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Research article

The vegetative buds of *Salix myrsinifolia* are responsive to elevated UV-B and temperature

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ABSTRACT

The predicted rise in temperature and variable changes in ultraviolet-B radiation will have marked effects on plant growth and metabolism. Different vegetative parts of trees have been studied to detect the impacts of enhanced temperature and UV-B, but the effects on buds have rarely been considered. In the present study, *Salix myrsinifolia* clones were subjected to enhanced UV-B and temperature over two growing seasons starting from 2009, and measured springtime bud development and concentrations of phenolic compounds. In 2010 and 2011 the buds under increased temperature were up to 30% longer than those in control plots. On the other hand, UV-B combined with elevated temperature significantly decreased bud length by 4–5% in 2010. This effect was stronger in males than in females. The vegetative buds contained high constitutive amounts of chlorogenic acid derivatives, which may explain the weak increase in hyperin and chlorogenic acid that are usual UV-B sheltering compounds. The elevated temperature treatment significantly increased salicin content (about 18% in males and 22% in females), while triandrin concentration decreased by only 50% in females. Our results indicate that vegetative bud size is highly affected by seasonal temperature, while UV-B induced a weaker and transient effect.

1. Introduction

Generally, it is predicted that the global mean temperature (T) will rise by 3–4.5 °C by the end of this century due to the man-made greenhouse effect (Jacob et al., 2013; IPCC, 2013), which may have significant effects on plant growth and metabolism. In addition, climate change predictions suggest a potential delay in the recovery of the stratospheric ozone level and concomitant variable changes in ultraviolet radiation (UV-B) levels (e.g. Ballaré et al., 2011; Mckenzie et al., 2011). Thus the biosphere may still be exposed to unpredictable levels of UV-B over the growing seasons.

Elevated temperature and UV-B radiation can affect plant growth and secondary chemistry in different directions, and their combined effects can result in numerous responses (e.g. Nybakken et al., 2012; Randriamanana et al., 2014; Ballaré et al., 2011). As a natural phenomenon, plants need to acclimate to simultaneous changes of UV-B and temperature in their environments. One tool enabling plants to resist UV-B is the accumulation of UV-B absorbing phenolic compounds in the epidermis of leaves, which reduces UV-B penetration and thereby protects the leaf structures from damage (e.g. Day et al., 1992; Bornman and Sundby-Emanuesson, 1995). A general increase in protective compounds has also frequently been reported in response to UV-B in various other tissues (Warren et al., 2003; Virjamo et al., 2014; Lavola et al., 2013). Temperature, in addition to increased growth, also induces changes in defensive chemistry as well as in the expression of metabolite biosynthesis genes in plants (e.g. phenolics, fatty acids, antioxidant proteins) (e.g. Peñuelas et al., 2013; Sardans et al., 2011). For instance, it has been found that quercetin flavonoids are reduced by temperature enhancement in mature leaves of *Betula pendula* (Lavola et al., 2013), while decreases in total phenolics have been shown in *Salix myrsinifolia* leaves (Paajanen et al., 2011).

The dioecious shrub *S. myrsinifolia* (dark-leaved willow) belongs to the family *Salicaceae* and is an important food source for several insects and mammals (Argus, 2009). It is a northern representative of the *Salix*-genus, which consists of approximately 330–500

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woody species representing various life forms, such as trees, shrubs, prostrate plants and ground covers (e.g. Argus, 1997). *S. myrsinifolia* is a very common shrub in Finland, growing mainly in road ditches and marshy fields. It grows rapidly, and the leaves and bark contain various phenolic metabolites, including phenolic acids, salicylates, flavonoids and condensed tannins (e.g. Julkunen-Tiitto and Meier, 1992). Earlier studies with *S. myrsinifolia* under elevated temperature have shown gender-specific changes in *S. myrsinifolia*, e.g. in concentrations of chlorogenic acids (Nybakken et al., 2012) and luteolins (Nybakken and Julkunen-Tiitto, 2012).

The dormant buds of *S. myrsinifolia* are hairy and have a blunt apex bearing either generative or vegetative buds covered with a single scale (e.g. Saska and Kuzovkina, 2010). Understanding the phenological responses of a plant is a key aspect in unravelling the impact of climate warming (Lechowicz, 1995) and apical buds generally play an important role in the growth and development of the plant. As a winter-dormant species, the willow bud break in springtime is a critical phenological event that determines later plant growth and development during the growing season (Weih, 2009). Generally, bud break, or dormancy release, is considered to be the first appearance of growth (Hänninen, 1990). The bud break and bud set of *Populus tremula*, also a member of the *Salicaceae*, were significantly influenced by elevated UV-B and temperature (StrØmme et al., 2014). Moreover, early bud burst resulted in an increase in the plant biomass of *Salix viminalis* (Ronnberg-Wastljung and Gullberg, 1999). Although the defensive chemistry of leaves and stems are widely studied, little preference has been given to vegetative buds and even less to the possible effects of climate change on these parameters. Since previous studies reported significant effects of UV-B and temperature on growth, and on concentrations of some phenolic compounds in other plant organs, we expect that UV-B and temperature might also affect the growth and bud chemistry of *S. myrsinifolia*.

Studying the pattern of defensive chemistry in buds should be of particular ecological interest. In northern areas, the development of frost resistance of overwintering plant parts is of utmost importance. Moreover, buds and expanding tissues are particularly vulnerable to herbivores since they may have low levels of quantitative (lignins, tannins) and structural defences (e.g. Herms and Mattson, 1992). In addition, in deciduous species such as *Salix*, developing buds and new leaves are also more nutritious to herbivores than other plant parts, as substantial amounts of nutrients such as nitrogen, phosphorus and potassium are transported into these structures from the plant's stored reserves (Chapin et al., 1980). Male and female plants sometimes invest their resources differently (Sánchez-Vilas et al., 2012). Male plants often invest more in growth, while female plants tend to focus on reproduction (Lloyd and Webb, 1977).

Both sexes of dioecious species may also differ along abiotic stress gradients as well as with the availability of resources (Laporte and Delph, 1996). This leads us to speculate that the climate change responses of the sexes could differ, which could be of importance for future sex ratios and thus the possibilities for sexual reproduction and genetic diversity.

In the present study, our aim was to investigate the responses of the vegetative buds of *S. myrsinifolia* genders grown over two growing seasons in the field under enhanced T and UV-B. We wanted to answer the following questions: (1) Are there any individual or combined effects of enhanced temperature and UV-B on the development of vegetative buds during bud break? (2) How do the enhancements of temperature and UV-B radiation affect the defensive chemistry of vegetative buds? Our hypotheses were (1) Both temperature and UV-B may affect the development and chemistry of vegetative buds, (2) enhanced temperature treatment alone may have a stronger effect when compared with UV-B, and (3) Temperature may mask the effect of UV-B or combined treatment. In all cases, we expect variable responses in overall performance of *S. myrsinifolia* genders.

2. Materials and methods

2.1. Plant material

We planted cuttings (20 cm in length) of four male and female clones of *S. myrsinifolia* originating from the Joensuu and Kaavi districts (Eastern Finland). The cuttings were collected from individuals several kilometres apart to ensure that they belonged to different genotypes.

2.2. Experimental setup

The four male and four female *S. myrsinifolia* clones were planted in the experimental field located in the Botanical Garden, Joensuu, Finland (62°60′ N, 29°75′ E) in 36 plots (6 replicated plots for each treatment). The plants within each plot received one of the six different treatments and treatment combinations: increased temperature, enhanced UV-B, and enhanced UV-A, UV-B + T, UV-A + T and control with ambient temperature and UV radiation. Five cuttings of each clone were randomly planted in four rows in each treatment plot on June 17, 2009. At the beginning the cuttings were watered every day to enhance rooting. Each plot had an aluminium frame (1.5 × 2 m) which was attached to a metal post enabling height adjustments. The spacing between each plot was 3 m in all directions. The experimental site was fenced, using a 1.5 m fence to secure the field and a 2 mm metal sheet shelter to prevent the intrusion of any kind of animal underneath the fence.

The enhanced levels of temperature and UV radiation in the field were modulated to +2 °C and +30% compared to ambient, respectively. The achieved average levels of temperature and UV-B for 2009, 2010 and 2011 can be found in Nybakken et al. (2012) and Randriamanana et al. (2014). On each aluminium frame six 40 W UV-B-313-fluorescent lamps 1.2 m in length (Q-panel Co, Cleveland, Oh, USA) were mounted. Cellulose diacetate (0.115 mm, Koteloraurna, Finland) filters were wrapped around each UV-B lamp in order to reduce the radiation below 290 nm. Because the UV-B lamps emit small amounts of UV-A, we also used additional UV-A plots, where the UV-B lamps were wrapped with polyester film (0.175 mm, Kariplast, Finland) to exclude radiation below 315 nm (UV-A). To maintain equal levels of shading, UV-untreated plots were also provided with unenergized UV lamps.

The temperature enhancements were achieved by using two infrared heaters (CIR 105, FRICO, Partille, Sweden) fixed along the middle axis of the aluminium frames. In the temperature control plots the IR radiators were replaced by wooden boards of the same size, in order to maintain the same shading pattern. The frames were raised at regular time intervals to maintain a distance of 60 cm between the highest shoot tip and the radiators. In 2009 the temperature treatment started on June 15, the UV treatment on July 10, and both were stopped on September 15. In 2010, both temperature and UV treatments started on June 3 and ended on October 15, and in 2011 the treatments were started on May 24 and shut off on August 6. UV-B radiation was measured using four sensors (Thies Clima, Göttingen, Germany). These sensors measured the radiation between 250 and 325 nm, with a peak of 300 nm. Two sensors were placed above the control frames for ambient UV-B levels, and two were placed under the frames of the UV-B enhancement plots for set-point values. Self-made linear temperature sensors modulated the enhancement of temperature using four PT1000 probe elements each having a cable connected to four sensors. Two sensors were placed above the control frames, while two were placed under