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Elevated Carbon Dioxide only Partly Alleviates the Negative Effects of Elevated Temperature on Potato Growth and Tuber Yield

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Abstract

The current rapid increase in ambient carbon dioxide concentration ([CO_2]) and global temperatures have major impacts on the growth and yield of crops. Potato is classified as a heat-sensitive temperate crop and its growth and yield are expected to be negatively affected by rising temperatures, but it is also expected to respond positively to increasing ambient [CO_2]. In this study, we investigated the physiological, growth, and yield responses of two potato cultivars to elevated temperature (eT) and the possible role of elevated [CO_2] (e[CO_2]) in counteracting the negative effects of eT. Two growth chamber trials (trials 1 and 2) were conducted using two temperature regimes: ambient temperature (aT, $T_{min}/T_{max} = 12/25$ ℃) and eT ($T_{min}/T_{max} = 15/38$ ℃), and two [CO_2]: ambient (a[CO_2]) = 415 ppm and e[CO_2] = 700 ppm. Temperatures gradually rose from the minimum at 6.00 AM to reach T_{max} at noon, then T_{max} was maintained for 1 h in trial 1 and for 4 h in trial 2. Elevated [CO_2] increased photosynthesis (*Anet*) in both cultivars at aT and eT. Elevated temperature also stimulated *Anet* compared to aT. Elevated [CO_2] significantly reduced stomatal opening size, while eT resulted in larger stomata openings and higher stomatal conductance. Elevated [CO_2] increased tuber yields at aT in both trials. Tuberisation was delayed by eT in trial 1, and completely inhibited in trial 2 even at e[CO_2], resulting in no tuber yield. The two cultivars responded similarly to treatments, but Mondial initiated more tubers and had higher tuber yield than BP1. The results suggest that potato will benefit from e[CO_2] in future, even when exposed to high T_{max} for a short period of the day, but the benefit will be eroded when the crop is exposed to high T_{max} for an extended period of the day.

Keywords Photosynthesis · Stomatal conductance · Starch grain · Stomata size · Tuberisation

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Introduction

Potato, a C_3 crop, is regarded as a temperate crop with optimal above-ground growth at 20 to 25 ℃ and optimal below-ground growth at 15 to 20 ℃ (Van Dam et al. 1996; Hastilestari et al. 2018). The Intergovernmental Panel on Climate Change's (IPCC) 6th assessment report (Pielke et al. 2022) forecasts increases in global temperatures of between 3.3 and 6 ℃ by 2099 as a worst-case scenario. This will substantially impact potato growth and tuber yields in the course of this century (Hijmans 2003; Wang et al. 2022). A reduction in potato yields will negatively impact food security and human nutrition as it is a vital food security crop in developing countries, as marked by a ~ 70% rise in consumption in Africa and Asia between 1961 and 2013 (Wijesinha-Bettoni and Mouillé 2019). The negative impact of high temperature on potato yields has been attributed to inhibition of tuberisation, reduced carbon transport to sink organs, and reduced photosynthetic performance of plants (Hancock et al. 2014). Temperatures above 25 ℃ have been found to delay tuberisation in potato (Dahal et al. 2019) and higher temperatures (35 ℃ day and 28 ℃ night) may completely inhibit tuberisation in some cultivars (Tang et al. 2018). Lafta and Lorenzen (1995) found that at 31/29 ℃ day/night temperature, more dry matter was allocated to the shoots at the expense of tuber growth. The delay in or inhibition of tuberisation is reported to be due to disruptions in molecular pathways (Singh et al. 2020), which include the tuberisation signal (*StSP6A*) (Hancock et al. 2014). This results in poor tuber cell division and expansion (Lee et al. 2020; Chen and Setter 2021). It is not only the absolute temperature that is important but the sensitivity of potato to high temperatures also depends on the duration of exposure (Ahn et al. 2004) and the growth stage, with the tuberisation stage being the most sensitive (Rykaczewska 2015; Tang et al. 2018).

It is thus widely expected that elevated temperatures (eT) will negatively impact growth and yield of potato. Despite the expected negative impact of eT, potato is currently being grown successfully in unfavourably warm temperatures in parts of South Africa (Steyn et al. 2016), with T_{max} occasionally rising to 40 ℃, and yet high potato yields (>90 t ha^{-1} fresh tuber) are regularly obtained (Steyn et al. 2016). In addition, actual yields are occasionally higher than potential yields as calculated by crop simulation models (ten Den et al. 2022), which assume heat stress at temperatures above 30 ℃. This is supported by the work of Tang et al. (2018), who found differences in the physiological and growth responses of 55 potato cultivars to 35/28 ℃ day/night conditions.

Contrary to eT, elevated CO_2 concentration (e[CO_2]) is expected to enhance plant growth through photosynthetic stimulation (Tausz-Posch et al. 2020), because the present-day atmospheric [CO_2] limits the ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) catalysed carboxylation reaction in C_3 species (Long et al. 2004). An increase in [CO_2] will therefore enhance photosynthesis (*Anet*) by increasing substrate availability (Thompson et al. 2017), while reducing photorespiratory carbon loss by suppressing the oxygenation reaction (Thompson et al. 2017). In potato, e[CO_2] has been found to have mixed effects on tuber yield. For example, Lee et al. (2020) observed no yield effect, while increased tuber yields were reported by Dahal et al. (2023). Miglietta et al. (2002) reported a ~ 10% increase in yields per 100 ppm rise in [CO_2] due to enhanced *Anet* and therefore more assimilates available for translocation to the tubers.

Despite the concomitant nature of increases in [CO_2] and temperature, it is particularly challenging to assess the e[CO_2] and eT interaction in free air CO_2 enrichment (FACE) or open-top chamber (OTC) trials. This is because it is difficult to manipulate temperatures and adequately control [CO_2] due to turbulent and oscillating air which causes significant temperature fluctuations (Allen et al. 2020). In addition, OTC and FACE experiments have in general been carried out in temperate regions under low temperature stress conditions; hence, the available datasets are highly restricted and are largely biased towards the Northern Hemisphere (Leakey et al. 2012; Kimball 2016). Therefore, the question arises whether the rather strong impacts of e[CO_2] on crop performance also hold in high-stress environments. A meta-analysis of the combined effect of e[CO_2] and eT has shown that plant responses tend to mimic those of e[CO_2] as a single factor (Dieleman et al. 2012), suggesting that e[CO_2] can generally alleviate the negative effects of eT on crops. However, the concomitant elevation in [CO_2] and temperature has only been assessed in a limited number of potato studies. At moderate eT (25 ℃ vs. 21 ℃), e[CO_2] (804 ppm) increased potato tuber yields by increasing tuber size (Lee et al. 2020). In another study, both temperature and [CO_2] were increased at either tuber initiation or tuber bulking, with eT of 35 ℃ and e[CO_2] of 700 ppm (Chen and Setter 2021). When treatments were applied at tuber initiation, dry matter accumulation was reduced at elevated temperatures, but when [CO_2] was increased in combination with eT, the accumulation of dry matter was not significantly different from the ambient control. At tuber bulking, e[CO_2] was also able to alleviate the negative impact of high temperatures on dry matter accumulation (Chen and Setter 2021). However, studies exposing potato crops to concurrent elevation in [CO_2] and temperature throughout the growth cycle are very limited, leaving the effects of long-term exposure to e[CO_2] and eT uncertain.

The present study therefore sought to elucidate the physiological, growth, and yield responses of two popular commercially grown cultivars in South Africa, Mondial and BP1, to varying [CO_2] and temperature combinations applied in growth chambers from planting until harvesting. The first aim was to determine whether potato yields are negatively impacted by eT, and what mitigating role e[CO_2] may play through the enhancement of *Anet*. Secondly, the impact of the duration of the daily eT on growth and yield of the two cultivars was investigated when grown at a[CO_2] or e[CO_2]. Firstly, we hypothesised that potato yield would be negatively impacted by suboptimal high day temperatures, but the decline in yield at eT would be mitigated by e[CO_2] due to increased photosynthesis. Secondly, we hypothesised that if elevated maximum temperatures are maintained for either short (1 h) or long (4 h) periods within a day, tuberisation would still occur and tuber yields would result.

Materials and Methods

Planting and Plant Growth Conditions

Two trials (trials 1 and 2) were conducted in four CONVIRON® growth chambers, allowing for light intensity and duration, temperature, [CO_2], and relative humidity control, with one $T \times CO_2$ treatment combination per chamber. In both trials, the [CO_2] (maintained day and night) was set at 415 ppm (a[CO_2]) or 700 ppm (e[CO_2]) from planting to crop end. Minimum (T_{min}) and maximum temperatures (T_{max}) were set at 12 and 25 ℃ for the ambient temperature regime (aT) and at 15 and 38 ℃ for the elevated temperature regime (eT). Temperatures rose gradually from 6.00 AM and reached a maximum at noon. aT was set to mimic growth conditions optimal for potato and eT was set to mimic a hot summer day in the North West Province potato growing region of South Africa. During trial 1, the maximum temperature was maintained for 1 h (T_{max}: 1 h), while in trial 2, T_{max} was maintained for 4 h (T_{max}: 4 h), followed by a gradual decline to reach the T_{min} at 5:00 AM. Cultivars Mondial, a very high yielding, medium to late maturing, and early tuberising cultivar and BP1, a medium-to-high yielding cultivar with a medium-to-long maturity and extended tuberisation period (Du Raan 2015) were used. As BP1 is an older cultivar than Mondial, it was suspected that it might be more heat sensitive than Mondial. The different treatment factors resulted in the following treatment combinations: i) a[CO_2]aT Mondial, ii) a[CO_2]aT BP1, iii) a[CO_2]eT Mondial, iv) a[CO_2]eT BP1, v) e[CO_2]aT Mondial, vi) e[CO_2]aT BP1, vii) e[CO_2]eT Mondial, and viii) e[CO_2]eT BP1. One minituber (± 20–30 g each) was planted in 6 kg of sterilized compost and silica sand mixture (1:1, v:v) in 9-L plastic planting bags, followed by further addition of 2 kg compost and silica sand mixture at 28 days after emergence (DAE).

The day length was set at 14 h with the light intensity increasing gradually from 06:00, to reach a maximum of ~ 1000 µmol m^{-2} s^{-1} at noon. The maximum light intensity was maintained for the same duration as T_{max} in the respective trials, thereafter, gradually decreasing to reach darkness at 8:00 PM. Humidity was set at 60–70%, while irrigation was done using a pressure-compensated drip irrigation system which maintained the available soil water content > 75% to ensure the plants were under no water stress. Fertigation was done twice a week using Hygroponic® (Hygrotech Properties LTD) combined with AG® calcium nitrate (AGROMATE) fertiliser. The air circulation system in the growth chambers injected/blew air from below the platform holding the planting bags, which resulted in considerably higher root zone temperatures for the eT treatments (32 ℃ when $T_{max} = 38$ ℃) than the aT treatments (21 ℃ when $T_{max} = 25$ ℃).

Gas Exchange Parameters

Gas exchange measurements (net photosynthesis and stomatal conductance) were taken on the youngest fully expanded and light-exposed leaf in trial 2 when T_{max} was reached (12:00–16:00) on four time points, namely 21, 42, 63, and 84 DAE, using a portable LI-6800 photosynthesis system (LI-COR Biosciences, NE, USA). The LI-6800 (small light chamber model 6800–02) had an artificial blue/red light source with sensors to monitor leaf temperature (T_{leaf}), and internal and external photosynthetically active radiation (PAR). Temperature, [CO_2], and relative humidity within the sample chamber were set to reflect the growth chamber conditions, while the flow rate was set at 500 µmol s^{-1} and PAR at 1000 µmol m^{-2} s^{-1}. Measurements were taken inside the growth chambers on four plants per treatment.

Leaf Scanning and Transmission Electron Microscopy (SEM and TEM)

The apical leaflet of the first fully expanded leaf was sampled at 63 DAE in trial 2 and immediately placed in formaldehyde, acetic acid, and ethanol solution (FAA) for scanning electron microscopy (SEM) to determine the length and width of the stomatal aperture and transmission electron microscopy imaging (TEM) to determine the starch grain area. Sample dehydration was done according to Murtey and Ramasamy (2016).

The SEM samples for the measurement of the length and width of stomatal pores were transferred into ethanol and hexamethyldisilazane (HMDS) in a 1:1 ratio for 1 h, following which they were transferred into 100% HMDS, for another 1 h. Lastly, fresh 100% HMDS was applied and left until the HMDS had completely evaporated. The samples were thereafter mounted on aluminium stubs and carbon coated using a Quorum Q150T Coating Unit. Imaging was done using a Zeiss Gemini Ultra Plus field emission gun (FEG) SEM with energy dispersive spectroscopy (EDS) and electron backscatter diffraction (EBSD) detectors. The length and width of the stomatal aperture (excluding guard cells) on both the top and bottom surfaces of the leaves were determined using ZEN 3.3 (blue edition) software (Fig. S1) (Jena, Germany).

After dehydration, TEM samples for starch grain area determination were subsequently transferred into 20, 40, 60, and 80% polyhydroxy-aromatic acrylic (LR white) resin in 100% ethanol for 1 h each, followed by 100% LR white resin for 4 h (X2). The samples were thereafter left overnight in fresh resin at 4 ℃ after which the resin was replaced with fresh LR white resin and allowed to stand for 4 h. Lastly, the samples were transferred into gelatine capsules filled with resin and polymerised at 60 ℃ for 36 h. Samples were sectioned using an ultramicrotome (Reichert-Jung Ultracut E) (Eindhoven, the Netherlands) and embedded on copper grids followed by double staining using uranyl acetate and lead citrate. Imaging was done using a Jeol 2100F FEG transmission electron microscope with an energy dispersive spectroscopy (EDS) detector. The length, width, and therefore surface area of 35 starch grains per sample were determined using ImageJ2 software (Fig. S2) (Rueden et al. 2017). The surface area of starch grains was then used as an indicator of starch grain size.

Plant Growth and Yield Parameters

Plant growth parameters were assessed through destructive sampling of four plants per treatment combination at 21, 42, and 63 DAE and a final harvest at 140 DAE in both trials. The number of tubers initiated was counted at 21 and 42 DAE. After sampling, plants were separated into leaves, stems, tubers, and roots. Leaf area was measured using an LI 3100C area meter (LI-COR Biosciences, NE, USA). The leaves and stems were dried at 75 ℃, while tubers (thinly sliced) were dried at 65 ℃ to a constant mass for dry mass determination. Final tuber fresh and dry mass were determined at 140 DAE. The harvest index (HI) was expressed as the fraction of tuber dry mass to the total dry plant biomass (tubers + haulms) at 140 DAE.

Statistical Analysis

The data were analysed separately for trials 1 and 2 as well as for the different sampling time points at 21, 42, 63, 84 (gaseous exchange measurements only), and 140 DAE. Data qualitative checks were done using the Grubbs' test for outliers and the Shapiro–Wilk test for normality was applied. When the data deviated from a normal distribution, the Box-Cox procedure to fit the most appropriate lambda function was used prior to analyses. The single factor effect was assessed followed by interaction effect of [CO_2], temperature, and cultivar on the recorded parameters using Minitab® statistical analysis software version 19 (PA, USA). Where the three-way interaction was not significant, two-way interaction analysis between temperature and [CO_2] was performed. Mean comparisons were done using Tukey's honest significant differences (HSD) test at a 0.05 level of probability.

Results

Leaf Photosynthesis and Stomatal Conductance

Considering the single-factor effects, e[CO_2] significantly increased leaf photosynthesis rate (*Anet*) at all assessment dates in trial 2 (Table 1). While eT had a negative impact on *Anet* at 21 DAE, it had a positive impact at 42 and 63 DAE and no effect at 84 DAE. Differences in *Anet* between cultivars were inconsistent throughout the trial. While the two cultivars had comparable *Anet* at 21 DAE, Mondial had higher *Anet* at 42 and 84 DAE, and BP1 had higher *Anet* at 63 DAE.

The interactions between [CO_2], temperature, and cultivar significantly impacted *Anet* at 42 and 63 DAE, but the response of *Anet* was not significant at 21 and 84 DAE. As expected, e[CO_2] increased *Anet* at aT, except at 21 DAE for Mondial (Table 1). The impact of eT on *Anet* at a[CO_2] was inconsistent across the measurement dates and cultivars. Although the three-way interaction was not significant at 21 DAE, a clearer pattern as a result of elevated temperatures emerged for both cultivars when [CO_2] was elevated. There was no effect of eT on *Anet* at 21 DAE, but for the rest of the trial *Anet* was significantly higher at e[CO_2]eT for both cultivars as compared to e[CO_2]aT. At e[CO_2]eT *Anet* was also significantly higher from 42 to 84 DAE for both cultivars when compared to a[CO_2]aT. At 21 DAE *Anet* of BP1 at e[CO_2]eT was also significantly higher than at a[CO_2]aT.

Table 1 Photosynthesis rate (*Anet*) and stomatal conductance (*gsw*) as affected by temperature, CO_2 concentration ($[CO_2]$), and cultivar in trial 2

	21 DAE	42 DAE	63 DAE	84 DAE
Anet ($\mu mol\ m^{-2}\ s^{-1}$)				
a$[CO_2]$	15.78	17.96	23.03	21.63
e$[CO_2]$	29.98	33.97	35.57	34.79
aT	24.90	23.86	25.85	28.06
eT	20.85	28.07	32.75	28.36
Mondial	22.57	26.66	28.02	29.04
BP1	23.18	25.27	30.58	27.38
a$[CO_2]$ aT Mondial	21.75	19.98 c	19.64 f	24.19
a$[CO_2]$ eT Mondial	13.32	19.25 c	23.70 e	21.90
e$[CO_2]$ aT Mondial	27.64	31.64 b	30.08 c	30.90
e$[CO_2]$ eT Mondial	27.58	35.75 a	38.66 b	39.17
a$[CO_2]$ aT BP1	19.48	11.29 d	22.65 e	25.48
a$[CO_2]$ eT BP1	8.57	21.31 c	26.15 d	14.95
e$[CO_2]$ aT BP1	30.74	32.52 b	31.03 c	31.65
e$[CO_2]$ eT BP1	33.94	35.96 a	42.50 a	37.43
$[CO_2]$	$p=0.000$	$p=0.000$	$p=0.000$	$p=0.000$
Temperature	$p=0.001$	$p=0.000$	$p=0.000$	$p=0.686$
Cultivar	$p=0.524$	$p=0.004$	$p=0.000$	$p=0.040$
$[CO_2]$ * Temp * Cultivar	$p=0.147$	$p=0.000$	$p=0.001$	$p=0.071$
gsw ($mol\ m^{-2}\ s^{-1}$)				
a$[CO_2]$	0.28	0.80	0.81	0.66
e$[CO_2]$	0.34	0.79	0.88	0.47
aT	0.20	0.43	0.58	0.32
eT	0.41	1.16	1.11	0.81
Mondial	0.31	0.90	0.91	0.57
BP1	0.30	0.69	0.78	0.56
a$[CO_2]$ aT	0.22 bc	0.34 d	0.50 c	0.49 b
a$[CO_2]$ eT	0.34 b	1.27 a	1.11 a	0.84 a
e$[CO_2]$ aT	0.18 c	0.53 c	0.65 b	0.16 c
e$[CO_2]$ eT	0.49 a	1.06 b	1.10 a	0.77 a
$[CO_2]$	$p=0.112$	$p=0.683$	$p=0.059$	$p=0.000$
Temperature	$p=0.000$	$p=0.000$	$p=0.000$	$p=0.000$
Cultivar	$p=0.903$	$p=0.000$	$p=0.001$	$p=0.928$
$[CO_2]$ * Temp	$p=0.014$	$p=0.000$	$p=0.025$	$p=0.001$

a, ambient; *e*, elevated; *T*, temperature. Numbers followed by the same letter in the same column do not differ significantly. $n=16$ for single factor, $n=8$ for two-way interaction, $n=4$ for three-way interaction, $p=0.05$, *DAE*, days after emergence

Analysis of the single-factor effects revealed that e$[CO_2]$ only had a significant effect on leaf stomatal conductance (*gsw*) at 84 DAE when *gsw* was significantly reduced as compared to a$[CO_2]$ (Table 1). In contrast, elevated temperature more than doubled *gsw* at all dates. Mondial and BP1 had comparable *gsw* at 21 and 84 DAE, but at 42 and 63 DAE Mondial had higher *gsw* than BP1.

The interaction effect of cultivar, [CO$_2$], and temperature on *gsw* was only significant at 42 DAE (data not shown), and therefore, only the two-way interactions between [CO$_2$] and temperature are reported. At a[CO$_2$] *gsw* was significantly increased by eT from 42 to 83 DAE as compared to aT, while at e[CO$_2$]eT *gsw* was significantly higher than at e[CO$_2$]aT at all time points (Table 1). Stomatal conductance at e[CO$_2$]eT was also significantly higher than a[CO$_2$]aT at all time points, but when compared to a[CO$_2$]eT, e[CO$_2$]eT did not result in significantly higher *gsw* throughout the trial, with differences only observed during the first two measurements. Initially *gsw* was increased at e[CO$_2$]eT relative to a[CO$_2$]eT, but at the second time point *gsw* was decreased at e[CO$_2$]eT, with no differences from 63 DAE.

Leaf Stomatal Dimensions

The single-factor responses of stomata size on the adaxial and abaxial leaf surfaces to treatments were comparable (Table 2). While e[CO$_2$] significantly reduced the

Table 2 Response of leaf stomatal aperture (length and width) to CO$_2$ concentration ([CO$_2$]) and temperature in trial 2 at 63 days after emergence

Treatment	Stomata width (μm)		Treatment	Stomata length (μm)	
	Adaxial	Abaxial		Adaxial	Abaxial
a[CO$_2$]	5.0	3.7	a[CO$_2$]	15.7	14.0
e[CO$_2$]	2.5	3.6	e[CO$_2$]	11.8	12.6
aT	0.7	1.0	aT	4.0	5.4
eT	6.8	6.2	eT	23.5	21.2
Mondial	2.5	3.8	Mondial	14.2	14.8
BP1	5.0	3.5	BP1	13.6	11.8
a[CO$_2$] aT Mon	0.7 c	0.9 d	a[CO$_2$] aT$^{\#}$	4.4 c	5.4 c
a[CO$_2$] eT Mon	4.8 b	5.5 b	a[CO$_2$] eT$^{\#}$	27.0 a	22.6 a
e[CO$_2$] aT Mon	0.7 c	0.7 d	e[CO$_2$]aT$^{\#}$	3.6 c	5.3 c
e[CO$_2$] eT Mon	3.8 b	8.0 a	e[CO$_2$]eT$^{\#}$	20.1 b	19.8 b
a[CO$_2$] aT BP1	0.7 c	0.9 d			
a[CO$_2$] eT BP1	13.7 a	7.4 a			
e[CO$_2$] aT BP1	0.6 c	1.5 d			
e[CO$_2$] eT BP1	4.8 b	4.0 c			
[CO$_2$]	$p < 0.001$	$p = 0.484$	[CO$_2$]	$p < 0.001$	$p < 0.001$
Temperature	$p < 0.001$	$p < 0.001$	Temperature	$p < 0.001$	$p < 0.001$
Cultivar	$p < 0.001$	$p = 0.092$	Cultivar	$p = 0.041$	$p < 0.001$
[CO$_2$] * Temp * Cultivar	$p < 0.001$	$p < 0.001$	[CO$_2$] * Temp	$p < 0.001$	$p = 0.001$

a, ambient; *e*, elevated; *T*, temperature. Numbers followed by the same letter in the same column do not differ significantly. Stomatal aperture length and width $n = 40$ for single factor, $n = 20$ for two-way interaction, $n = 10$ for three-way interaction, $p = 0.05$), $^{\#}$Applicable to the stomatal length parameters only size of stomata, eT significantly increased the size of stomata. BP1 had shorter but wider stomata aperture on the adaxial surface than Mondial.

Stomatal aperture width on both leaf surfaces was influenced by the three-way interaction between [CO_2], cultivar, and temperature, but stomatal length was only influenced by a two-way interaction between [CO_2] and temperature (Table 2). On both leaf surfaces, eT resulted in increased stomatal width and length, irrespective of [CO_2] or cultivar, but shorter stomatal were found on both surfaces at e[CO_2]eT as compared to a[CO_2]eT. An impact of e[CO_2] at eT was also evident for stomatal width, but this effect varied with leaf surface and cultivar. For Mondial, stomatal width was reduced at e[CO_2]eT as compared to a[CO_2]eT on the abaxial surface, but had no effect on stomatal width on the adaxial surface. In contrast, stomatal width was significantly reduced at e[CO_2]eT compared to a[CO_2]eT for BP1 on both surfaces. Importantly, at aT, an increase in [CO_2] had no effect on stomatal size (width and length) on both leaf surfaces.

Starch Grain Size

An analysis of the single-factor effects found that e[CO_2] did not affect starch grain size (Table 3), while eT significantly reduced starch grain size and Mondial

Table 3 Starch grain size (surface area) in response to CO_2 concentration ([CO_2]) and temperature in trial 2 at 63 days after emergence

Treatment	Starch grain area (μm^2)
a[CO_2]	0.59
e[CO_2]	0.61
aT	0.81
eT	0.39
Mondial	0.63
BP1	0.57
a[CO_2] aT Mondial	1.12 a
a[CO_2] eT Mondial	0.48 d
e[CO_2] aT Mondial	0.80 b
e[CO_2] eT Mondial	0.14 e
a[CO_2] aT BP1	0.58 cd
a[CO_2] eT BP1	0.18 e
e[CO_2] aT BP1	0.76 bc
e[CO_2] eT BP1	0.75 bc
[CO_2]	$p = 0.524$
Temperature	$p = 0.000$
Cultivar	$p = 0.027$
[CO_2] * Temp * Cultivar	$p = 0.001$

a, ambient; *e*, elevated; *T*, temperature. Numbers followed by the same letter in the same column do not differ significantly. Starch grain area $n = 140$ for single factor, $n = 70$ for two-way interaction, $n = 35$ for three-way interaction, $p = 0.05$

had slightly larger leaf starch grains than BP1. The interaction between temperature, e[CO_2], and cultivar significantly affected the starch grain size. In Mondial, eT reduced starch grain size at both a[CO_2] and e[CO_2] when compared to aT. In addition, for Mondial starch grains were smaller when [CO_2] was increased at both aT and eT, with starch grains size reduced at e[CO_2]eT as compared to e[CO_2]aT. The impact of treatments on starch grain size in BP1 was slightly different, with eT only decreasing starch grain size at a[CO_2], but having no effect at e[CO_2] when compared to aT conditions. In contrast to Mondial, e[CO_2]eT resulted in larger starch grains than a[CO_2]eT in BP1, but these starch grains were of similar size to the e[CO_2]aT treatment.

Leaf Area

Elevated [CO_2] significantly increased leaf area (LA) in both trials, while eT significantly reduced LA in both trials (Table 4). Mondial had a higher LA than BP1 for the first two measurement dates in trial 1, but later in the growing season (63 and 140 DAE) LA did not differ between the two cultivars. In trial 2, differences in LA between cultivars were inconsistent across the measurement dates.

In trial 1 the three-way interaction between cultivar, [CO_2], and temperature was only significant at 21 and 42 DAE, while in trial 2 only the two-way interaction between [CO_2] and temperature was significant at three of the four measurements dates (Table 4). Across all measurement dates in trial 1, and in both cultivars, the highest LA was found at e[CO_2]aT. Elevated temperatures reduced LA at both a[CO_2] and e[CO_2] in Mondial, but only consistently at e[CO_2] for BP1. In BP1 eT only reduced LA at a[CO_2] at the last two measurement dates. When comparing the impact of e[CO_2] at eT and aT, it was evident that e[CO_2] increased LA at both temperature regimes in Mondial. In BP1, e[CO_2] increased LA at aT at all measurement dates, but when e[CO_2] and eT were combined, LA was only significantly higher than a[CO_2]eT in the later stages of the trial.

In trial 2, significant differences in the two-way interaction were only found at 21, 63, and 140 DAE. As in trial 1, the highest LA was observed at e[CO_2]aT and eT reduced LA at both a[CO_2] and e[CO_2]. By elevating e[CO_2], the negative impact of eT on LA was partly reduced, as at 63 and 140 DAE LA at e[CO_2]eT was higher than at a[CO_2]eT. However, in trial 2 LA at e[CO_2]eT was significantly lower than at a[CO_2]aT.

Haulm Dry Mass

Evaluation of the single-factor effects of treatments on haulm dry mass (HDM) in both trials revealed that e[CO_2] increased HDM and that BP1 generally had higher HDM than Mondial (Table 5). However, the impact of eT on HDM depended on the duration of the maximum temperature. In trial 1 HDM was significantly increased at eT after 140 DAE as compared to aT, but in trial 2 HDM was significantly reduced by eT across all measurement dates.

Table 4 Leaf area (cm^2 $plant^{-1}$) as affected by CO_2 concentration ([CO_2]), temperature, and variety

	Treatment	21 DAE	42 DAE	63 DAE	140 DAE
Trial 1 (T_{max}: 1 h)	a[CO_2]	1791	2285	3637	7553
	e[CO_2]	5078	5640	7861	14,656
	aT	4570	5248	7399	13,177
	eT	2298	2676	4100	9032
	Mondial	3731	4238	5749	11,212
	BP1	3138	3687	5749	10,997
	a[CO_2] aT Mondial	1985 de	2394 d	4748	9989
	a[CO_2] eT Mondial	969 e	1571 e	2426	5246
	e[CO_2] aT Mondial	8257 a	9124 a	10,192	16,282
	e[CO_2] eT Mondial	3711 c	3862 c	5631	13,331
	a[CO_2] aT BP1	2561 cd	2891 d	4846	9989
	a[CO_2] eT BP1	1647 de	2283 de	2529	4989
	e[CO_2] aT BP1	5478 b	6585 b	9808	16,448
	e[CO_2] eT BP1	2866 cd	2987 d	5814	12,564
	[CO_2]	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Temperature	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Cultivar	$p=0.009$	$p<0.001$	$p=1.000$	$p=0.580$
	[CO_2] * Temp * Cultivar	$p=0.039$	$p=0.004$	$p=0.427$	$p=0.662$
Trial 2 (T_{max}: 4 h)	a[CO_2]	964	2821	3363	7090
	e[CO_2]	1205	3628	6810	11,990
	aT	1575	4782	7504	13,285
	eT	594	1667	2669	5795
	Mondial	1133	3613	4991	8084
	BP1	1036	2836	5182	10,997
	a[CO_2] aT	1310.0 b	4431.2	4933.2 b	9240.3 b
	a[CO_2] eT	618.1 c	1211.6	1792.4 d	4940.5 d
	e[CO_2] aT	1840.3 a	5133.3	10,074.4 a	17,329.7 a
	e[CO_2] eT	570.0 c	2123.0	3545.5 c	6651.1 c
	[CO_2]	$p=0.006$	$p<0.001$	$p<0.001$	$p<0.001$
	Temperature	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Cultivar	$p=0.241$	$p<0.001$	$p=0.543$	$p<0.001$
	[CO_2] * Temp	$p=0.001$	$p=0.555$	$p<0.001$	$p<0.001$

a, ambient; *e*, elevated; *T*, temperature. Numbers followed by the same letter in the same column do not differ significantly. $n=16$ for single factor, $n=8$ for two-way interaction, $n=4$ for three-way interaction, $n=12$ $p=0.05$, *DAE*, days after emergence

In trial 1 the two-way interaction of [CO_2] and temperature was only significant at 140 DAE, while in trial 2 the three-way interaction was significant at the last two measurement dates. In trial 1 eT had no impact on HDM at a[CO_2] and only increased HDM at 140 DAE at e[CO_2] when compared to a[CO_2]aT. However, e[CO_2] increased HDM at both aT and eT. In trial 2, the highest HDM was found at e[CO_2]aT for both cultivars. The impact of a longer duration of maximum

Table 5 Haulm dry mass (g plant^{-1}) response to CO_2 concentration ([CO_2]) and temperature treatment combinations in trials 1 and 2

	Treatment	21 DAE	42 DAE	63 DAE	140 DAE
Trial 1 (T_{max}: 1 h)	a[CO_2]	19.5	29.4	61.4	127.1
	e[CO_2]	40.3	54.6	112.4	210.2
	aT	30.6	42.5	83.3	160.9
	eT	29.3	41.6	90.6	176.4
	Mondial	28.1	38.4	78.7	157.4
	BP1	31.7	45.7	95.1	179.9
	a[CO_2] aT	21.29	30.15	59.21	131.35 c
	a[CO_2] eT	17.75	28.73	63.68	122.80 c
	e[CO_2] aT	39.84	54.84	107.31	190.35 b
	e[CO_2] eT	40.77	54.40	117.42	229.95 a
	[CO_2]	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Temperature	$p=0.334$	$p=0.666$	$p=0.943$	$p=0.034$
	Cultivar	$p=0.012$	$p=0.002$	$p=0.035$	$p=0.034$
	[CO_2]*Temp	$p=0.102$	$p=0.820$	$p=0.117$	$p<0.001$
Trial 2 (T_{max}: 4 h)	a[CO_2]	6.3	31.8	50.7	189.3
	e[CO_2]	9.9	50.2	101.8	409.6
	aT	11.3	51.1	98.5	336.3
	eT	4.9	30.9	54.0	262.5
	Mondial	8.0	40.1	68.0	282.1
	BP1	8.1	41.8	83.8	316.8
	a[CO_2] aT Mondial	8.0	41.7	64.7 cd	176.4 de
	a[CO_2] eT Mondial	4.9	18.0	28.3 e	147.9 e
	e[CO_2] aT Mondial	14.9	61.6	131.0 a	462.5 a
	e[CO_2] eT Mondial	4.2	39.1	50.8 cde	341.4 b
	a[CO_2] aT BP1	7.1	40.1	71.0 c	212.5 cd
	a[CO_2] eT BP1	5.0	27.4	38.9 de	220.2 c
	e[CO_2] aT BP1	15.0	60.9	127.3 a	493.8 a
	e[CO_2] eT BP1	5.3	38.9	98.1 b	340.6 b
	[CO_2]	$p=0.006$	$p<0.001$	$p<0.001$	$p<0.001$
	Temperature	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Cultivar	$p=0.881$	$p=0.390$	$p=0.001$	$p<0.001$
	[CO_2] * Temp * Cultivar	$p=0.999$	$p=0.193$	$p=0.007$	$p=0.004$

a, ambient; *e*, elevated; *T*, temperature. Numbers followed by the same letter in the same column do not differ significantly. $n=16$ for single factor, $n=8$ for two-way interaction, $n=4$ for three-way interaction $p=0.05$, *DAE*, days after emergence

temperature was very evident in trial 2, with HDM reduced at 42 and 63 DAE in Mondial at a[CO_2]eT when compared to a[CO_2]aT, while at e[CO_2] eT reduced HDM at each time point when compared to e[CO_2]aT. By elevating [CO_2] at eT, HDM increased relative to a[CO_2]eT at 42 and 140 DAE. Haulm dry mass was only significantly increased at 140 DAE at e[CO_2]eT, when compared to a[CO_2]aT. The impact of eT on HDM for BP1 was similar to Mondial, with HDM reduced at every time point for e[CO_2]eT compared to e[CO_2]aT. However, for BP1 HDM was only decreased at 63 DAE in a[CO_2]eT when compared to a[CO_2]aT. When comparing the effect of eT at a[CO_2] and e[CO_2], it was evident that at 63 and 140 DAE e[CO_2] increased HDM when compared to a[CO_2]. At these two time points, e[CO_2]eT also significantly increased HDM relative to that at ambient conditions (a[CO_2]aT).

Tuber Initiation, Yield, Harvest Index, and Total Plant Biomass

Elevated [CO_2] increased the number of tubers initiated at 21 and 42 DAE in both trials and both cultivars under aT (Table 6), which resulted in increased tuber fresh and dry mass (Table 6). As a result, total plant dry mass and harvest index increased under e[CO_2]. Mondial produced significantly more tubers than BP1 at 42 DAE, which was reflected in the higher yields for Mondial (Table 6). Although eT inhibited tuberisation at 21 and 42 DAE, some tubers were produced at the end of trial 1 at eT. However, in trial 2 the longer duration of maximum temperatures completely inhibited tuberisation and no tuber yield was realised.

In trial 1, eT reduced potato fresh and dry mass and total plant dry mass at both a[CO_2] and e[CO_2] when compared to aT (Table 7). As a result, harvest index was also reduced when temperatures were elevated. The opposite was true when [CO_2] was increased, with a positive effect of e[CO_2] on tuber fresh and dry mass at both eT and aT when compared to a[CO_2]. The highest yields were achieved at e[CO_2]aT.

Table 6 The number of tubers initiated at 21 and 42 DAE as affected CO_2 concentration ([CO_2]) and cultivar

	Trial 1 (T_{max}: 1 h)		Trial 2 (T_{max}: 4 h)	
Treatment	21 DAE	42 DAE	21 DAE	42 DAE
a[CO_2]	2	10	16	30
e[CO_2]	6	24	28	56
aT	4	17	22	43
eT[1]	-	-	-	-
Mondial	6	20	33	66
BP1	1	13	12	21
a[CO_2] Mondial	7 b	24	24 b	46 b
e[CO_2] Mondial	18 a	56	42 a	85 a
a[CO_2] BP1	0 d	15	8 d	14 d
e[CO_2] BP1	4 c	39	15 c	27 c
[CO_2]	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Cultivar	$p<0.001$	$p=0.009$	$p<0.001$	$p<0.001$
[CO_2] * Cultivar	$p=0.001$	$p=0.409$	$p=0.018$	$p<0.001$

[1] No data given for eT due to a complete inhibition of tuberisation. *a*, ambient; *e*, elevated; *T*, temperature. Numbers followed by the same letter in the same column do not differ significantly. $n=16$ for single factor, $n=8$ for two-way interaction, $p=0.05$, *DAE,* days after emergence

Table 7 Fresh and dry tuber yield (g $plant^{-1}$), harvest index and total plant dry mass (g $plant^{-1}$) at 140 DAE as affected by CO_2 concentration ($[CO_2]$), temperature, and variety

		Tuber yield (g $plant^{-1}$)		Harvest index	Total plant dry mass (g $plant^{-1}$)
		Fresh yield	Dry mass		
	Treatment	140 DAE	140 DAE		
Trial 1 (T_{max} duration = 1 h)	$a[CO_2]$	309	61	0.30	187.7
	$e[CO_2]$	1291	300	0.56	513.2
	aT	1114	251	0.53	416.0
	eT	486	110	0.33	285.0
	Mondial	889	204	0.45	370.3
	BP1	711	156	0.41	330.7
	$a[CO_2]$ aT	457 c	87 c	0.39	221.8 c
	$a[CO_2]$ eT	161 d	33 d	0.21	153.6 d
	$e[CO_2]$ aT	1770 a	412 a	0.67	610.2 a
	$e[CO_2]$ eT	812 b	187 b	0.45	416.2 b
	$[CO_2]$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Temperature	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	Cultivar	$p<0.001$	$p<0.001$	$p=0.047$	$p<0.001$
	$[CO_2]$ * Temp	$p<0.001$	$p<0.001$	$p=0.335$	$p<0.001$
Trial 2 (T_{max} duration = 4 h)	$a[CO_2]$	1019	200	0.51	289.1
	$e[CO_2]$	2377	516	0.50	668.7
	aT	2208	358	0.51	694.2
	eT	-	-	-	-
	Mondial	1961	436	0.57	500.2
	BP1	1434	280	0.44	456.6
	$a[CO_2]$ Mondial	1117	229 c	0.56	276.8 c
	$e[CO_2]$ Mondial	2804	643 a	0.58	723.6 a
	$a[CO_2]$ BP1	920	170 c	0.44	301.4 c
	$e[CO_2]$ BP1	1949	389 b	0.44	611.8 b
	$[CO_2]$	$p<0.001$	$p<0.001$	$p=0.824$	$p<0.001$
	Cultivar	$p=0.014$	$p=0.004$	$p<0.001$	$p=0.069$
	$[CO_2]$ * Cultivar	$p=0.095$	$p=0.045$	$p=0.616$	$p=0.007$

a, ambient; *e*, elevated; *T*, = temperature. Numbers followed by the same letter in the same column do not differ significantly. $n=16$ for single factor, $n=8$ for two-way interaction $n=4$ for three-way interaction, $p=0.05$, *DAE*, days after emergence

Importantly, tuber yields were significantly higher at $e[CO_2]$eT than $a[CO_2]$aT. Differences in harvest index and total plant dry mass between treatments mirrored the differences in yield.

In trial 2, due to the absence of tuber formation at eT, only the $[CO_2]$ and cultivar interaction was considered at aT. As in trial 1, tuber fresh yield, dry mass yield, and total plant dry mass were increased by $e[CO_2]$. The two cultivars had comparable fresh and dry yield when grown at $a[CO_2]$; however, at $e[CO_2]$ Mondial tended to have higher tuber dry yield than BP1. Despite an impact on yield, $[CO_2]$ had no effect on harvest index, but Mondial had a higher harvest index than BP1 regardless of the [CO_2]. The two-way interaction between [CO_2] and temperature was significant for total plant dry mass. In both trials e[CO_2] increased total plant dry mass at aT and eT, but at the same [CO_2] eT reduced total plant dry mass, with the lowest total plant dry mass found at a[CO_2]eT. In trial 1, plants grown at e[CO_2]eT had significantly higher total plant dry mass compared to a[CO_2]aT, but in trial 2 when the maximum temperature was sustained for longer, these two treatments had comparable total dry matter.

In both trials, e[CO_2] led to an increase in total plant dry mass (TPDM) relative to a[CO_2]. Comparing the two cultivars in trial 1 revealed that Mondial exhibited higher TPDM compared to BP1, whereas the cultivars displayed similar TPDM in trial 2. During trial 1, where the eT effect was evaluated, it was observed that eT resulted in a reduction in TPDM as compared to aT.

In trial 1, it was observed that eT negatively affected TPDM at both a[CO_2] and e[CO_2] levels. However, e[CO_2] exhibited a significant ameliorative effect that resulted in higher TPDM at e[CO_2]eT as compared to a[CO_2]eT. The lower TPDM at a[CO_2]eT as compared to a[CO_2]aT further emphasised the impact of eT on TPDM. Notably, the plants grown at e[CO_2]eT accumulated higher TPDM compared to those grown at a[CO_2]aT, indicating a positive impact of e[CO_2] on TPDM at eT. In the second trial, e[CO_2] significantly increased TPDM in both cultivars, with Mondial having higher TPDM compared to BP1. It was observed that the TPDM of the two cultivars was comparable at a[CO_2].

Discussion

Elevated temperature had a negative impact on tuber yield at both a[CO_2] and e[CO_2]. Although tuber initiation was delayed when the maximum temperature of 38 ℃ was sustained for 1 h during the day, tubers were still produced. However, when the maximum temperature of 38 ℃ was sustained for 4 h, tuber initiation was completely inhibited. Both cultivars therefore produced tubers under elevated growth temperatures, which were far above the optimum temperatures reported for tuber initiation in potato of between 15 and 19 ℃ (Van Dam et al. 1996). The susceptibility of tuberisation to high temperatures has been widely reported (Hancock et al. 2014; Paul et al. 2016; Kim et al. 2017; Dahal et al. 2019; Al Mahmud et al. 2021) and is largely attributed to the inhibition of the tuberisation signal (Hancock et al. 2014; Zhou et al. 2023). The substantially higher soil temperature in the planting bags in trial 2 (±32 ℃ at $T_{max}=38$ ℃, compared to 21 ℃ at $T_{max}=25$ ℃) could have resulted in the inhibition of the tuberisation signal, explaining why no tubers were formed at eT in trial 2. The importance of below ground temperatures on the inhibition of tuber formation was reported by Zhou et al. (2023). Furthermore, these authors also found that if only shoots or below ground parts were exposed to high temperatures, then tubers were still formed; however, if the entire plant was exposed to high temperatures, then tuber formation was inhibited (Zhou et al. 2023).

Elevated [CO_2] significantly increased tuber yields at aT in both trials, as was previously reported for potato (Donnelly et al. 2001; Miglietta et al. 2002; Bishop et al. 2014). However, the ability of e[CO_2] to alleviate the negative effects of eT on yield was dependent on the duration of the T_{max} period. Although significantly higher yields were found at e[CO_2]eT in trial 1, as compared to a[CO_2]eT, when T_{max} was sustained for 4 h (trial 2), the ameliorating effect of elevated CO_2 was completely lost and no tubers were formed. In addition, in trial 1 e[CO_2] could only partially alleviate the negative effect of eT on yield, as plants grown under e[CO_2]aT had higher tuber yields than the e[CO_2]eT treatment. However, in trial 1 yield of the e[CO_2]eT treatment was still higher than under ambient conditions (a[CO_2]aT). The ability of e[CO_2] to alleviate the negative effects of moderately high temperatures on potato yield was also reported by Lee et al. (2020), but in their study the maximum temperature was 25 ℃ and this was only implemented after emergence. When maximum temperature was increased to 35 ℃, with e[CO_2] at 700 ppm (Chen and Setter 2021), a reduction in tuberisation and yield was observed as compared to 25 ℃ and a[CO_2], suggesting e[CO_2] could only partially alleviate the impact of high temperature on tuberisation. However, Chen and Setter (2021) only implemented treatments during either tuber production or tuber bulking. There is therefore no comparative study where treatments were applied through all stages of potato growth, and at 38 °C, with evidence from the current study indicating that the amelioration effect of e[CO_2] on yield is possible at higher maximum temperatures ($T_{max} = 38$ °C) and for the duration of the growth cycle, provided the duration of the maximum temperature during the day is quite short.

Similar observations were reported of increased yield in wheat (Cai et al. 2016) and rice (Yoshinaga et al. 2020; Gao et al. 2021) grown in FACE studies. While there are limited reports of FACE studies where elevated temperature was applied concurrently with e[CO_2] (T-FACE), Cai et al. (2016) reported that e[CO_2] did not compensate for the negative eT effects in rice, contrasting the present evidence of partial alleviation of the negative eT effects on potato tuber yield by e[CO_2]. Therefore, while the response to e[CO_2] in growth chambers was comparable to FACE studies, contrasting results were observed for T-FACE.

FACE experiments were designed to overcome a majority of the challenges similar to those reported in the present study, by allowing for plant assessment under field conditions compared to growth chambers, which are deemed unnatural due to pot restrictions as well as artificial lighting conditions (Allen et al. 2020). Despite their advantages, FACE trials have often been found to exhibit poor crop performance due to fluctuating [CO_2] (Allen et al. 2020; Dahal et al. 2023). Although with some limitations, growth chamber trials allow for more accurate control of environmental factors such as [CO_2] and temperature (Ziska and Bunce 2007).

Both temperature and [CO_2] impacted final total plant dry mass in both trials. Plants grown at e[CO_2]aT had the highest total plant dry mass, haulm dry mass, leaf area, and tuber fresh and dry mass, supporting previous findings of an overall stimulation of potato growth at e[CO_2] (Donnelly et al. 2001; Miglietta et al. 2002; Bishop et al. 2014). In contrast, eT decreased total dry matter when compared to aT, irrespective of [CO_2] in both trials, with plants grown at a[CO_2]eT having the lowest total plant dry mass. The ability of e[CO_2] to partially alleviate the negative effect of eT on dry matter accumulation was evident in both trials, where by the end of both trials total plant dry mass was either comparable (trial 2) or higher (trial 1) than total plant dry mass at a[CO_2]aT. As with yield, this amelioration effect was greater when temperatures were only elevated for 1 h of the day. High temperatures also reduced the HI in trial 1 at a[CO_2], where both tuber yield and total plant dry mass were significantly reduced at eT relative to aT. However, the HI of plants grown at e[CO_2]eT was comparable to that of plants grown at a[CO_2]aT, despite yields and total plant dry mass being higher at e[CO_2]eT. This can be attributed to a change in partitioning of assimilates under e[CO_2] and eT, which resulted in taller plants with greater HDM and leaf area, as previously reported by Wolf et al. (1990) and Mokrani et al. (2023). The HI values observed in trial 1 were all lower than the reference value of 0.75 for potato (Kooman and Haverkort 1995; Molahlehi et al. 2013), which could largely be attributed to the lower-than-expected yield per plant for both cultivars and much taller plants than usual, resulting from increased stem elongation in all treatments. This could be due to the very close plant spacing in the growth chambers resulting in increased stem elongation (data not presented), thus increasing the above-ground growth and consequently resulting in a general reduction in HI. The increased elongation under densely spaced potato plants has previously been reported (Van der Zaag et al. 1990).

Although a positive link between *Anet* and biomass accumulation was observed at aT, in trial 2 the same was not found at eT, where despite higher *Anet* rates at e[CO_2]eT than e[CO_2]aT from 42 DAE onwards, significantly lower total dry mass was accumulated. While part of the differences in total plant dry mass between these two treatments can be explained by the lack of the tubers, HDM was also significantly lower at e[CO_2]eT compared to e[CO_2]aT. A similar lack of correlation between *Anet* rates and dry matter accumulation was observed between the temperature treatments at a[CO_2]. This suggests that even though e[CO_2] can alleviate the negative effect of eT on photosynthesis of potato, the increase in *Anet* at eT cannot meet the increase in whole plant maintenance respiration at eT (Timlin et al. 2006), resulting in a decline in total plant dry mass production.

The increase in *Anet* at e[CO_2]aT, as compared to a[CO_2]aT, was associated with an increase in *gsw* from 42 to 63 DAE, while at 84 DAE *gsw* was decreased at e[CO_2]. Typically, reduced *gsw* under e[CO_2] has been reported before (Leakey et al. 2006; Ainsworth and Rogers 2007; Habermann et al. 2019; Lee et al. 2020; Ahmadi-Lahijani et al. 2021), as well as down-regulation in *Anet* following long-term exposure to e[CO_2] (Ludewig et al. 1998; Schapendonk et al. 2000). This was not observed in the current study and after 84 DAE *Anet* rates were still higher at e[CO_2] when compared to a[CO_2] despite lower *gsw*, which could be attributed to the high CO_2 diffusion gradient in the leaf at e[CO_2] (Pastore et al. 2019). Although Ludewig et al. (1998) and Schapendonk et al. (2000) also reported increased starch accumulation in potato leaves in response to e[CO_2], a change in average starch grain size under e[CO_2] was not observed in the present study, suggesting rapid transport of assimilates away from leaves in plants exposed to e[CO_2] (Ainsworth and Bush 2010; Ainsworth and Lemonnier 2018), even when no tubers were present under eT. The high *Anet* under e[CO_2] can be explained as follows. Since the current [CO_2] in the atmosphere is a limiting factor for *Anet* in C_3 plants, e[CO_2] increases the substrate for Rubisco, thereby enhancing potato *Anet* (George et al. 2017). This is achieved through the e[CO_2] driven inhibition of the oxygenation reaction of

Rubisco, which reduces photorespiratory carbon loss in favour of the carboxylation reaction (Long et al. 2004).

Temperatures above the optimum (29 ℃) have been reported to negatively impact *Anet* in heat-sensitive potato cultivars (Hastilestari et al. 2018). The impact of eT on *Anet* at a[CO_2] was inconsistent in the current study, with a decline noted at 21 DAE, but an increase at 42 and 63 DAE, while chlorophyll fluorescence measurements (data not presented) did not indicate damage to PSII in these plants (Fleisher and Timlin 2006; Timlin et al. 2006). This suggests that under simulated field conditions, short exposure to high temperatures might not have such a negative impact of *Anet* in potato, but this does not necessarily translate into any positive effects on yield. Elevated temperature was associated with increased *gsw* and increased stomatal width and length, but only at e[CO_2] was this increase in *gsw* associated with an increase in *Anet*. Increased *gsw* and stomatal pore size in response to elevated temperatures were previously reported (Radin et al. 1994; Zhou et al. 2015; Fauset et al. 2019) and perhaps reflect the need for increased evaporative cooling to prevent leaf temperatures from reaching dangerously high levels (Julia and Dingkuhn 2013; Urban et al. 2017). It is unlikely that stomatal responses were driven by either water-deficit stress or high VPD, as the plants were well watered and relative humidity in the chamber was maintained between 60 and 70%.

While the study confirmed that eT negatively impacts yield of potatoes, it also demonstrated that for the cultivars used in the present study, tuberisation can occur at temperatures far higher than the optimum range for tuberisation reported in literature, despite a slight time delay in initiation. Acceptable tuber yields can still be realised with substantial elevation in growth temperature, but only when the daily exposure to eT is relatively short. The current study also provides evidence of an e[CO_2] amelioration effect on eT in terms of potato yields at 1 h of exposure to high temperatures (38 °C). However, in regions where potato is currently grown under relatively hot weather, sustained high temperatures during tuberisation will likely lead to greatly reduced yields and even crop failures. This study was conducted in growth chambers with relatively high relative humidity and in future it will be important to assess the impact of hot and dry climatic conditions on plant growth, yield, and *Anet*, as these conditions often occur under field conditions. Under such conditions, *gsw* could be reduced because of high vapour pressure gradients between the atmosphere and the leaf, which could impact leaf temperatures and *Anet*.

Conclusions

In conclusion, potato yield was negatively impacted by eT in the current study, but the negative impact of eT on yield was mitigated by e[CO_2], although tuberisation was delayed when maximum temperatures were maintained for 1 h (trial 1). However, tuber initiation in both cultivars used was negatively impacted by exposure to eT for a longer duration of the day ($T_{max} = 4$ h; trial 2), which resulted in no tuber yields at both ambient and elevated [CO_2]. It is proposed that the amelioration effect of e[CO_2] on plant growth and yield is most likely due to increased *Anet* rates, which availed more photo-assimilates for both tuber bulking and plant growth.

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Declarations

Conflict of Interest The corresponding author is an editorial board member of *Potato Research*.

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