histochemical analysis and anatomical measurements

Leaves collected from plants growing in the greenhouse in Kórnik, and at the forest site, were longitudinally sectioned (2 × 3 mm) from the central part of the lamina, and placed in a fixative (2% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer). After 24 h, specimens were rinsed three times with cacodylate buffer (0.05 M, pH 6.8; Polysciences, Warrington, PA, USA) and dehydrated in a graded ethanol series (10%–100%). Samples were then embedded in Technovit 7100 and sectioned with a rotary microtome (Leica RM2265, Wetzlar, Germany). The resulting 5-μm-thick sections were stained with 0.5% toluidine blue