

Article

Role of Tuber Developmental Processes in Response of Potato to High Temperature and Elevated CO₂

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Abstract: Potato is adapted to cool environments, and there is concern that its performance may be diminished considerably due to global warming and more frequent episodes of heat stress. Our objectives were to determine the response of potato plants to elevated CO₂ (700 μmol/mol) and high temperature (35/25 °C) at tuber initiation and tuber bulking, and to elucidate effects on sink developmental processes. Potato plants were grown in controlled environments with treatments at: Tuber initiation (TI), during the first two weeks after initiating short-day photoperiods, and Tuber bulking (TB). At TI, and 25 °C, elevated CO₂ increased tuber growth rate, while leaves and stems were not affected. Whole-plant dry matter accumulation rate, was inhibited by high temperature about twice as much at TI than at TB. Elevated CO₂ partially ameliorated high temperature inhibition of sink organs. At TI, with 25 °C, elevated CO₂ primarily affected tuber cell proliferation. In contrast, tuber cell volume and endoreduplication were unaffected. These findings indicate that the TI stage and cell division is particularly responsive to elevated CO₂ and high temperature stress, supporting the view that attention should be paid to the timing of high-temperature stress episodes with respect to this stage.



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Keywords: *Solanum tuberosum*; tuber; sink organ; ambient temperature; cell proliferation

1. Introduction

Atmospheric CO₂ levels and average global temperature have risen in the last decades and earth air temperature is predicted to continue to increase as a result of the rise in the levels of CO₂ and other greenhouse gases [1,2]. While it is recognized that these trends will impact crop production and food security [3], crop growth models of potato (*Solanum tuberosum*) response to environmental variables, suggest that our understanding of the effects of elevated atmospheric CO₂ and temperature remains far from complete and in need of improvement [4]. Findings from eight global climate models that were used to simulate potato agronomic and climate responses indicate that model variation was modest for predicted CO₂ but was particularly uncertain for temperature [5]. Furthermore, climate predictions indicate that accompanying an increase in global average temperatures of 1.5 °C, will be a sharp rise in the likelihood of extreme heat stress events [6].

Atmospheric CO₂ enrichment can affect potato growth and productivity by directly increasing leaf photosynthesis, in accordance with leaf photosynthetic CO₂ response, and also by eliciting partial stomatal closing and increasing water use efficiency [7–9]. In addition, altered rates of photosynthate availability can modify partitioning of photosynthate among plant parts, which in turn can have feedback effects on photosynthesis [10–12]. These effects may lessen the potential benefit of elevated CO₂ on potato photosynthesis, as has been found in season-long free air CO₂ enrichment (FACE) [11,13,14]. In potato, elevated CO₂ can lead to greater stimulation of tuber development than above-ground plant parts such that harvest index is increased [15].

Consistent with its Andean origin, potato is a heat-sensitive crop, which performs best at relatively cool temperatures [3]. For example, tuber dry matter partitioning in the temperate-adapted cultivar Katahdin was decreased 65% when day/night temperature was increased from 24/15 °C (max/min) to 30/21 °C [16]. Increases in temperature have the potential of affecting growth and development in several ways. High temperature inhibits photosynthesis in potato, as is generally the case in cool-adapted C3 plants [17]. In support of this explanation, some studies have reported that above-ground temperature has a greater effect than either root or stolon temperature on tuber growth [18]. Moreover, studies to determine the cause of poor yield of heat-susceptible potato genotypes grown at high temperature suggest it is related to inhibited photosynthesis and insufficient availability of the transportable sugar, sucrose [19]. In addition, warm temperature can have direct effects that inhibit tuber initiation and growth [20,21], thereby diverting photosynthate from tubers to shoots [15,22,23]. High temperature in the tuber zone inhibits tuber development [24], and studies have indicated that below-ground high temperature has greater effects than above-ground temperature due to inhibition of tuber development and sink strength, which shifts partitioning to above-ground shoots [25]. Studies have indicated that the negative effects of high temperature on potato growth and yield is dependent on the growth stage and is especially great at the early stages of tuber growth [26].

Potato is adapted to relatively cool growing environments, and it has been predicted that its yield will diminish due to global warming and more frequent episodes of heat stress [3]. It is possible that elevated atmospheric CO₂ concentration, may partially ameliorate negative effects of heat stress by enhancing photosynthesis and lessening negative effects on potato tuber growth. Our objectives were to determine the response of potato plants to elevated CO₂ and high temperature treatment at tuber initiation and tuber bulking, and to elucidate effects on sink developmental processes, including tuber cell division and expansion, levels of sugars, and certain carbohydrate-metabolizing enzymes. Our findings indicate that cell proliferation is particularly responsive to elevated CO₂ and high temperature treatment, and that elevated CO₂ partially ameliorates the effects of high temperature.

2. Results

2.1. The Effects of High Temperature and Elevated CO₂ on Potato Plant Growth

2.1.1. Tuber Initiation Stage

Dry matter accumulation rate (DMAR) during each treatment period was estimated from the difference in dry matter of plants harvested at the onset of a treatment, and those harvested 2 weeks later at the conclusion of the treatment. At the tuber initiation stage, high temperature (35 °C) substantially decreased tuber growth compared to normal temperature (25 °C) in both low (Low_{CO₂}) and elevated (Elev_{CO₂}) CO₂ environments (Figure 1). High temperature under Low_{CO₂} decreased tuber DMAR by 82%, and had similar effects (about 60% decrease) on DMAR of leaves and stems. Under Elev_{CO₂}, high temperature decreased DMAR somewhat less: by 63% in tubers and by about 25% in leaves and stems. On a whole plant basis (sum of the three plant parts), high temperature decreased DMAR by 56 and 35% in plants at Low_{CO₂} and Elev_{CO₂}, respectively (Table 1). In contrast to temperature, elevated CO₂ provided distinctly greater benefit to tubers compared to other plant parts. Elevated CO₂ at 25 °C increased DMAR 60% in tubers, but did not affect DMAR in leaves and stems. At 35 °C, elevated CO₂ appeared to increase DMAR, and thereby partially ameliorate high temperature inhibition, in all three plant parts, but did not achieve statistical significance ($p \leq 0.05$).

2.1.2. Tuber Bulking Stage

At the tuber bulking stage, tuber growth predominated, as there was negligible leaf and stem growth (Figure 2). Elevated CO₂ increased tuber DMAR about 30 to 40% at both temperatures (Figure 2), and this was reflected in whole plant DMAR (the sum of tuber, leaf and stem) (Table 1). Comparing across growth stages, high temperature had greater effects on whole plant DMAR (Table 1) at the tuber initiation stage than at the

tuber bulking stage (overall about -45 and -24% , respectively). In contrast, the effects of elevated CO_2 on whole-plant DMAR were fairly similar at the two stages. Elevated CO_2 at 25°C increased whole plant DMAR by 29% at tuber initiation and by 34% at tuber bulking. Given that whole plant DMAR is a measure of net whole-plant photosynthesis in excess of respiration, this indicates that elevated CO_2 provided consistent benefit throughout these phases of development. The main difference for CO_2 effects across development stages was the case with high temperature during tuber initiation, where elevated CO_2 benefitted leaf and stem growth in addition to tuber growth, whereas only tubers benefitted at the tuber bulking stage. This suggests that growing plant parts have a development window within which they are capable of responding to improved photosynthate supply.

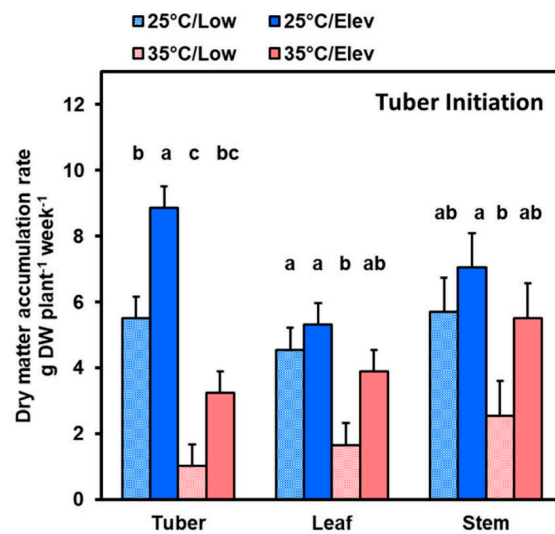


Figure 1. Tuber, leaf, and stem dry matter accumulation rate at the tuber initiation stage in plants that were given temperature (25 or 35°C) and CO_2 (Low CO_2 or Elev CO_2) treatments during the 2-week tuber initiation period. To initiate tuberization, plants that were grown in long days were transferred to short-day growth chambers and exposed to treatments for 2 weeks, then harvested. Bars represent averages \pm SEM of 6 replicates; values labeled with different letters are significantly ($p \leq 0.05$).

Table 1. Total plant dry-matter accumulation rate during temperature and CO_2 treatments at the tuber initiation or tuber bulking stage. CO_2 treatments were Low and Elevated (Elev). The pooled standard error of the design (SE) are shown ($n = 6$); values labeled with different letters are significantly ($p \leq 0.05$) different.

Treatment		Stage of Development			
		When Treatment Was Imposed			
temperature	atm CO_2	Tuber Initiation		Tuber Bulking	
		dry-matter accumulation rate			
$^\circ\text{C}$	$[\text{CO}_2]$			$\text{g DW plant}^{-1} \text{wk}^{-1}$	
25	Elev	24.3	c	37.7	c
25	Low	18.9	cb	28.0	b
35	Elev	15.8	ba	31.9	cb
35	Low	8.3	a	19.0	a
SE		1.6		1.8	

2.2. Tuber Numbers and Size

High temperature at the tuber initiation stage substantially decreased the number of tubers per plant (-37% at Low CO_2 , and -53% at Elev CO_2) (Figure 3). Plants receiving treatments during tuber bulking were exposed to 25°C and Low CO_2 during their tuber

initiation stage, and they did not further increase their tuber numbers during tuber bulking. Hence, there were no treatment effects on tuber numbers at tuber bulking (Figure 3). Elevated CO₂ did not affect tuber number at either stage. Histograms of the distribution of biomass among individual tubers at tuber initiation and tuber bulking, appeared to show that temperature × CO₂ treatment interactions affected the proportions of tubers in various size classes (Supplementary Figure S1).

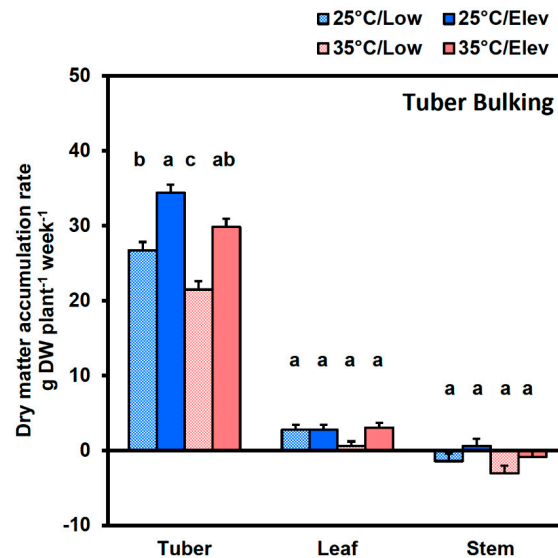


Figure 2. Tuber, leaf, and stem biomass accumulation rate at the tuber bulking stage following temperature (25 or 35 °C) and CO₂ (LowCO₂ or ElevCO₂) treatments during the 2 week tuber bulking stage. Plants were given two weeks of shortday conditions at 25 °C with low CO₂ to induce tuberization, then maintained at short days and exposed to treatments for 2 weeks, and then harvested. Bars represent averages ± SEM of 6 replicates; values labeled with different letters are significantly ($p \leq 0.05$) different.

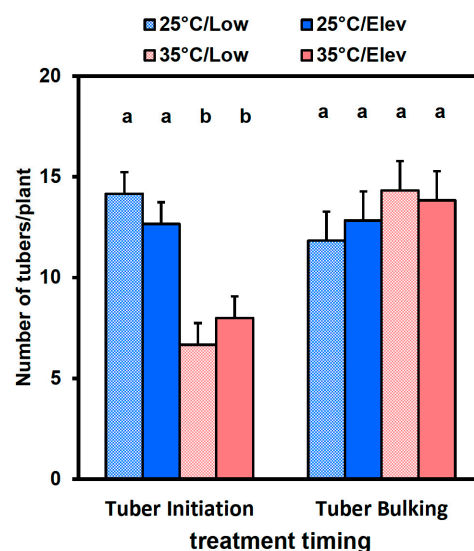


Figure 3. Tuber number in response to temperature (25 or 35 °C) and CO₂ (LowCO₂ or ElevCO₂) treatments at stages of tuber initiation and tuber bulking. Plants were harvested at 2 weeks after starting short-day exposure (tuber initiation period), or 4 weeks after starting short-day exposure and receiving temperature and CO₂ treatments for the latter 2 weeks of the period (tuber bulking period). Tubers exceeding 1 cm diameter were counted. Bars represent averages ± SEM of 6 replicates; values labeled with different letters are significantly ($p \leq 0.05$) different.

2.3. The Effects of High Temperature and Elevated CO₂ on Potato Tuber Cell Properties

Given that plant growth responses to CO₂ and temperature treatments primarily involved changes in tuber growth, we determined the extent to which each aspect of tuber growth was altered. Three aspects were evaluated: (1) cell division, which we evaluated by counting cell nuclei with flow cytometry; (2) cell expansion growth, which we evaluated by calculating average cell volume; and (3) post-mitotic DNA replication (endoreduplication), which we evaluated from the nuclear DNA analysis as the count of nuclei with DNA content $\geq 8C$ (where C is the haploid DNA content). The data in Figures 4 and 5 are shown as a proportion of the LowCO₂/25 °C control so that treatment effects on various tuber properties can be readily compared. At tuber initiation (Figure 4), high temperature substantially decreased tuber fresh weight, and correspondingly, total tuber cell number per plant. Elevated CO₂ significantly ($p \leq 0.05$) increased cell proliferation at 25 °C, while at 35 °C the number of cells produced was very low and effects of elevated CO₂ could not be discerned (Figure 4). In contrast, cell volume was not affected by CO₂ and temperature treatments, except at high temperature and elevated CO₂, where average cell volume was increased. The extent of endoreduplication, which reflects nuclear DNA reduplication in the absence of intervening mitosis, was not affected by treatments. Similarly, at tuber bulking (Figure 5), elevated CO₂ and high temperature affected cell proliferation in some treatment combinations, and at elevated CO₂ and temperature cell volume increased; however, the magnitude of treatment effects was less than during tuber initiation.

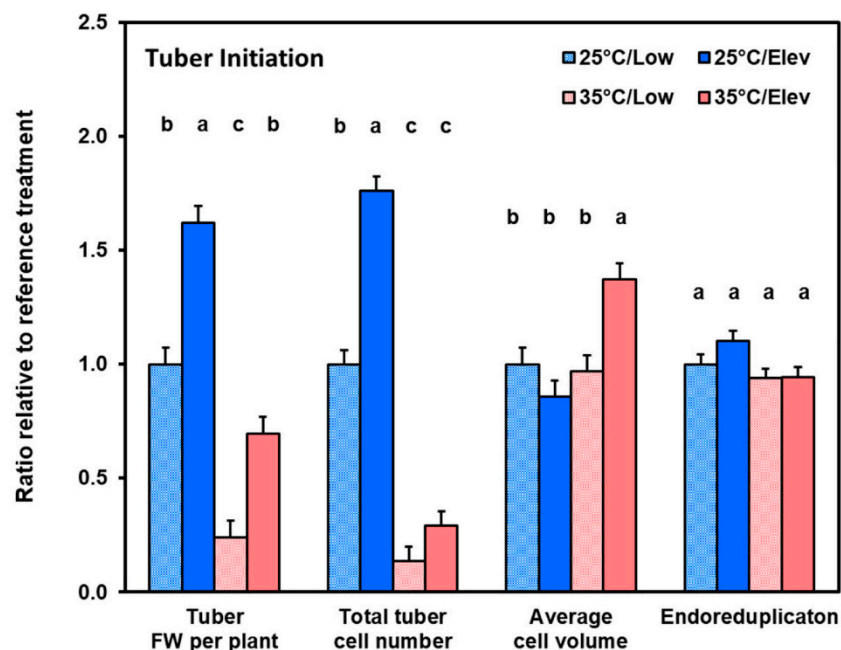


Figure 4. Relative increase in tuber properties with respect to 25 °C/LowCO₂ control in response to temperature (25 or 35 °C) or CO₂ treatments (LowCO₂ or ElevCO₂) at the tuber initiation stage. Tuber weight and tuber cell number were summed for each plant and represent the increments of tuber fresh-weight growth and cell proliferation during the treatment periods. Data are expressed relative to the 25 °C/LowCO₂ reference which was: 80.5 g tuber fresh weight plant⁻¹, 1.25×10^8 tuber cells plant⁻¹, $7.9 \times 10^5 \mu\text{m}^3$ average tuber cell volume, and 31.3% average proportion of tuber cells in endoreduplication categories ($\geq 8C$ nuclear DNA content). See Materials and Methods for details. Bars represent averages \pm SEM of 6 replicates; values labeled with different letters are significantly ($p \leq 0.05$) different.

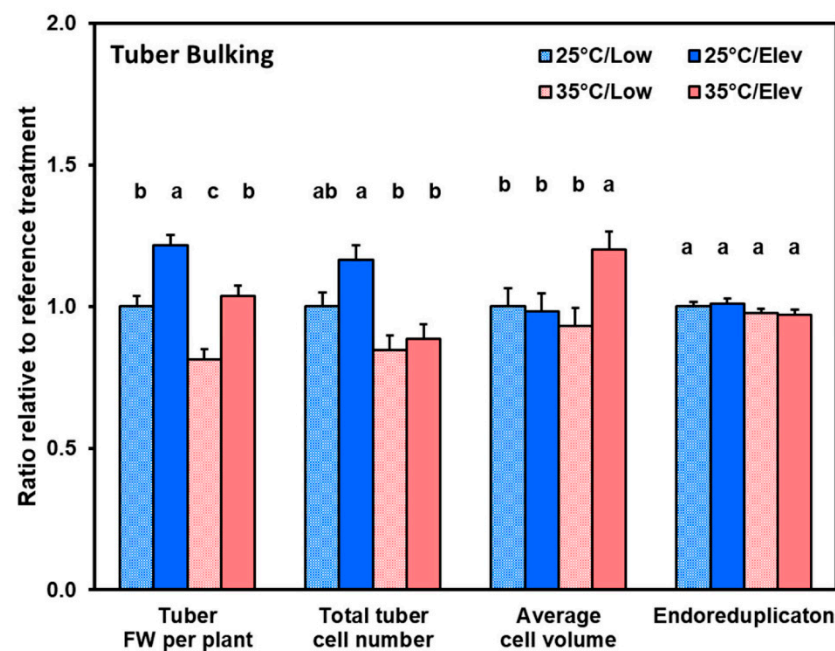


Figure 5. Relative increase in tuber properties with respect to 25 °C/Low_{CO₂} control in response to temperature (25 or 35 °C) or CO₂ treatments (Low_{CO₂} or Elev_{CO₂}) at the tuber bulking stage. Tuber weight, and tuber cell number were summed for each plant and represent the increments of tuber fresh-weight growth and cell proliferation during the treatment periods. Data are expressed relative to the 25 °C/Low_{CO₂} reference which was: 529 g tuber fresh weight plant⁻¹, 6.12 × 10⁸ tuber cells plant⁻¹, 1.17 × 10⁶ μm³ average tuber cell volume, and 48.2% average proportion of tuber cells in endoreduplication categories (≥8C nuclear DNA content). See Materials and Methods for details. Bars represent averages ± SEM of 6 replicates; values labeled with different letters are significantly ($p \leq 0.05$) different.

2.4. The Effects of High Temperature and Elevated CO₂ on Sugar Levels and Invertases

2.4.1. Sugars

Given the evidence that cell proliferation in tubers responded substantially to treatments, we considered the hypothesis that CO₂ and temperature treatments could affect photosynthate supply or consumption in the phloem-rich zone of tubers. Phloem-containing perimedullary-zone tissues were sampled to provide an indication of carbohydrate status in the region of abundant phloem and rapid cell proliferation. At the tuber initiation stage with elevated CO₂, high temperature treatment decreased hexose (glucose + fructose) concentrations (Figure 6a). A decrease in sugar concentration would be expected if high temperature increases the rate of metabolism and use of sugar. However, high temperature increased sucrose concentration at tuber initiation (Figure 6b), suggesting that high temperature did not have a net effect on sugar import/utilization, but might affect conversion of one form to the other. There were no treatment effects on sugars at the tuber bulking stage. Trends in sugar concentrations were toward higher values in elevated CO₂, though the differences were not significant ($p \leq 0.05$). This finding suggests that the conversion of sucrose to hexoses might be affected by the treatments.

2.4.2. Invertases

To evaluate whether treatments affected enzymes for converting imported sucrose into hexoses, we measured cell-wall-bound (CWB) and soluble invertase activities in tubers. Activities of CWB and soluble invertases were analyzed in perimedullary zones where phloem is abundant and is delivering photosynthate to growing tissues. In the elevated CO₂ treatment at tuber initiation, and at both CO₂ treatments at tuber bulking, CWB invertase activity was slightly decreased in response to high temperature (Figure 7a). Though slight,

the direction of this inhibition is consistent with it playing a role in the observed increase in sucrose and decrease in hexose in response to high temperature (Figure 6a,b). In contrast, at tuber initiation with low temperature, the elevated CO₂ treatment increased both CWB invertase and soluble invertase activity (Figure 7a,b). The direction of this stimulation of invertase activity is consistent with it playing a role at low temperature for maintaining low sucrose and a tendency for higher hexose in response to elevated CO₂ (Figure 6).

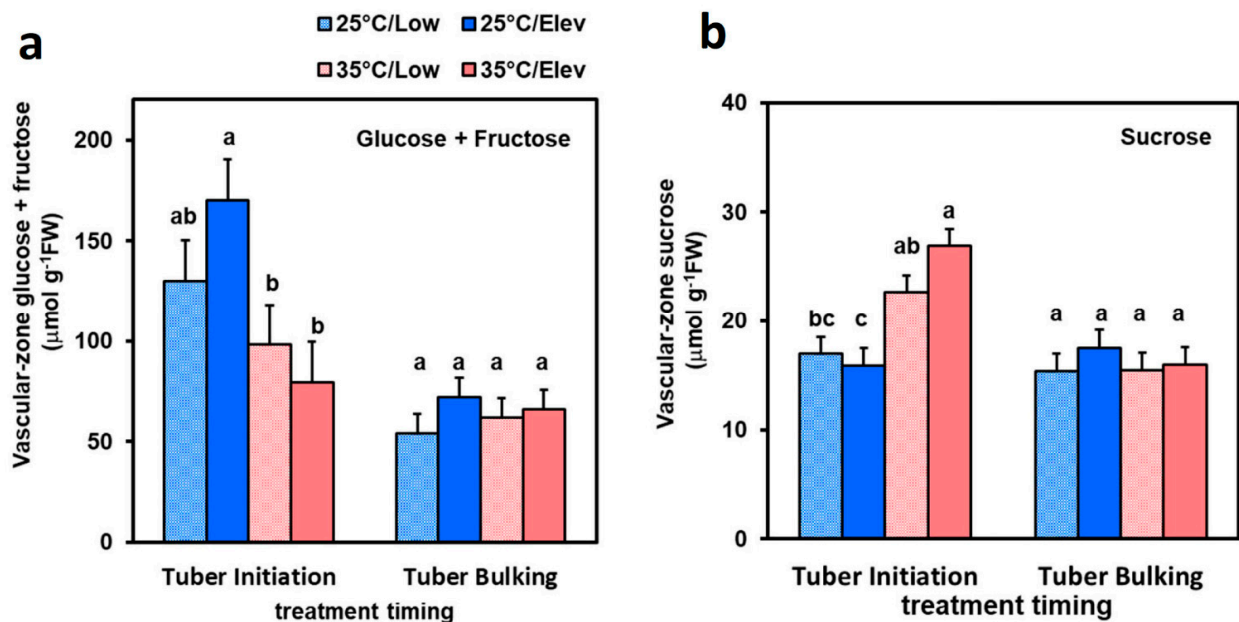


Figure 6. Tuber phloem-rich perimedullary-zone hexose (glucose + fructose) (a) and sucrose (b) in plants exposed to temperature (25 or 35 °C) or CO₂ (LowCO₂ or ElevCO₂) treatments at the tuber initiation stage (left of each figure) or tuber bulking stage (right). Plants were harvested after exposure to treatments for 2 weeks. Bars represent averages ± SEM of 6 replicates; values labeled with different letters are significantly ($p \leq 0.05$) different.

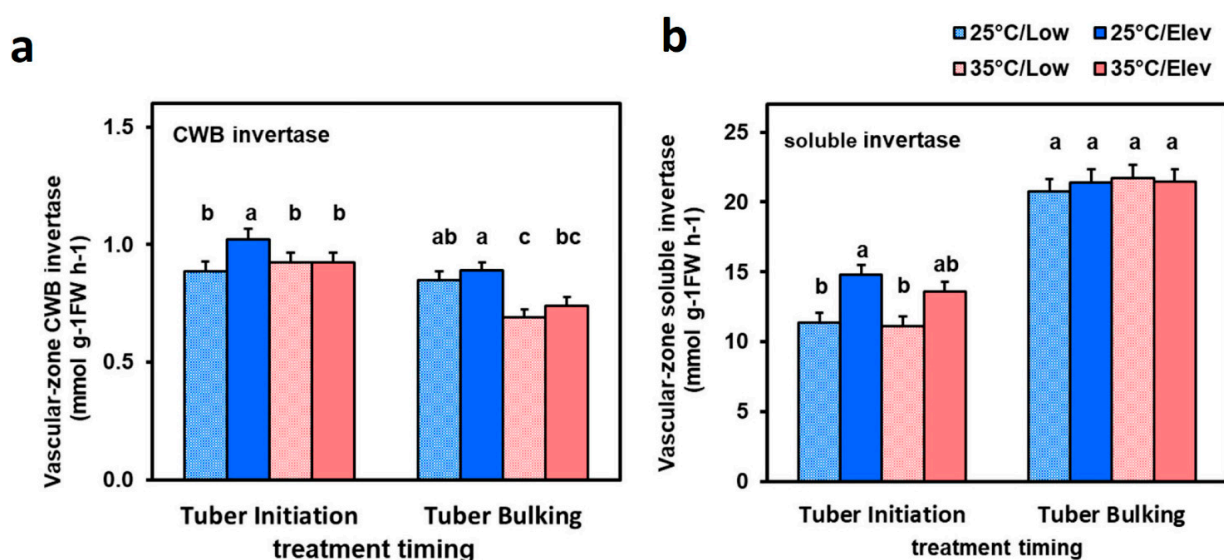


Figure 7. Tuber phloem-rich perimedullary-zone (a): cell wall bound (CWB) acid invertase activity and (b): soluble acid invertase activity in plants exposed to temperature (25 or 35 °C) or CO₂ (LowCO₂ or ElevCO₂) treatments at the tuber initiation stage (left of each figure) or tuber bulking stage (right). Plants were harvested after exposure to treatments for 2 weeks. Bars represent averages ± SEM of 6 replicates; values labeled with different letters are significantly ($p \leq 0.05$) different.

3. Discussion

3.1. Elevated Atmospheric CO₂ Effect on Whole Plant Biomass Accumulation

The current findings indicated that two-week treatments involving elevated atmospheric CO₂ concentration at 25 °C increased dry matter accumulation rate by about 30%. This is consistent with previous findings involving short-term elevated CO₂ treatments which have been conducted in growth chambers and controlled environments [27,28]. However, studies involving long-term exposure to elevated CO₂ in open-top and FACE experiments have indicated that such increases in whole-plant photosynthesis are not generally sustained, such that benefit from CO₂ enrichment is typically only about half as much in the long term [11,13,14]. Several factors are involved in this phenomenon, among them partial stomatal closing, limitations in nitrogen supply, and acclimation as plants adjust their development of sink organs in response to an incremental increase in photosynthate availability, and feedback inhibition of photosynthesis [7,9,29]. One of the objectives of the current study was to determine the extent to which potato plants respond to short-term elevated CO₂ by stimulating growth of sink organs: tubers, stems, and expanding leaves. In previous full-season studies of potato exposed to CO₂ enrichment with open-top chambers, elevated CO₂ increased tuber growth and yield, and tubers were preferentially favored, as the partitioning ratio of below-ground to above-ground biomass was increased [15]. Similar findings have been reported for experiments in growth chambers where precise control of the timing of treatments can be achieved [30]. Consistent with this, the current study indicated that elevated CO₂ at 25 °C during tuber initiation increased tuber dry matter accumulation rate (DMAR) by more than 60%, while leaf and stem DMAR was not significantly ($p \leq 0.05$) affected, even though these organs collectively represented almost two thirds of the sink-organ DMAR (Figure 1). At the tuber bulking phase, when most of plant photosynthate is used for starch storage in tubers, leaves had very low DMAR and stems had a net loss in dry matter, so almost all the benefit from elevated CO₂ was obtained by tubers.

3.2. High Temperature Stress Effect on Whole Plant Biomass Accumulation

Potato grows optimally in cool climates (15 to 22 °C) whereas its growth is inhibited in warm or hot environments [10,21,31]. Heat stress can exert detrimental effects on many growth and development processes, so the current studies, involving 2-week treatments at two discrete developmental stages, provide information on the relative susceptibilities of various developmental processes in tubers. Whole-plant dry matter accumulation rate, a measure of whole plant photosynthesis in excess of respiration, was inhibited by high temperature about twice as much at the stage of tuber initiation than at tuber bulking (Table 1; Figures 1 and 2). Similar findings were obtained in studies of elevated temperature under field conditions with temporary transparent chambers in which temperature was increased by about 4 °C for discrete periods of time [32,33]. Moreover, studies that involved treatments with 35 °C stress, similar to the present investigation, showed that tuber growth was strongly decreased by exposure to 35 °C at early phase of tuberization but had a diminishing effect at later stages [26]. In contrast, in a greenhouse study to test the effect of high temperature (30 °C) before tuber initiation versus after tubers had begun bulking, both treatments reduced tuber growth to a similar extent [34]. However, this experiment involved plants that were exposed starting at the date of planting, prior to exposure to tuber-inducing photoperiods, whereas in the current study, plants were first grown with long days, which is a non-inductive photoperiod, then tuber induction was begun at a discrete experimentally determined time. To separate high temperature effects on above-ground and below-ground plant parts, investigators have used growth chambers with root-zone temperature control [25]. After tuber induction, high temperature whole-plant or below-ground treatments both resulted in a reduction in tuber growth and decreased photosynthetic rates [25]. This suggests that communication of root-zone environment or sink-strength status is conveyed to leaves and this may determine the extent to which plant biomass is diminished. Hence, potato response to high temperature appears to depend

on differences in sensitivity of various plant parts and of the developmental processes at different stages.

Amelioration of high temperature effects by Elevated CO₂. In the current study, the percentage inhibition of dry matter accumulation rate (DMAR) by high temperature was somewhat greater in LowCO₂ than in ElevCO₂ treatments (56 and 35%, respectively, at tuber initiation; and 32 and 15%, respectively, at tuber bulking), consistent with earlier findings [33]. In several studies, elevated CO₂ has been found to partly ameliorate high temperature stress [35,36]. Although the 35 °C environment decreased both tuber and whole plant DMAR at both tuber initiation and tuber bulking stages (Figures 1 and 2), the elevated CO₂ treatment (35 °C/ElevCO₂) at tuber initiation eased some of the impact of heat stress so that the DMAR of tubers and whole plants recovered to rates comparable to the normal treatment (25 °C/LowCO₂). At tuber bulking, elevated CO₂ eased most of the heat stress, such that tuber DMAR at 35 °C /ElevCO₂ was mid-way between 25 °C/LowCO₂ and 25 °C/ElevCO₂.

3.3. Tuber Growth and Development

3.3.1. High Temperature Effects on Components of Tuber Development

Both tuber cell proliferation and cell expansion (and associated organelle and enzyme development) are needed to develop starch storage capacity for tuber bulking in potato tubers [37]. In previous studies, high temperature suppressed tuber initiation so that tuber numbers per plant were decreased [33,38,39]. Consistent with this, we found that tuber numbers were decreased by high temperature when applied at tuber initiation (Figure 3). However, the lower number of tubers may have been due, in part, to the poorer cell division and expansion growth of tubers at high temperature (Figure 4) such that more of the initiated tubers were smaller than the 1 cm diameter cutoff used for tuber counts. Outcomes similar to this were observed in studies with other treatments that were unfavorable to tuber development and growth, such as shade and long-day photoperiods during tuber bulking [30,40]. Moreover, in the present study photoperiod was switched from 14 to 10 h day length, which provides a very strong tuber inductive signal that may have been sufficient to overcome inhibitory influences due to high temperature [41–44].

3.3.2. Elevated CO₂ Effects on Components of Tuber Development

We obtained data on three components of early tuber development: cell proliferation, cell expansion, and post-mitotic DNA endoreduplication (Figures 3 and 4). At the tuber initiation stage, with 25 °C, elevated CO₂ had a substantial stimulative effect on cell proliferation such that tuber cell numbers in the elevated CO₂ treatment were 62% greater than with the corresponding low CO₂ control (Figure 4). In contrast, cell volume and the proportion of cells that had undergone endoreduplication were unaffected. Cell proliferation continued during the tuber bulking stage and cell numbers responded to ElevCO₂ in proportion to tuber weight, while average cell volumes and endoreduplication were not affected (Figure 5). These data are in agreement with previous studies which have shown that cell proliferation is highly responsive to elevated CO₂ while cell expansion growth is less responsive at the early phase of tuber development [30]. Transcriptome studies on potato tubers show that genes associated with cell division are highly upregulated during tuber initiation [45].

3.3.3. Sugar Signaling in Tuber Development

It is plausible that sugar status in tubers serves as a component of signaling pathways for stimulating enhanced growth in the tubers. At both tuber initiation and tuber bulking, high temperature decreased tuber dry matter (Figures 1 and 2) and fresh weight (Figures 4 and 5) growth. The percentage inhibition at each stage was approximately the same as that for respective whole-plant DMAR, so it is plausible that the inhibitory response in tubers involved photosynthate status as a cue. This interpretation is also supported by the approximately similar pattern of CO₂ benefit in tubers compared with that in whole

plants in response to the Elev_{CO₂} treatments, although the magnitude of response was greater in tubers than leaves and stems, as discussed above. Studies have identified sugar signaling in potato tubers that regulate patatin storage-protein synthesis [46] and expression of starch pathway enzymes [25,47,48], which are thought to involve sucrose sensing. Consistent with this, when the levels of Trehalose-6-phosphate (T6P), a sugar-signaling metabolite, was elevated with tuber-specific overexpression of the T6P synthase, or lowered with expression of T6P phosphatase, expression of genes involved in tuber cell proliferation were affected [47]. Studies have shown linkages between sugar signaling pathways and enhanced cell proliferation [49]. In the current study, tuber hexose concentrations tended to increase in the 25 °C/Elev_{CO₂} treatment while sucrose was unaffected (Figure 6). Invertase activities were increased in response to Elev_{CO₂} (Figure 7), consistent with potential involvement in increasing hexose levels and sugar signaling through a hexose signaling pathway [50]. In tomato (*Solanum lycopersicum*) fruit, evidence from gene silencing indicates that cell wall invertase is essential for sugar signaling and development [51]. While sugar signaling has been studied with respect to potato tuber carbon flux and starch synthesis, it merits further attention with respect to regulation of cell proliferation.

Contrary to the general pattern with Elev_{CO₂}, in the high temperature treatments, at both tuber initiation and tuber bulking, partial amelioration provided by elevated CO₂ was substantially due to enhanced cell expansion in the high temperature treatments, whereas cell expansion was not involved in the normal temperature treatments (Figures 4 and 5). In studies of the effects of shade on tuber development, the extent of endoreduplication usually increased where average cell volume was higher [40]. However, in the current study, even though high temperature enhanced cell expansion, it did not affect endoreduplication (Figures 4 and 5). Endoreduplication is common in plants, and in the present study a high proportion of cells were endoreduplicated to nuclear DNA contents $\geq 8C$; they were 35 and 49% at tuber initiation and tuber bulking, respectively. The roles of endoreduplication are still a matter of speculation, though in other plant systems it has been suggested that endoreduplication has a role in stress response [52]. In the present system there was no evidence for an endoreduplication response to Elev_{CO₂} or high temperature. So, the mechanism by which environment affects tuber growth and development may be different from that involved in other systems.

3.4. Global Climate Change

In relation to global climate change, the current study provides insight into potato's sensitivity to various combinations of CO₂ and temperature at two discrete developmental phases. In the field, although elevated CO₂ is a constant condition throughout a crop season, high temperature stress often occurs for relatively short periods of time, such as one or several days. Given the short time over which heat stress episodes commonly occur in the field, it is of interest to determine whether there are particular stages of potato development that are more sensitive to heat stress. Evaluations of simulation models for potato agronomic responses and global climate change indicate that model variation is modest for predicted CO₂ but is particularly uncertain for temperature [5]. This suggests that more knowledge about the nature of heat susceptibility in potato would be valuable in guiding future efforts to improve the crop genetically and to optimize management [12,21,53]. In regard to whole plant DMAR, the current studies indicate that the tuber initiation stage may be somewhat more sensitive to stress than tuber bulking. This may be a consequence of the considerably greater sensitivity of cell proliferation and organelle developmental processes at tuber initiation, whereas starch accumulating cells at tuber bulking may be more robust and less responsive to stress. Moreover, plants have numerous feedbacks in their regulatory systems such that changes in tuber sink capacity can provide feedback regulation of photosynthesis [25].

4. Materials and Methods

4.1. Plant Material

Potato plants (cv. Katahdin) were grown from tuber cuttings in a greenhouse with 14 h supplemental lighting and were watered with nutrient solution (Peter's 15-16-17 fertilizer, W.R. Grace and Co., Fogelsville, PA, USA) which supplied the following elemental nutrients: 33 mg L⁻¹ of NO₃-N, 86 mg L⁻¹ of NH₄-N, 42 mg L⁻¹ of urea-N, 74 mg L⁻¹ of P, 151 mg L⁻¹ of K. Pots (6 L) contained peat, vermiculite, and perlite (1:1:1 v/v/v) with 3 g of pulverized limestone, 17 g of CaSO₄, 21 g of powdered FeSO₄, 0.5 g of fritted trace elements (Peters FTE 555, Scotts Co., Marysville, OH, USA), and 1.5 g of wetting agent (AquaGro G, Aquatrols, Pennsauken, NJ, USA). After two weeks, young plants were trimmed to include one shoot and re-sown in new 12 L pots with one plant/pot. After transplanting, plants were grown in the greenhouse for an additional 4 weeks, then the plants were transferred to four matched growth chambers where short days (10 h photoperiod) were imposed to initiate tuber formation, as described below. Nutrient solution and watering regime were the same as in the greenhouse.

On the day that plants were transferred to growth chambers for imposition of temperature × CO₂ treatments, plants from a set of uniform material were randomly assigned to the treatments, as described below; concurrently, a subset was designated for immediate harvest at time = 0 for estimating the dry matter of plants parts at the onset of treatments.

4.2. Temperature and CO₂ Control

Controlled-environment chambers (Model CEL-63-10, Sherer Inc, Marschall, MI, USA) had interior dimension of 112 × 74 cm (width × depth) and 600 μmol photons (photosynthetically active radiation, 400–700 nm) m⁻² s⁻¹ at the top of the canopy, supplied by fluorescent lamps. Temperature regime was 25/18 °C (day/night) in the normal temperature treatment and 35/25 °C (day/night) in the high temperature treatment. CO₂ treatments were either 700 μmol CO₂ mol⁻¹ air (elevated CO₂) or between 350 and 400 μmol CO₂ mol⁻¹ air (low CO₂). Chamber CO₂ concentration was monitored with a calibrated infrared gas analyzer [30]. The four growth chambers were used to impose a 2 × 2 matrix of temperature × CO₂ treatments at two stages of development: (1) during the first 2 weeks after switch from 14 to 10 h days (weeks after short d; WASD), which stimulates tuber production [hereinafter described as the tuber initiation stage (TI)], or (2) during the next two weeks after switch to short days, when starch accumulation predominates [hereinafter described as the tuber bulking (TB) stage]. Plants for the tuber bulking treatments were first given a short-day (10 h photoperiod) treatment for two weeks in chambers at the normal conditions of 25/18 °C (day/night) and low CO₂.

4.3. Tuber Sampling Method

To representatively sample tubers for analyses, all tubers within each plant were characterized into three groups by size, each group containing about one third of the tuber fresh biomass in the entire plant. The grouping started by ranking tubers within a plant by size and, starting with the largest tuber, assigning it to the largest size-category, then continuing to the next smaller tuber until the sum of tuber fresh biomass in the category accounted for about one third of the tuber biomass of the entire plant. In this way, at least two tubers were chosen for this size category. Then tubers were assigned to the next size category until the summed biomass in each of the three categories accounted for one third of the total. In each category, three tubers (or two, depending on availability) were randomly chosen for sugar levels or enzymes activities determination.

4.4. Assay Methods

From representative tubers, two cores, longitudinal and transverse, were immediately withdrawn with a 0.5 cm diameter cylindrical borer and fixed in ethanol/acetic acid (3:1) solution for flow cytometry. Then the apical-bud end (rose end) of the tuber was cut off 1 cm proximal from the apex, and a 0.5 cm thick slice was obtained from the apical end,

frozen in liquid nitrogen, and stored at $-20\text{ }^{\circ}\text{C}$ for further determination of sugars and related enzyme activities. Leaves, stems, and remaining portions of tubers were dried at $50\text{ }^{\circ}\text{C}$ to constant weight and weighed. Tuber dry weights were corrected for the fresh weight removed for cell cytometry and enzyme activity.

For flow cytometry analysis, core slices were digested with 0.5% (*w/v*) pectinase (EC 3.2.1.15, MP Biomedicals, Solon, OH, USA) for 18 h without shaking at $37\text{ }^{\circ}\text{C}$, then cooled in a $4\text{ }^{\circ}\text{C}$ refrigerator overnight before mechanically disrupting cell walls and releasing nuclei into the medium. After about 18 h of shaking (150 rpm) the tubers were stored in a $4\text{ }^{\circ}\text{C}$ refrigerator then filtered through nylon-mesh fabric with $100\text{ }\mu\text{m}$ openings (Nitex, Teco Inc, Briarcliff Manor, NY, USA). The DNA-binding fluorochrome propidium iodide was added to the filtrate to a final concentration of $90\text{ }\mu\text{M}$ in a 10 mM Tris-HCl buffer (pH 7.4). A $100\text{ }\mu\text{L}$ aliquot of filtrate was counted by flow cytometry using either a FACScan analyzer (Becton Dickinson, Mountain View, CA, USA), or an Epic Profile (Coulter Electronics, Hialeah, FL, USA) operating with an argon-neon laser (488 nm).

For determination of sugars and related enzymes, 0.5 cm diameter disks were cut out from the zone rich in vascular bundles in the perimedullary zone near the perimedulla/cortex interface of slices with a cylindrical borer and then homogenized with 50 mM Hepes-KOH (pH 7.4) buffer (tissue:buffer 1:2(*w/v*)) containing 5 mM MgCl_2 , 1 mM EGTA, 1 mM EDTA, 40% (*v/v*) glycerol, 0.1% bovine serum albumin, 0.5 mM dithioerythritol and 2% insoluble polyvinylpyrrolidone and stored at $-20\text{ }^{\circ}\text{C}$. Ten microliter aliquots of supernatant were mixed with $90\text{ }\mu\text{L}$ ethanol and stored for sugar assay. The concentrations of glucose, fructose and sucrose were determined using an enzyme-coupled assay based on hexokinase (EC 2.7.1.1, 0.14 unit) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49, 0.07 unit), and using phosphoglucosomerase (EC 5.3.1.9, 0.2 unit) and invertase (EC 3.2.1.26, 80 units) for fructose and sucrose interconversions [54].

Acid invertase activities were determined in the sucrose hydrolysis direction. For soluble acid invertase, 0.5 mL of buffer-extract was centrifuged at $16,000\times g$ and desalted on a Sephadex G-25M to obtain enzyme extract. For cell-wall-bound acid invertase, 0.5 mL of buffer-extract was centrifuged at $16,000\times g$ and, then, the pellet was resuspended in 1 mL of 1 M NaCl and incubated at $4\text{ }^{\circ}\text{C}$ at least 12 h for salt extraction. After centrifugation at $16,000\times g$, the salt extract was desalted on a Sephadex G-25M to obtain enzyme extract. Both soluble and cell-wall-bound acid invertase activities were determined by mixing enzyme extract (150 μL) with 50 μL of 1 M sucrose to start the enzyme reaction. The reaction was incubated for 75 min at $24\text{ }^{\circ}\text{C}$ and the glucose + fructose produced was assayed as before [54]. All enzyme assays were linear with time and the amount of enzyme extract added.

4.5. Data Analysis

The experiments were conducted in three batches with two plants in each (6 biological replicates). A plant was considered an experimental unit. Each batch contained a complete set of treatments, which were randomly assigned to four matched chambers set at the two CO_2 concentrations and temperatures (a 2×2 set of treatments), as described above. Data were modeled using a simple linear model with batch, temperature treatment (T), CO_2 treatment, and $T\times \text{CO}_2$ interaction as sources of variation. Analysis of variance was conducted in R version 3.6.0 [55] using the *lm* function. Tukey HSD multiple-range significant-difference tests were used for multiple comparisons.

5. Conclusions

The current study elucidated the effects of elevated CO_2 and high temperature on potato tuber development at two stages: tuber initiation and tuber bulking. While whole-plant dry matter accumulation rate was increased by elevated CO_2 about 30% at both stages, the responses of plant parts differed considerably. At the tuber initiation stage, and at normal day-time temperature of $25\text{ }^{\circ}\text{C}$, elevated CO_2 increased tuber dry matter accumulation rate by more than 60%, while leaf and stem DMAR was not affected,

even though these organs collectively represented almost two thirds of the sink-organ DMAR. At the tuber bulking phase, when most of plant photosynthate is used for starch storage in tubers, leaves had very low DMAR and stems had a net loss in dry matter, so almost all the benefit from elevated CO₂ was obtained by tubers. The extent to which high temperature stress affected plant growth and sink development differed considerably between stages. Whole-plant dry matter accumulation rate, was inhibited by high temperature treatment about twice as much at the stage of tuber initiation than at tuber bulking. In contrast to CO₂ effects at 25 °C, where tubers primarily benefited, at high temperature stress, elevated CO₂ benefitted leaf and stem growth in addition to tuber growth, and hence partially ameliorated high temperature inhibition. This suggests that growing plant parts have a development window within which they are capable of responding to improved photosynthate supply. At the tuber initiation stage, with 25 °C, elevated CO₂ had its primary effect on cell proliferation such that tuber cell numbers in the elevated CO₂ treatment were 62% greater than with the corresponding low CO₂ control. In contrast, cell volume and the proportion of cells that had undergone endoreduplication were unaffected. These findings indicate that the tuber initiation stage and cell division process are particularly responsive to elevated CO₂ and high temperature stress. To fully understand impacts of climate change and to develop improved crops, attention should be paid to the timing of high-temperature stress episodes with respect to this stage.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/plants10050871/s1>, Suppl. Figure S1. Distribution of whole-plant tuber production among individual tuber size-classes in plants exposed to temperature (25 or 35 °C) or CO₂ treatments (LowCO₂ or ElevCO₂). (a) Tuber initiation stage; (b) tuber bulking stage. Plants were harvested after exposure to treatment for 2 weeks. Tubers exceeding 1 cm diameter were counted. Tuber production was the sum of tuber fresh weight within the defined size-range category. Data was from 6 replicate plants per stage of development.

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