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Impact of seasonal warming on overwintering and spring phenology of blackcurrant

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ABSTRACT

The rate of global warming varies on a seasonal timescale, with temperatures increasing faster in winter and spring than in summer or autumn. Temperature is a major driver of phenological events, but little information is available about the effects of non-uniform seasonal warming on plant development. Here we report phenological, metabolic and transcriptional responses of two *Ribes nigrum* cultivars to slightly elevated temperatures in winter and spring. Winter warming delayed floral bud break of 'Narve Viking', but advanced bud break of 'Zusha', implying genotypic differences in the magnitude of responses to warming during dormancy and in the heat accumulation phase in spring. Accordingly, more genes putatively associated with dormancy release, growth or development were up-regulated earlier in 'Zusha' than in 'Narve Viking' or showed significantly different transcript abundance in buds of plants at elevated or ambient temperatures. Primary metabolism of flower buds was largely unaffected by warming, but pronounced seasonal accumulation patterns of specific metabolites were evident. Especially bud break was associated with a dramatic remodeling of the metabolome, with differential seasonal regulation of metabolism between the two cultivars. Asymmetric warming did not increase the risk of freeze-induced damage to stems and flower primordia. The expression of several genes putatively related to freezing tolerance appeared coordinated during seasonal transitions in freezing tolerance. These data provide new insights into the timing of metabolic and transcriptional regulation during deacclimation, dormancy release and bud burst in a woody perennial, and contribute to our understanding of plant phenological responses to climate change.

1. Introduction

The Earth's mean global surface temperature is increasing and is projected to increase further in the foreseeable future (IPCC, 2013). Except for some tropical regions, meteorological records and climate model projections have shown that the rate of warming varies on a seasonal timescale, with temperatures increasing faster in winter and in spring than in summer or autumn (Xia et al., 2014). However, current research on plant responses to climate change remains seasonally biased towards the growing season and little effort has been directed towards understanding the effects of non-uniform seasonal warming on plant development (Liu et al., 2012; Ladwig et al., 2016). Temperature is a major driver of phenological events in temperate woody perennials (Fitter and Fitter, 2002; Parmesan, 2006), including chilling requirement for the transition from endodormancy to ecodormancy, induction of deacclimation with increasing temperatures and heat sum requirement to initiate bud break, which all takes place in winter and spring. Seasonal asymmetric warming is therefore likely to induce changes in most of these events. Proper timing of phenological events is especially important for perennial fruit crops where it plays a critical role in the selection of cultivars that are appropriate for a specific region and where the inability of a cultivar to adequately respond to environmental conditions may have severe consequences in terms of plant mortality, annual growth and fruit yield (Fu et al., 2012; Chung et al., 2013).

Woody perennials from temperate climate regions, such as blackcurrants (*Ribes nigrum*), develop endodormancy at the end of the growing season as an adaptive strategy to avoid growth and flowering under unfavorable conditions. Endodormancy is defined as the inability to initiate growth from meristems under growth promoting conditions

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and is gradually overcome by chilling (Rohde and Bhalerao, 2007). Thus, temperature has a dual effect on bud development. On the one hand, low temperatures are necessary to break bud dormancy and, on the other hand, higher temperatures are necessary to promote bud growth afterward. One likely impact of warming during dormancy is a delay in the fulfilment of chill requirements and consequently a delay in the time at which perennials become receptive to warm temperatures for spring growth (Luedeling et al., 2011). Insufficient chilling may additionally cause uneven bud break and a reduction in flowering and/or reproductive output of perennial fruit crops (Atkinson et al., 2013). Inversely, the advances in spring phenology, that have dominated climate-warming responses thus far, have been explained by increasing temperatures during ecodormancy leading to a more rapid fulfilment of the heat requirement (Cleland et al., 2007). Blackcurrant has a relatively high chilling requirement and is one of the fruit crops that is potentially at risk in parts of Europe due to the lack of winter chilling forecast in projections of future climatic conditions (Atkinson et al., 2013; Jones et al., 2013). This hypothesis is supported by a recent study, showing that seasonal asymmetric warming may specifically delay dormancy release of high-chilling requiring cultivars (Pagter et al., 2015).

Simultaneously with dormancy development, overwintering organs of temperate perennials increase their freezing tolerance through cold acclimation. Maximum freezing tolerance is reached mid-winter, while in spring plants lose acclimated freezing tolerance by deacclimation. Milder weather in winter and spring may decrease mid-winter freezing tolerance and directly accelerate the deacclimation process (Ogren et al., 1997; Taulavuori et al., 2004), increasing the risk of frost injury in buds and stems of deciduous species. Sugars and other compatible solutes exert cryoprotective properties and accumulate in overwintering organs during cold acclimation (Guy et al., 2008; Charrier et al., 2013). Consequently, one explanation for reduced mid-winter freezing tolerance and accelerated deacclimation at elevated temperatures is the consumption of soluble carbohydrates due to increasing respiration rates (Ögren, 1996; Taulavuori et al., 1997). During spring, these stored carbohydrate reserves support metabolic activity of flushing buds until new leaves develop (Cheng and Fuchigami, 2002). Warming-induced changes in carbohydrate metabolism may therefore also alter the availability of carbohydrates for spring growth. Consistent with studies of other woody perennials (Ögren, 1996; Taulavuori et al., 1997; Riikonen et al., 2013) elevated winter temperatures affect carbohydrate metabolism of blackcurrant stems, although without causing a decrease in freezing tolerance (Pagter et al., 2015). It remains largely unknown, however, whether warming alters biochemical responses associated with phenological traits other than carbohydrate metabolism.

The molecular events that regulate cold acclimation have been well studied and numerous reviews on this topic have been published (Welling and Palva, 2006; Fennell, 2014; Wisniewski et al., 2014; Shi et al., 2015). In comparison, little is known about the molecular mechanisms underlying deacclimation (Zuther et al., 2015). *CBF* genes, a sub-family of the *APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF)* transcription factors, play an integral, regulating role in both cold acclimation and deacclimation (Wisniewski et al., 2014; Zuther et al., 2015). Functional studies of *CBF* genes in woody plants indicate that their regulation and impact on abiotic stress resistance are more complex than in herbaceous plants and may include a role in the regulation of dormancy (Wisniewski et al., 2014). Cold acclimation is associated with induction of cold-regulated (*COR*) genes, which play a fundamental role in stress tolerance and are targets of CBF transcription factors (Jaglo-Ottosen et al., 1998; Welling and Palva, 2006). In particular, transcripts encoding LEA proteins or the corresponding proteins accumulate during endodormancy or in response to chilling (Welling and Palva, 2008; Ueno et al., 2013; Falavigna et al., 2014; Shin et al., 2015). Reconfiguration of carbohydrate metabolism in cold acclimating plants is correlated with the expression of genes encoding enzymes in the respective pathways, such as raffinose biosynthesis, consistent with regulation by transcriptional activation (Guy et al., 2008; Maruyama et al., 2009). A variety of genetic components that contribute to the intricate regulation of dormancy release, bud break and flowering have been reported (Rohde et al., 2007; Cooke et al., 2012; Falavigna et al., 2014; Howe et al., 2015). In blackcurrant, Hedley et al. (2010) identified three genes which co-localise with previously characterized blackcurrant bud break QTL and have probable roles in the dormancy release and bud break processes. They encode proteins with homology to acetyl CoA carboxylase, calmodulin-binding protein and beta-tubulin. The regulation of dormancy and freezing tolerance in woody perennials has received increasing attention in recent years. Nevertheless, there is still only limited information regarding the role of various genes associated with overwintering or the regulation of seasonal transitions in freezing tolerance and dormancy status. In addition, molecular knowledge of the potential effects of winter warming on the processes of deacclimation, dormancy release and bud break is completely lacking.

Blackcurrant is grown in a relatively small area, but is an economically high value crop that is increasingly recognized as a rich source of vitamin C and anthocyanins (Vagiri et al., 2012). In Europe, where blackcurrant is mainly grown, production is rising and there is increasing interest in expanding it to countries and regions that currently do not cultivate *R. nigrum* (Mitchell et al., 2011). We have previously shown that mild winter warming may alter phenological traits in blackcurrant, depending on genotype specific differences in chilling requirement (Pagter et al., 2015). Here, evaluation of the effects of asymmetric seasonal warming on phenological traits in blackcurrant flower buds was supplemented with GC–MS based metabolite profiling and expression analyses of endogenous genes homologous to genes associated with overwintering or spring phenology in other plant species. It was hypothesized that warming induced phenological changes are associated with alterations in primary metabolism and transcript levels of genes associated with overwintering and/or spring phenology and that the timing of metabolic and transcriptional regulation during deacclimation, dormancy release and bud burst varies between cultivars with different chilling requirements.

2. Materials and methods

2.1. Experimental set-up and plant material

The experimental area was established at the Department of Food Science, Aarhus University in Denmark (55° 18′ N 10° 26′ E) and consisted of a control plot (ambient temperature) and a warming plot (elevated temperature), which were adjacent to each other and had identical sizes (5 × 5.5 m). Warming was conducted from October 22nd 2014 to April 17th 2015 with 240 m of temperature-controlled heating cable (producing a maximum of 83 W m^{-2}) distributed between the plants at a distance of ca. 10 cm from the plants. The cables were resting on metal holders five cm above the soil surface. The air temperature in the plots was monitored with Pt100 temperature sensors covered by radiation shields at 20 cm, 50 cm and 80 cm above the soil surface. Temperature means over five-min intervals were logged throughout the treatment period. Whenever the temperature at 20 cm in the warmed plot was more than 2 °C higher than the corresponding temperature in the control plot the heating cables were switched off until the temperature difference fell below 2 °C. The soil temperature was measured in each plot at a depth of ca. 15 cm using Tinytag Talk temperature loggers (Gemini Data Loggers, Chichester, England). From October onwards, after leaf abscission, both plots were covered with a transparent polyethylene net tunnel providing some wind shelter. The net had a shading effect of 15% and was removed during bud break on April 17th. The net tunnel allowed the plants to be exposed to the natural rainfall.

The experiment was carried out using two-year old vegetatively