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High temperatures limit plant growth but hasten flowering in root chicory (*Cichorium intybus*) independently of vernalisation

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ABSTRACT

An increase in mean and extreme summer temperatures is expected as a consequence of climate changes and this might have an impact on plant development in numerous species. Root chicory (*Cichorium intybus* L.) is a major crop in northern Europe, and it is cultivated as a source of inulin. This polysaccharide is stored in the tap root during the first growing season when the plant grows as a leafy rosette, whereas bolting and flowering occur in the second year after winter vernalisation. The impact of heat stress on plant phenology, water status, photosynthesis-related parameters, and inulin content was studied in the field and under controlled phytotron conditions. In the field, plants of the Crescendo cultivar were cultivated under a closed plastic-panelled greenhouse to investigate heat-stress conditions, while the control plants were shielded with a similar, but open, structure. In the phytotrons, the Crescendo and Fredonia cultivars were exposed to high temperatures (35 °C day/28 °C night) and compared to control conditions (17 °C) over 10 weeks. In the field, heat reduced the root weight, the inulin content of the root and its degree of polymerisation in non-bolting plants. Flowering was observed in 12% of the heat stressed plants during the first growing season in the field. In the phytotron, the heat stress increased the total number of leaves per plant, but reduced the mean leaf area. Photosynthesis efficiency was increased in these plants, whereas osmotic potential was decreased. High temperature was also found to induced flowering of up to 50% of these plants, especially for the Fredonia cultivar. In conclusion, high temperatures induced a reduction in the growth of root chicory, although photosynthesis is not affected. Flowering was also induced, which indicates that high temperatures can partly substitute for the vernalisation requirement for the flowering of root chicory.

Introduction

The global climate change that is expected to occur during the next few decades will have an impact on crop yields as a consequence of the increased surface temperature (Peng et al., 2004). Heat stress can cause an array of morpho-anatomical, physiological and biochemical changes in plants (Wahid et al., 2007) and can drastically affect the plant phenology (Wollenweber et al., 2003; Snider et al., 2009). It is thus important to evaluate the impact of temperature increases on the development and yields of crop plants. Root chicory (*Cichorium intybus* L.) is a major crop in north-western Europe, where increases in the mean and extreme summer temperatures are expected as a consequence of climate changes.

Root chicory is a biennial species that produces a tap root and a leaf rosette during the first year of growth. The root accumulates inulin, which consists of one terminal α-glucose and a variable number of β-fructose moieties exclusively linked by 2 → 1 bonds (Hendry, 1993; Vergauwen et al., 2003). These stored reserves are mobilised by the plant during the second year to sustain the reproductive phase of development. Inulin is used in many applications by the food and non-food industries (Stevens et al., 2001) and has

Abbreviations: A, instantaneous CO_2 assimilation; Chl*a*, chlorophyll a; Chl*b*, chlorophyll b; *CiFL1*, *Cichorium intybus FLC-LIKE1*; DP, mean inulin degree of polymerisation; DW, dry weight; *E*, instantaneous transpiration; 1-FEH, fructan 1-exohydrolase (EC 3.2.1.153); 1-FFT, 1-kestose fructan:fructan 1-fructosyltransferase (EC 2.4.1.100); FM2, fluorescence monitoring system II; *FLC*, *FLOWERING LOCUS C*; *FLM*, *FLOWERING LOCUS M*; Fs, steady state level of fluorescence; FW, fresh weight; IP, inulin percentage; φ_{PSII}, photosystemII efficiency; g_s, leaf stomatal conductance; NPQ, non-photochemical-quenching; PAR, photosynthetically active radiation; qP, photochemical quenching; WC, water content; Ψs, osmotic potential.

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also been shown to have numerous health-promoting properties (Roberfroid and Delzenne, 1998; Kaur and Gupta, 2002). These plants are therefore harvested at the end of the first growing season, with the optimal date being defined on the basis of the total root yield and qualitative aspects related to the degree of fructan polymerisation (Wilson et al., 2004).

Bolting in root chicory is controlled by low temperatures and this species requires a defined period of vernalisation for the initiation of the flowering stalk and for this stem elongation (Harrington et al., 1959; Dielen et al., 2005). As a consequence, precocious sowing in early spring should be avoided, as the low temperature might induce flowering during the first year of plant development, thus diverting the sugars from the root accumulation and lowering the final inulin yield. Resistance to bolting is genetically controlled, and is thus an important trait used by plant breeders for the selection of new cultivars (Baert and Van Bockstaele, 1993).

Dielen et al. (2005) also reported that there is bolting in root chicory fields during hot summers, which has suggested that heat might also induce bolting, independent of vernalisation. Numerous studies have been devoted to the impact of cold stress and drought on the physiological behaviour of root chicory during its vegetative phase of development (Schittenhelm, 1999; De Roover et al., 2000; Monti et al., 2005; Mingeot et al., 2009; Devacht et al., 2009; Vandoorne et al., 2012), but surprisingly, the impact of high temperatures on root chicory growth and development has not yet been systematically studied.

In the present study, the impact of high temperatures was analysed in the field and under controlled conditions in the phytotron. The root chicory growth, water status, sugar content, and photosynthesis-related parameters were followed throughout the exposure to heat under both conditions (i.e. field, phytotron), and the inulin yield was analysed after this heat stress in the field. The rate of flowering was also monitored throughout the growing period, under both conditions. Comparisons between two root chicory cultivars that differ in their sensitivities to bolting was also carried under the controlled conditions.

Materials and methods

Plant material and growing conditions

The trial fields were located at Pecq, Belgium (50°40′N–3°25′E) and had a sandy silt soil that was fertilised (N–P–K 4–5–24 + MgO:8) at a rate of 750 kg ha^{-1} before sowing. The seeds of the root chicory (*Cichorium intybus* L. var *sativum*) cultivar Crescendo (Chicoline, Cosucra Warcoing S.A., Belgium) were sown at the end of March in lines, at 15 cm intervals, and with a line-to-line distance of 45 cm. Fifty days after sowing, greenhouses with in a metal framework and plastic sheeting were assembled in the fields. The greenhouses were 4.5 m × 4.5 m and covered eigth plant lines. The control greenhouses remained open along the edges, while the greenhouses for the heat treatment remained completely closed, to provide higher temperatures during the day than for the controls. The temperatures and relative hymidity were recorded in each greenhouse every 15 min, using a data logger (Hobbo®, Onset Computer Corporation, Bourne, MA, USA). These experiments were conducted in the field in 2006 and 2009, and they gave similar trends; thus for the sake of clarity, only the data from 2006 are presented here. The mean, maximum, and minimum temperatures, and relative humidity per week are shown in Fig. 1. The mean and maximum temperatures were higher in the heat-treated greenhouses than in the control greenhouses throughout the experimental period (Fig. 1A and C). The mean and maximum relative humidities were similar in the control and heat-treated greenhouses (Fig. 1D and F), while the minimum relative humidity was slightly higher in the control greenhouses. The mean photosynthetically active radiation (PAR) in the greenhouses fluctuated between 150 and 450 μmol m^{-2} s^{-1}, depending on the external climatic conditions, although it was always similar in the control and heat-treated greenhouses. Drip irrigation was provided throughout the growing season to avoid any water stress.

Seeds of root chicory cultivars Crescendo (Chicoline, Warcoing S.A., Belgium) and Fredonia (SAREA, Austria) were used for the experiments under controlled conditions in the phytotron. Two independent experiments were performed that gave similar results, so again we present here only the data from one experiment. Crescendo is classified as resistant to vernalisation, while Fredonia is prone to premature flowering and is thus considered as sensitive to vernalisation (Dielen et al., 2005). These seeds were sown on substrate containing sand and loam (3:1, v/v) and incubated in a growth chamber under a constant temperature of 17 °C at 70% relative humidity, with a photoperiod of 16 h light with a mean PAR of 300 μmol m^{-2} s^{-1}, provided by lighting (Sylvania VHO 250 W). Three weeks after sowing, the young seedlings were transplanted in pots containing 2 L of the same substrate. Four weeks after sowing, when the seedlings were at four-leaf stage, half of the plants were maintained in the phytotron under control conditions, with a constant temperature of 17 °C and a photoperiod of 16 h light while the other half were exposed to high temperatures, at 35 °C, during the photoperiod of 16 h light, and with a night temperature of 28 °C (8 h). In both cases, the plants were maintained at 70% relative humidity and exposed to 300 μmol m^{-2} s^{-1} PAR from the top of the canopy (provided by Sylvania VHO 250 W lamps). The total duration of the experimentation was 14 weeks.

Plant growth and development

For the experiment in the phytotron, the numbers of rosette leaves were recorded every two weeks for six plants per treatment, and the data were used to calculate the plastochron. The area of the youngest fully expanded leaf was measured with a leaf-area meter (ADC, Bioscientific Ltd.) every two weeks, on six plants per treatment. The same analyses were carried out in the field once a month. The visually detectable bolting plants were recorded regularly in the field and in the phytotron.

The physiological measurements taken for the root chicory maintained in the phytotron included water status, photosynthesis-related parameters, and total soluble sugars, and these were performed on the youngest fully expanded leaf every two weeks, for six plants per treatment. For the root chicory in the field, the water status, total soluble sugars contents, and chlorophyll fluorescence were determined on the same leaf once a month. At the end of the experimental period in the field, the root-yield-related parameters (root weight, inulin percentage, and mean inulin degree of polymerisation) were measured.

Plant water status

The central vein of the youngest fully expanded leaf was discarded, and a portion of the lamina was cut into small segments that were placed into microcentifuge tubes that were perforated with four small holes; these samples were immediately frozen in liquid nitrogen. After being encased individually in a second intact microcentrifuge tube, the samples were left to thaw for 30 min and centrifuged at 15,000 × *g* for 15 min at 4 °C. The collected tissular sap was analysed in terms of its osmotic potential (Ψs) estimation. The osmolarity (c) was also assessed, using a vapour pressure osmometer (Wescor 5500). This was then converted from mosmoles kg^{-1} to MPa, using the formula: $\Psi s\,(MPa) = -c\,(\text{mosmoles kg}^{-1}) \times 2.58 \times 10^{-3}$ according to the Van't Hoff equation (Lefèvre et al., 2009). This osmotic potential of the heat-treated plants was adjusted to the water content of the control plants, according to Lefèvre et al. (2009): $\Psi s_{adjusted}\,(MPa) = \Psi s$