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Long-term UV-B and temperature enhancements suggest that females of *Salix myrsinifolia* plants are more tolerant to UV-B than males

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ABSTRACT

Males of sexually dimorphic trees and shrubs are hypothesized to have lower reproduction costs, to grow faster and to be less well defended than females. Sex-related differences in sexual reproduction, photosynthesis, growth, phenolic concentrations and rust infection in field-grown *Salix myrsinifolia* plants were investigated after 3-year exposure to modulated UV and temperature enhancements. UV-B increased leaf hyperin and salicortin, while elevated temperature increased chlorogenic acid concentrations in females only. The UV-induced accumulation of leaf flavonoids, condensed tannins and salicylates was facilitated by warming to a greater degree in females than in males. The UV treatment had only minor effects on shoot biomass, photosynthesis and reproduction, while temperature significantly increased all the variables related to growth and sexual reproduction. Enhanced UV-B tended to decrease biomass and leaf thickness in males, which, along with the UV-induced increase in leaf phenolics in females, suggests that the females have greater tolerance to UV-B compared to males. Irrespective of the UV and temperature treatments, no apparent trade-off between growth, reproduction and allocation to defense was detected. Consequently, in the long-term, the genders of *S. myrsinifolia* might respond differently to future climate change which, in turn, might affect the sex ratio and spatial distribution of willow species.

Introduction

Salix myrsinifolia (dark-leaved willow) is a sexually dimorphic shrub that is native to Europe and western Siberia. The term sexual dimorphism is commonly used to refer to differences in morphological, ecological and behavioral attributes resulting from the differentiation of reproductive functions between males and females and their separation into separate individuals (Meagher, 1984). Males and females of sexually dimorphic species often respond in a different way to various environmental factors (Li et al., 2007; Xu et al., 2008; Xu et al., 2010; Zhao et al., 2009, 2012; Zhang et al., 2010, 2011; Peng et al., 2012; Han et al., 2013; Randriamanana et al., 2014). Apart from being sexually dimorphic, the genus *Salix* is, in most instances, female-biased in nature, with a sex ratio of 2:1 (Alliende and Harper, 1989; Dawson and Bliss, 1989; Dudley, 2006; Ueno et al., 2007; Hughes et al., 2010; Myers-Smith and Hik, 2012). Many hypotheses have been invoked to explain the asymmetry of *Salix* as regards to sex ratio: these hypotheses include an inherent bias in seed populations (Ueno et al., 2007), early-life factors that may affect the seedling's establishment (Myers-Smith and Hik, 2012), differences in herbivory pressure (Danell et al., 1985), or spatial segregation of the sexes (Dawson and Bliss, 1989; Dormann and Skarpe, 2002; Hughes et al., 2010).

Although no unanimity was found to explain such a skewed sex ratio, according to the conventional theory of sex allocation, favoring the sex that requires lower costs seems to be an adaptive trait for dioecious species, aimed at increasing the population's overall reproductive success and maximizing fitness (Sinclair et al., 2011 and references therein). Moreover, in perennial trees and shrubs, females are expected to be more expensive to maintain because of their higher sexual reproductive effort (Lloyd and Webb, 1977; Ågren et al., 1999; Obeso, 2002). If females are indeed more expensive than males, it remains enigmatic why *Salix* populations should be female-biased. Furthermore, such sexual dimorphism in life history traits might be accompanied by gender specialization in physiology and eventually niche partitioning, in order to meet their resources requirements and/or to alleviate intraspecific

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competition, as was the case with *Salix arctica* and *Salix sachalinensis* (Dawson and Bliss, 1989; Ueno and Seiwa, 2003). Spatial segregation of the sexes does, however, pose the risk of ultimately increasing the distance between males and females and thus, decreasing the likelihood of successful sexual reproduction (Meagher, 1984; Bierzychudek and Eckhart, 1988). Hence, the fitness return gained from favoring females should be sufficient to meet such costs. Moreover, due to the plants' finite resources and the physiological trade-off between reproduction, growth and maintenance, higher reproduction costs in one of the genders might incur costs in other life history traits, such as growth and allocation to defense (Lloyd and Webb, 1977; Ågren et al., 1999; Delph, 1999). The present study attempts to elucidate the trade-off between growth, reproduction and allocation to defense in dark-leaved willows and how it will be influenced by long-term UV-B and temperature enhancements.

Thanks to successful implementation of the Montreal protocol, the stratospheric ozone hole is expected to recover. However, its recovery is not expected to materialize before several decades and will depend on various tropospheric factors (aerosols, clouds, etc.), which in turn are influenced by climate change factors (Hegglin and Shepherd, 2009; Ballaré et al., 2011). Plants engage a range of protective methods of counteracting the damaging effects of high doses of UV-B, such as efficient antioxidation systems, DNA repair mechanisms and increases in the concentrations of certain phenolic compounds, particularly flavonoids and hydroxycinnamic acids (Tegelberg et al., 2001; Searles et al., 2001; Tegelberg et al., 2002; Britt, 2004; Newsham and Robinson, 2009; Nybakken et al., 2012; Hideg et al., 2013). Flavonoids and cinnamic acids are mainly acknowledged for their ability to screen UV-B, but they are also known for their possible role as antioxidants (Rice-Evans et al., 1997; Agati and Tattini, 2010; Agati et al., 2012, 2013). However, it is also suggested that other phenolic compounds, such as salicylates and condensed tannins, protect plants from photodamage (Close and McArthur, 2002; Warren et al., 2003; Virjamo et al., 2013). Moreover, the ubiquitously present phenolic compounds are reported to play defensive roles against *Melampsora* (rust) fungi (Miranda et al., 2007). In addition, genders might differ in their responses to rust disease (Zhang et al., 2010).

The effects of UV-B on plants also vary with species, the plant's degree of adaptation/acclimation to UV-B, and with the nature of UV-B treatment (fluence rate, duration and wavelength, methodologies), as well as interacting closely with other environmental factors (Jenkins, 2009; Newsham and Robinson, 2009). Temperature is among the key environmental factors that will determine a plant's reaction to climate change. Global temperatures have increased by 0.85 °C over the period 1880–2012 and it is likely that they will reach 1.5 °C above pre-industrial levels by the end of the 21st century (IPCC, 2013). Given that climate change factors act in concert to shape terrestrial ecosystems, in order to reflect as extensively as possible the biological processes occurring in nature, it is of particular importance to implement a multifactor study. Yet very few have studied the long-term effects of combined UV-B and temperature on woody species. Moreover, there are very few studies dealing with plants at the reproductive stage, which would give more insights into the influence of climate change on the sexual reproduction of woody species.

The main goal of the present study was to investigate sexual dimorphism in the three competing processes: growth, defense and reproduction of *S. myrsinifolia* under long-term UV and temperature treatments in the season when the species start intensive sexual reproduction. The following questions were addressed: (i) In terms of biomass, how will sexual reproduction efforts differ between males and females of *S. myrsinifolia*? (ii) How will long-term UV and temperature enhancements affect the dynamics between the three foremost life history traits of *S. myrsinifolia* : growth, chemical defense and reproduction? (iii) What effects will UV- and temperature-mediated changes in plant growth and phenolics have on rust infection? Based on the results of the first and the second growing seasons (the pre-reproductive phase), males seemed to be more growth-biased than females, although no differences in biomass, total low molecular weight phenolic and condensed tannin concentrations were detected (Nybakken et al., 2012). Given the small gender-based differences in growth rate and allocation to defense, it can be hypothesized that even though growth demands might not differ much between the genders, females of *S. myrsinifolia* would probably have an efficient mechanism, such as a higher photosynthetic rate, than males, enabling them to compensate for their higher sexual reproductive costs thus, favoring females can still maximize population overall reproductive fitness.

Materials and methods

Experimental setup

The experiment was set up in a field in the botanical garden in Joensuu, Finland (62 °N, 35 °E, 62 °N, 46 °E). The experimental layout is described in Nybakken et al. (2012) and Virjamo et al. (2013). Briefly, the setup consisted of 36 plots (3 m × 1.5 m each and with an effective area of 2.40 m × 0.80 m), randomly distributed to the treatments C, T, UV-B, UV-A, UV-B + T and UV-A + T (six replicates each), and situated 3 m away from each other. In 2009, each plot initially contained 40 individual cuttings of *S. myrsinifolia* Salisb. (five cuttings per genotype, four male genotypes and four female genotypes) originating from the Joensuu and Kaavi areas (Eastern Finland, 62 °N, 29 °E), which were directly planted in the soil.

Ultraviolet radiation and temperature were modulated to follow *in situ* climatic conditions. The supplemental UV-radiation was provided by six fluorescent UV-313 lamps (Q-Panel, Cleveland, OH, USA) supported by aluminum frames mounted above the plants and driven by three digital dimmable ballasts (Tridonic, Austria) per plot. The UV radiation was increased by 32% (unweighted), which corresponds to a 20% decrease in the ozone layer above central Finland (Björn, 1990, 1991). The lamps in the UV-B plots were wrapped with pre-burned 0.115 mm cellulose diacetate films (Kotelorauma, Finland) to remove radiation below 290 nm. The cellulose diacetate films were changed at three-week intervals. The lamps in the UV-A plots were wrapped with 0.175 mm polyester films (Kariplast, Finland) to remove radiation below 315 nm and served as controls for the UV-B treatment. The control plots (controls) had unenergized lamps mounted on aluminum frames in order to mimic the same shading as in the treated plots.

The performance of the system in the field for the growing seasons 2009 and 2010 was reported in Nybakken et al. (2012). The performance of the modulated system in 2011 is reported in Fig. A1. In plots with elevated temperature, the temperature was set to +2 °C above the ambient by using two infrared heaters (CIR 105, FRICO, Partille, Sweden) placed perpendicularly to the UV lamps, in the center of the frames. The actual temperature increase achieved by the IR heaters was on average +1.3 °C during the whole growing season. In the temperature control frames, the IR heaters were replaced by wooden boards of the same size. The lamps were kept at a distance of 60 cm from the top of the highest plants throughout the experiment.

In 2009, the temperature treatment was started on June 15, the UV treatment on July 10, and both were stopped on September 15. In 2010, both UV and temperature treatments started on June 3 and ended on October 15. In 2011, UV and temperature treatments started on May 24 and ended on August 6. Due to a power cut in July 2010, five frames were thereafter omitted from all the