Effects of Elevated CO₂ and Temperature on Yield and Fruit Quality of Strawberry (Fragaria × ananassa Duch.) at Two Levels of Nitrogen Application

Peng Sun¹, Nitin Mantri², Heqiang Lou¹, Ya Hu¹, Dan Sun¹, Yueqing Zhu¹, Tingting Dong¹, Hongfei Lu¹*

¹ College of Chemistry and Life Science, Zhejiang Normal University, Jinhua, China, ² School of Applied Sciences, Health Innovations Research Institute, RMIT University, Melbourne, Victoria, Australia

Abstract

We investigated if elevated CO₂ could alleviate the negative effect of high temperature on fruit yield of strawberry (Fragaria × ananassa Duch. cv. Toyonoka) at different levels of nitrogen and also tested the combined effects of CO₂ temperature and nitrogen on fruit quality of plants cultivated in controlled growth chambers. Results show that elevated CO₂ and high temperature caused a further 12% and 35% decrease in fruit yield at low and high nitrogen, respectively. The fewer inflorescences and smaller umbel size during flower induction caused the reduction of fruit yield at elevated CO₂ and high temperature. Interestingly, nitrogen application has no beneficial effect on fruit yield, and this may be because of decreased sucrose export to the shoot apical meristem at floral transition. Moreover, elevated CO₂ increased the levels of dry matter-content, fructose, glucose, total sugar and sweetness index per dry matter, but decreased fruit nitrogen content, total antioxidant capacity and all antioxidant compounds per dry matter in strawberry fruit. The reduction of fruit nitrogen content and antioxidant activity was mainly caused by the dilution effect of accumulated non-structural carbohydrates sourced from the increased net photosynthetic rate at elevated CO₂. Thus, the quality of strawberry fruit would increase because of the increased sweetness and the similar amount of fruit nitrogen content, antioxidant activity per fresh matter at elevated CO₂. Overall, we found that elevated CO₂ improved the production of strawberry (including yield and quality) at low temperature, but decreased it at high temperature. The dramatic fluctuation in strawberry yield between low and high temperature at elevated CO₂ implies that more attention should be paid to the process of flower induction under climate change, especially in fruits that require winter chilling for reproductive growth.

Introduction

The Intergovernmental Panel on Climate Change (IPCC) reported that rising temperatures, drought, floods, desertification and weather extremes will severely affect agricultural production, especially in developing countries [1]. The CO₂ concentration near the ground level has risen from 280 mmol mol⁻¹ in the pre-industrial times to the present 390 mmol mol⁻¹ [1]. At the present rate of emission, CO₂ concentration is projected to be in the range of 500–1000 mmol mol⁻¹ by the end of this century, which will potentially increase global temperature by 1.8–5.8°C [1]. Higher temperature individually or along with the ongoing global increase of atmospheric CO₂ could affect various physiological and morphological traits of crops that subsequently influence crop growth and final yield. As estimated by Xiong et al. [2], in China, without the CO₂ fertilization effect, grain yields of rice, wheat and maize would fall consistently if temperature rises by 2.5°C; even taking the CO₂ fertilization effect into account, the yield reductions of these crops would still occur if temperature rises by 3.9°C. Therefore, it’s necessary and important to conduct research focusing on the combined effect of elevated CO₂ and increased temperature on crop yield.

Strawberry (Fragaria × ananassa Duch.) is one of the most important fruit crops that is widely planted in North America, Mediterranean Europe, Southwest Asia, and Australia [3]. Shortened photoperiod and low temperature are known to induce flower formation for June-bearing strawberries [4]. Kumakura and Shishido [5] suggested that maximum strawberry yields are associated with a narrow range of temperatures between 15 and 20°C. The yield is reduced when the day temperature exceeded 25°C, even if the diurnal mean temperature is maintained below 20°C. Therefore, in the event of increased temperatures due to global warming, strawberry production would be severely affected. Currently, there is little knowledge of the combined effects of high temperature and elevated CO₂ on strawberries or other crops, although published data suggest that such interaction is critical. Chen et al. [6] reported that elevated CO₂ levels greatly improved yield and fruit quality of strawberry by increasing the total fruit number per plant, average fruit fresh weight, dry matter content, fruit total sugars and sugar/acid ratio. On the contrary, combined effect of elevated CO₂ and temperature on other C₃ crops such as rice, soybean, dry bean, peanut, cowpea, wheat and cotton cultivated in different growth conditions, including growth...
chambers, open-top chambers and plastic tunnels, showed no beneficial effect on yield [7–13]. However, strawberries require much lower temperature than these crops, and it is important to test whether elevated CO₂ will ameliorate the negative effects of the increased temperature on its reproductive development.

Nitrogen is one of the most important resources limiting plant growth and seed production in natural and agricultural ecosystems [14]. An increase in carbon availability due to elevated CO₂ may enhance nitrogen limitation, leading to a reduction in plant nitrogen concentration [15]. Studies on spring wheat and rice suggested that under elevated CO₂ concentration, nitrogen fertilization had important influence on the maintenance and continuing increase of crop yield [16–18]. The deeper and larger root system with nitrogen fertilization, which is of benefit to the use of soil moisture and nutrient, is thought to be the reason of continuing increase of crop yield [16]. Deng and Woodward [19] reported high CO₂ increased the strawberry fruit yield by 42% at high nitrogen supply and 17% at low nitrogen supply through an increase in flower and fruit number of individual plants. However, they did not analyze the effect of high temperature, elevated CO₂ and nitrogen supply on strawberry fruit production and quality.

Strawberries are a good source of natural antioxidants [20]. In addition to the usual nutrients, such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic acids [20]. Strawberries have shown a remarkably high scavenging activity toward chemically generated radicals, thus making them effective in inhibiting oxidation of human low-density lipoproteins [20]. At elevated CO₂, decrease, no change, and an increase in fruit antioxidant activity have been reported [21–24]. Levine and Paré [24] showed in scallions that both, total phenol and total antioxidant activity decrease under elevated CO₂. They suggested that besides species differences, in the absence of stress, plant grew with minimum investment in antioxidant compounds to maintain a basal defense level under elevated CO₂. Contrastingly, the increase of fruit antioxidant activity may stem from the reduction of fruit nitrogen concentration induced by the elevated CO₂. As a 'physiological trade-off', the amount of secondary metabolites like phenolics increases at low nitrogen to maintain the growth-differentiation balance (GDB) framework [25]. Further, the antioxidant activity of plant tissues also increases as reactive oxygen species (ROS) that are involved in the signaling and perception of nitrogen deficiency increase [26].

Due to the prediction of climate change, a number of studies have examined the effects of rising CO₂ and/or temperature on yield characteristics, notably quantity and nutrition of food crops [7–13,16–19]. However, almost nothing is known regarding the concurrent interaction of CO₂, temperature and nutrition (e.g. N) on reproductive biology of fruit crops. This is the first study to undertake an assessment of these potential interactions. Further, unlike the crops that have been studied, the temperature requirement for strawberry cultivation is quite low and the response of strawberry to the increased temperature may therefore be different to other crops. Therefore, we assessed the combined effects of CO₂ concentration, air temperature and nitrogen application on the fruit yield and quality of strawberry. Firstly, we examined the fruit yield under these abiotic factors, and tested whether elevated CO₂ can modify the response of fruit yield to elevated temperature. The effects of nitrogen supply on the response to fruit yield at elevated CO₂ concentration and temperature were also studied. Secondly, we examined the combined effects of CO₂ concentration, temperature and nitrogen supply on fruit quality such as carbohydrate accumulation, nitrogen content and antioxidant levels.

Results

Variation in Fruit Weight and Yield

The abbreviations for the combined treatments of different CO₂ concentrations, temperatures and nitrogen concentrations reported below are explained in Table 1. Elevated CO₂ increased fruit yield (viz. total fruit dry weight per plant) at low temperature, but decreased it at high temperature, when compared to the corresponding treatments in ambient CO₂ (Figure 1a). The greatest fruit yield was in high CO₂, low temperature and low nitrogen treatment (C), while the least was in high CO₂, high temperature and high nitrogen treatment (CTN). The plants grown at low nitrogen concentration had greater yield than those grown at high nitrogen concentration, except for plants grown in low CO₂, low temperature and low nitrogen treatment (ck).

Strawberries are also rich in anthocyanins, flavonoids, and phenolic acids [20]. At elevated CO₂, decrease, no change, and an increase in fruit antioxidant activity have been reported [21–24]. Levine and Paré [24] showed in scallions that both, total phenol and total antioxidant activity decrease under elevated CO₂. They suggested that besides species differences, in the absence of stress, plant grew with minimum investment in antioxidant compounds to maintain a basal defense level under elevated CO₂. Contrastingly, the increase of fruit antioxidant activity may stem from the reduction of fruit nitrogen concentration induced by the elevated CO₂. As a 'physiological trade-off', the amount of secondary metabolites like phenolics increases at low nitrogen to maintain the growth-differentiation balance (GDB) framework [25]. Further, the antioxidant activity of plant tissues also increases as reactive oxygen species (ROS) that are involved in the signaling and perception of nitrogen deficiency increase [26].

Due to the prediction of climate change, a number of studies have examined the effects of rising CO₂ and/or temperature on yield characteristics, notably quantity and nutrition of food crops [7–13,16–19]. However, almost nothing is known regarding the concurrent interaction of CO₂, temperature and nutrition (e.g. N) on reproductive biology of fruit crops. This is the first study to undertake an assessment of these potential interactions. Further, unlike the crops that have been studied, the temperature requirement for strawberry cultivation is quite low and the response of strawberry to the increased temperature may therefore be different to other crops. Therefore, we assessed the combined effects of CO₂ concentration, air temperature and nitrogen application on the fruit yield and quality of strawberry. Firstly, we examined the fruit yield under these abiotic factors, and tested whether elevated CO₂ can modify the response of fruit yield to elevated temperature. The effects of nitrogen supply on the response to fruit yield at elevated CO₂ concentration and temperature were also studied. Secondly, we examined the combined effects of CO₂ concentration, temperature and nitrogen supply on fruit quality such as carbohydrate accumulation, nitrogen content and antioxidant levels.

Variation in Taste and Health-related Compounds

Compared to the corresponding treatments in ambient CO₂, elevated CO₂ decreased the antioxidant compounds and total...
antioxidant capacity (in simple terms, antioxidant activity) of strawberry fruit in both high-temperature and low-temperature treatments (Table 3). As expected, the response of antioxidant capacity to the CO$_2$ and temperature treatments was altered by nitrogen application, which increased at elevated CO$_2$ but decreased in ambient CO$_2$ with increasing nitrogen supply (Table 3). CO$_2$ and nitrogen both significantly affected the total antioxidant capacity and all antioxidant compounds in strawberry fruit (Table 4). Compared to the corresponding treatments in ambient CO$_2$, anthocyanin (AC) content decreased 27% in CT treatment and 48% in C treatment, but decreased only 1% and 4% in CTN and CN treatments, respectively (Table 3). There were significant CO$_2$-temperature-nitrogen ($C \times T \times N$), CO$_2$-temperature ($C \times T$), CO$_2$-nitrogen ($C \times N$), and temperature-nitrogen ($T \times N$) interactions affecting AC (Table 4). The treatment effects on total phenolics (TP) closely matched that of AC. Strawberry fruits showed a 27% and 21% decline in TP levels in CT and C treatments, respectively, but decreased only 8% in CTN treatment and 10% in CN treatment, when compared to the corresponding treatments in ambient CO$_2$ (Table 3). There were significant CO$_2$ and nitrogen main effects, and significant $C \times T$, $C \times N$ and $T \times N$ interactions affecting TP (Table 4). The total flavonoid (TF) decreased 31% and 36% in CT and C treatments, respectively, but decreased only 13% in both CTN and CN treatments, when compared to the corresponding treatments in ambient CO$_2$ (Table 3). Besides significant CO$_2$, temperature and nitrogen main effects, all interactions also affected the TF levels under various treatments (Table 4). Total antioxidant capacity measured using the free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method decreased 28% in CT treatment and 20% in C treatment, but decreased 12% and 13% in CTN and CN treatments, respectively (Table 3). Comparatively, total antioxidant capacity measured using the 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method, which closely matched DPPH, decreased approximately 19% and 18% in CT and C treatments, respectively, but decreased 12% in CTN treatment and 8% in CN treatment, when compared with the corresponding treatments in ambient CO$_2$ (Table 3). There were significant $T \times N$, $C \times T$, and $C \times N$ interactions affecting DPPH levels (Table 4). Similarly, all interactions had significant effects on the levels of ABTS in different treatments (Table 4). Fruit nitrogen contents (FNC) at elevated CO$_2$ were similar to the corresponding treatments in ambient CO$_2$, except the one in C treatment (Table 3). Contrastingly, high temperature increased the levels of FNC among all treatments with only an exception of T treatment. Not surprisingly, nitrogen application significantly increased the levels of FNC. There were only significant nitrogen and temperature main effects on FNC level (Table 4).

The concentrations of three main sugars (viz., fructose, glucose and sucrose) were also determined for each treatment; fructose and glucose were quantitatively the most important in this study. The contents of fructose and glucose at elevated CO$_2$ were almost 1.3 times (1.29 and 1.35 times, respectively) higher than in ambient CO$_2$ regardless of temperature and nitrogen treatments (Table 5). There was no significant difference in sucrose concentration in different treatments. There was significant effect of CO$_2$, temperature, and $C \times T$, $C \times N$ and $C \times T \times N$ interactions on fructose concentration ($P<0.05$), whilst only CO$_2$, $C \times T$ and $C \times T \times N$ interactions significantly affected glucose concentration in different treatments (Table 6). Total sugars per gram fresh weight (TSW) averagely increased 43% under elevated CO$_2$ regardless of temperature and nitrogen treatments (Table 5), and CO$_2$ had a significant effect on TSW when compared to other factors (Table 6). Despite differences in the sugar distribution among the treatments, the ranking of sweetness index (SI) was similar to the ranking of total sugars (per fresh weight) from 86.4 to 128.8 relative units. CO$_2$ effect was significant as it resulted in a 49%, 38%, 45% and 36% increase in SI in CT, CTN, C and CN treatments, respectively (Table 5, 6), when compared to the corresponding treatments in ambient CO$_2$.

### Discussion

#### Variation in Fruit Number, Weight and Yield of Strawberry

Fruit yield of strawberry per plant is composed of fruit dry weight (FDW) and fruit number (FN), while FDW is affected by the total achene number (TAN) and dry matter accumulated per achene (DMA), since achenes (actual seeds) are considered to be involved in regulating strawberry fruit development [27]. Therefore, the treatment effects on either FDW or FN will highlight the effect on fruit yield under those treatments.

Compared to the corresponding treatments in ambient CO$_2$ (T and TN treatments), elevated CO$_2$ further reduced the fruit yield at high temperature (CT and CTN treatments). Yield reductions, which were further enhanced by elevated CO$_2$ at high temperature during flowering and fruit development, also have been documented in other crops such as rice, wheat, grain sorghum, kidney bean, dry bean, soybean, peanut and tomato, though the extreme temperatures were much higher than the one used in this study [7,9–10,28–31]. Commonly, the increased seed abortion caused by decreased pollen production [30], lower pollen

---

**Table 1. Treatments performed in controlled growth chambers that were applied to strawberry plants for nearly 6 months**.

| Description | Denoted |
|-------------|---------|
| Increase in CO$_2$ (720 ppm) | C |
| Increase in temperature (25°C/20°C; day temperature/night temperature) | T |
| Increase in nitrogen fertilizer input (50 ml of 0.1% NH$_4$NO$_3$ twice a week per plant ) | N |
| Increase in CO$_2$ and temperature and nitrogen input | CTN |
| Increase in CO$_2$ and nitrogen input | CN |
| Increase in temperature and nitrogen input | TN |
| Control (360 ppm × 20°C/15°C × without nitrogen input) | ck |

*Values in round brackets indicate the detailed factors designed in the experiment.

doi:10.1371/journal.pone.0041000.t001
reception by stigma due to anther indehiscence [32], and lower pollen viability due to degeneration of tapetum layer and decreased carbohydrate metabolism [33–35] during flower development and opening, resulted in the reduction of crop yield at high temperature. The exact mechanism of the increased susceptibility of these processes to high temperature at elevated CO₂ is still unclear, but the small increase in tissue temperatures (owing to decreased leaf conductance) which reduces the ceiling temperatures for seed-set by about 2°C is one possible explanation [9]. However, in this study, achene abortion (as seed abortion in other crops) caused by the negative impacts of warmer tissue temperatures on flower development and opening were insufficient.

Figure 1. Total fruit dry weight (a), total fruit number (b) and fruit grades (c, d, e) of strawberry plants cultivated under different conditions (mean ± SD, n = 4). The berries were graded in three size classes depending on fruit dry weight (FDW; FDW < 0.4 g, grade 1; 0.4 ≤ FDW ≤ 0.7 g, grade 2; FDW > 0.7 g, grade 3). The frequency distribution of grade 1, 2 and 3 is showed in figures c, d and e, respectively. Bars indicate standard deviation, while * and ** indicate significant differences at P < 0.05 and 0.01, respectively.
doi:10.1371/journal.pone.0041000.g001
to explain the reduction of strawberry yield under high CO₂ concentration. Nitsch [36] reported that fruit production of strawberry was proportional to the extent of achene fertilization, and strawberry fruit size was positively related to the number of

![Figure 2. Correlations between fruit dry weight and total achene number (TAN) of strawberry fruits for plants grown in different conditions (a, b, c and d). The linear regression: y = a x + b.](image)
doi:10.1371/journal.pone.0041000.g002

### Table 2. Correlations between fruit dry weight and total achene number (TAN) of strawberry fruits grown in different conditions.

| Treatments | a    | b    | r²     |
|------------|------|------|--------|
| CT         | 0.0165 | -0.539 | 0.913** |
| CTN        | 0.0150 | -0.541 | 0.657** |
| C          | 0.0159 | -0.463 | 0.771** |
| CN         | 0.0177 | -0.617 | 0.815** |
| T          | 0.0117 | -0.360 | 0.808** |
| TN         | 0.0104 | -0.020 | 0.725** |
| ck         | 0.0136 | -0.611 | 0.906** |
| N          | 0.0093 | -0.148 | 0.563** |

*the linear regression: y = a x + b, a-slope of linear regression, b-increment of linear regression, r-correlation coefficient.

**indicate P<0.01.
doi:10.1371/journal.pone.0041000.t002

![Figure 3. Correlations between pooled fruit dry weight and total achene number (TAN, open square), total number of fertilized achenes (TFA, open circle) and total number of aborted achenes (TAA, open triangle) of strawberry fruits for plants grown in different conditions. Regression lines: solid line, TAN; broken line, TFA; dotted line, TAA. Regression lines: y = a x + b.](image)
doi:10.1371/journal.pone.0041000.g003
Effects of carbon dioxide, temperature and nitrogen treatments on anthocyanin (AC), total phenolic (TP), total flavonoid (TF), DPPH radical scavenging assay (DPPH), ABTS radical scavenging assay (ABTS) and fruit nitrogen content (FNC) of strawberry fruits.

Table 3. Effects of carbon dioxide, temperature and nitrogen treatments on anthocyanin (AC), total phenolic (TP), total flavonoid (TF), DPPH radical scavenging assay (DPPH), ABTS radical scavenging assay (ABTS) and fruit nitrogen content (FNC) of strawberry fruits.

| Effects | CO2 | Temperature | Nitrogen | CO2×Temp | CO2×N | Temp×N | 3-Way | MS error | R² |
|---------|-----|-------------|----------|-----------|-------|--------|-------|----------|-----|
| AC      | 243.1*** | 0.01 | 58.45*** | 7.64†    | 44.95*** | 76.88*** | 5.89† | 0.08 | 0.94 |
| TP      | 45.13*** | 7.02* | 43.93*** | 19.90*** | 118.58*** | 4.21   | 92.29*** | 0.05 | 0.92 |
| TF      | 807.89**** | 230.04*** | 52.05*** | 52.87**** | 4.36†   | 83.18*** | 69.21*** | 0.20 | 0.98 |
| DPPH   | 1660.9*** | 0.39 | 343.35*** | 7.41†    | 358.39*** | 0.72   | 22.13*** | 9.28 | 0.99 |
| ABTS   | 487.69**** | 0.59 | 160.50**** | 33.94**** | 270.70**** | 40.68**** | 50.84**** | 18.34 | 0.97 |
| FNC    | 2.31 | 4.55† | 18.40**** | 2.16    | 2.16 | 2.15   | 2.55 | 6.83 | 0.22 |

*Data are expressed as mean ± SD, n = 12, while *, ** and *** indicate P<0.05, 0.01 and 0.001, respectively. Abbreviations are: DW- dry weight.

doi:10.1371/journal.pone.0041000.t003

Table 4. MGLM analysis of treatment (CO2, temperature and nitrogen) main effects and their interactions on AC, TP, TF, DPPH, ABTS and FNC of strawberry fruits for plants cultivated at ambient (360 ppm) and elevated (720 ppm) CO2, high and low temperature, and high and low nitrogen.

| Effects | CO2 | Temperature | Nitrogen | CO2×Temp | CO2×N | Temp×N | 3-Way | MS error | R² |
|---------|-----|-------------|----------|-----------|-------|--------|-------|----------|-----|
| AC      | 243.1*** | 0.01 | 58.45*** | 7.64†    | 44.95*** | 76.88*** | 5.89† | 0.08 | 0.94 |
| TP      | 45.13*** | 7.02* | 43.93*** | 19.90*** | 118.58*** | 4.21   | 92.29*** | 0.05 | 0.92 |
| TF      | 807.89**** | 230.04*** | 52.05*** | 52.87**** | 4.36†   | 83.18*** | 69.21*** | 0.20 | 0.98 |
| DPPH   | 1660.9*** | 0.39 | 343.35*** | 7.41†    | 358.39*** | 0.72   | 22.13*** | 9.28 | 0.99 |
| ABTS   | 487.69**** | 0.59 | 160.50**** | 33.94**** | 270.70**** | 40.68**** | 50.84**** | 18.34 | 0.97 |
| FNC    | 2.31 | 4.55† | 18.40**** | 2.16    | 2.16 | 2.15   | 2.55 | 6.83 | 0.22 |

*Data are expressed as F values, and *, **, *** and **** indicate P<0.05, 0.01, 0.001 and 0.0001, respectively. Abbreviations are: FW- fresh weight; Temp- Temperature; N-Nitrogen; 3-Way- CO2×Temperature×Nitrogen.

doi:10.1371/journal.pone.0041000.t004
induction. Besides the vernalization pathway, GA biosynthesis and signaling, including genes such as GA1, GA3, GA20, GA53, and ARABIDOPSIS [37]. It was suggested that temperature effect may be mediated by changes in the level of active endogenous GAs [41]. Su et al. [42] reported that the flowering shoots of Phalaenopsis hybrida grown under high temperature contained lower levels of GA1, GA19, GA20, and GA53 than GA3-treated and cold-induced plants. They also found relatively low level of GA1 and high level of GA8 in shoots-tips of warm control (non-flowering) plants compared to plants whose flowering was promoted with GA3 or cool-temperature. Tayor et al. [43] studied the possible role of endogenous GAs in the control of flowering in strawberry and observed the increase in the level of active endogenous GAs 

Variation in Taste- and Health-related Compounds

The increased dry matter-content (DMC) of the fruits was probably due to the increased non-structural carbohydrates sourced from the increased net photosynthetic rate of strawberry at elevated CO2 [47–48]. The non-structural carbohydrates including fructose (the dominant sugar), glucose, and sucrose, contribute directly to the perceived sweetness of the fruit, and these sugars account for more than 990 g kg−1 of the total sugars in ripe strawberries [49]. Therefore, elevated CO2 which increased fructose, glucose and total sugar levels relative to other taste related compounds would improve the perception of fruit sweetness.

Table 5. Effects of CO2, temperature and nitrogen treatments on fructose (Fru), glucose (Glu), sucrose (Suc), total sugars (TSW), sweetness index (SI) and dry matter-content (DMC) of strawberry fruits.

|           | CT  | CTN | C   | CN  | T   | TN  | ck   | N   |
|-----------|-----|-----|-----|-----|-----|-----|------|-----|
| Fru (mg g⁻¹ DW) | 291.7±22.1** | 301.7±54.9** | 264.5±28.8* | 301.9±41.4** | 225.7±50.8 | 186.4±44.1 | 259.1±43.6* | 229.0±43.6 |
| Glu (mg g⁻¹ DW)  | 299.2±34.0** | 294.3±77.1* | 252.8±36.7* | 288.3±34.8** | 204.0±54.6 | 180.5±63.4 | 238.3±27.6 | 216.3±54.4 |
| Suc (mg g⁻¹ DW)  | 73.3±12.8** | 51.3±18.8** | 86.2±19.3** | 86.14±63.35 | 107.63±84.93 | 108.08±45.61 | 41.57±57.32 | 49.52±66.65 |
| TSW (mg g⁻¹ FW) | 80.26±15.91* | 77.19±18.57* | 79.29±7.84* | 75.77±9.82* | 53.54±10.90 | 57.14±10.85 | 53.67±10.62 | 54.80±15.24 |
| SI (mg g⁻¹ FW)   | 128.8±25.9* | 126.1±29.4* | 128.6±11.0 | 121.8±15.9* | 126.4±16.6 | 195.1±17.9 | 88.5±16.1 | 89.6±23.8 |
| DMC             | 0.127±0.014* | 0.132±0.017* | 0.131±0.015* | 0.114±0.010 | 0.099±0.019 | 0.115±0.004 | 0.098±0.013 | 0.100±0.072 |

Table 6. MGLM analysis of treatment (CO2, temperature and nitrogen) main effects and their interactions on Fru, Glu, Suc, TSW, SI and DMC of strawberry fruits for plants cultivated at ambient (360 ppm) and elevated (720 ppm) CO2, high and low temperature, and high and low nitrogen.

| Effects | CO2 | Temperature | Nitrogen | CO2 x Temp | CO2 x N | Temp x N | 3-Way | MS error | Whole model R² |
|---------|-----|-------------|----------|------------|----------|----------|-------|----------|--------------|
| Fru     | 24.79*** | 4.58* | 0.05 | 5.51* | 4.38* | 0.15 | 6.10* | 1464.02 | 0.58 |
| Glu     | 22.54*** | 1.94 | 0.05 | 5.37* | 1.31 | 0.52 | 7.92* | 2079.33 | 0.53 |
| Suc     | 0.2 | 0.34 | 0.67 | 2.47 | 1.15 | 0.26 | 0.65 | 3391.86 | −0.03 |
| TSW (FW) | 88.41*** | 3.29 | 0.47 | 1.02 | 0.15 | 0.05 | 0.37 | 120.90 | 0.64 |
| SI (FW) | 101.15*** | 2.92 | 0.52 | 1.68 | 0.03 | 0.08 | 0.29 | 277.05 | 0.67 |
| DMC     | 22.93*** | 2.22 | 0.08 | 0.01 | 2.5 | 3.76 | 0.14 | 0.00 | 0.46 |

*Data are expressed as mean ± SD, n = 12, while * and ** indicate P<0.05 and P<0.01, respectively. Abbreviations are: FW- fresh weight; Temp- Temperature; N- Nitrogen; 3-Way- CO2 x Temperature x Nitrogen.

doi:10.1371/journal.pone.0041000.t005

doi:10.1371/journal.pone.0041000.t006

doi:10.1371/journal.pone.0041000.t007
At elevated CO₂, decrease in tissue nitrogen content has been widely reported, but there was significant variation in different taxa [50]. In this study, fruit nitrogen content (FNC) decreased nearly 11% at elevated CO₂ and this value was in the range of the reduction of seed nitrogen content (15%) at elevated CO₂ [15]. Dilution hypothesis suggests that the decrease in tissue nitrogen content under elevated CO₂ results from the dilution due to accumulation of non-structural carbohydrates or plant secondary compounds [51]. In this study, the decline in FNC at elevated CO₂ may be caused by dilution effect of accumulated non-structural carbohydrates, since elevated CO₂ greatly increased leaf photosynthesis and accelerated the accumulation of these compounds [51]. In this study, the decline in FNC at elevated CO₂ may be caused by dilution effect of accumulated non-structural carbohydrates, since elevated CO₂ greatly increased leaf photosynthesis and accelerated the accumulation of these compounds. At elevated CO₂, the total antioxidant capacity and all antioxidant compounds in strawberry fruits decreased nearly 27.5% (from 19% to 37%) at low nitrogen and 9.5% (from 3% to 13%) at high nitrogen, whilst DMC increased 23.7% and 12.5% in the corresponding treatments, respectively. The increase of DMC was proportional to the decrease in total antioxidant capacity and all antioxidant compounds at elevated CO₂, which implied that the reduction of total antioxidant capacity and all antioxidant compounds in strawberry fruits were mainly caused by the dilution effect of accumulated non-structural carbohydrates, though the dilution effect on individual antioxidant compounds varied.

The extent of decrease in total antioxidant capacity and antioxidant compounds was greater at low nitrogen than at high nitrogen, implying that nitrogen application greatly modified the treatment effect of elevated CO₂ on these compounds. From the results, the greater decrease of antioxidant activity at low nitrogen mainly came from the higher antioxidant levels in ambient CO₂ and lower antioxidant levels at elevated CO₂, when compared to these compounds at high nitrogen. Commonly, the change of antioxidant activity results from the change of ROS, and these antioxidant compounds were evolved to protect plants from oxidative damage [52].

Environmental stresses, including nitrogen starvation [25], may increase the production of ROS. We have summarized four possible causes of the increase of ROS under nitrogen deficiency including: (1) "physiological trade-off" between plant growth and secondary metabolite production in GDB framework [25–26,53]; (2) accelerated senescence of plant tissues or organs [54–55]; (3) limitation of CO₂ uptake efficiency and accumulation of reducing power due to accumulation of H₂O₂ in nitrogen deficient plants, which is known to decrease stomatal opening [56–57]; (4) surplus electron flow leading to enhanced oxygen photo-reduction in the chloroplast via the Mehler reaction as the ratio of Rubisco activity declined under nitrogen deficiency [58].

![Figure 4. Strawberry fruits with similar fruit fresh weight (FSW) but different achene abortion rates (AAR).](image)

(a) strawberry fruits with low achene abortion rate; (b) part of the figure a is amplified to indicate the aborted achenes; (c) strawberry fruit with high achene abortion rate; (d) part of the figure c is amplified to indicate the aborted achenes. doi:10.1371/journal.pone.0041000.g004
In this study, the increased antioxidant activity in strawberry fruit at low CO₂ concentration and low nitrogen treatments could not be explained satisfactorily with the reasons mentioned above except the first one. Obviously, reason 3 and 4 were not suitably explained in fruit, while reason 2 contrasted with recent research that elevated CO₂ accelerated senescence of plant tissues or organs and would increase antioxidant level in them [59–61]. Therefore, reason 1 will be a possible explanation that secondary metabolites such as phenolics are accumulated at low nitrogen [25]. Meanwhile, ROS which is involved in the signaling and perception of nitrogen deficiency is also increased [26]. The antioxidant levels decreased in CT and C treatments (though the extent was rather small) suggesting that the effect of nitrogen deficiency on antioxidant level has been modified by the elevated CO₂. We speculate that the reduced FNC in these treatments may inhibit the activity and amount of relevant enzymes involved in perception of nitrogen deficiency and synthesis of secondary metabolites, and negatively affect the antioxidant levels.

Conclusions
Overall, our study illustrates the combined effects of elevated CO₂, nitrogen and temperature on strawberry yield and quality. At low temperature, elevated CO₂ greatly improved the fruit yield by increasing fruit number and fruit weight. However, at high temperature, elevated CO₂ decreased fruit yield. This decrease was mainly caused by the fewer induced inflorescences and smaller induced umbel size which eventually reduced fruit number and fruit weight, respectively. Moreover, elevated CO₂ increased the levels of dry matter-content, fructose, glucose, total sugar and sweetness index per dry matter, but decreased fruit nitrogen content, total antioxidant capacity and all antioxidant compounds per dry matter in strawberry fruit. The reduction of fruit nitrogen content and antioxidant activity was mainly caused by the dilution effect of accumulated non-structural carbohydrates sourced from the increased net photosynthetic rate during fruit development. Thus, the quality of strawberry fruit would increase because of the increased sweetness and the similar amount of fruit nitrogen content, DPPH, ABTS and all antioxidant compounds per fresh matter at elevated CO₂. Interestingly, nitrogen application had no beneficial effect on the fruit yield, but greatly increased fruit weight among all treatments. Fruit quality such as antioxidant activity increased at high nitrogen and elevated CO₂, but decreased at high nitrogen and low CO₂. Considering all treatment effects, we conclude that elevated CO₂ improved the production of strawberry (including yield and quality) at low temperature, but decreased it at high temperature. In addition, the dramatic fluctuation in strawberry yield between low and high temperature at elevated CO₂ implies that more attention should be paid to the process of flower induction under climate change especially in fruits that require winter chilling for reproductive growth, as chronic and steady reduction in winter chill is expected [62]. Therefore, efforts should be made to develop cultivars that require less winter chill for future climate.

Materials and Methods

Plant Material and Experimental Design
Four large growth chambers with an internal chamber height of 2.20 m and a growth area of 1.0 m² were used for the experiment. All chambers have air temperature, relative humidity and carbon dioxide control. Photosynthetic active radiation (PAR) was about 600 µmol m⁻² s⁻¹, and relative humidity was controlled at 80% by an air humidifier 24 hours a day. CO₂ was injected automatically into the chambers all day and night, and its concentration was controlled using a CO₂ delivery system and chamber vents. An individual LICOR infrared gas analyzer (LI-800 GasHound CO₂ Analyzer, LI-COR, Nebraska, USA) was used to monitor the CO₂ levels for each chamber independently, and the accuracy of the analyzer was ±2%. The experimental design consisted of a three-way randomized block with four replications. The treatments consisted of two day/night temperature levels [20/15°C (TA), 25/20°C (TA + 5°C)], two CO₂ concentrations [360 and 720 µmol CO₂ mol⁻¹ air], and two nitrogen application levels [0% (distilled water) and 0.01% NH₄NO₃]. The temperature and CO₂ treatments were randomly allocated in each of the four growth chambers as follows:

- Chamber 1-TA +5°C and 360 µmol CO₂ mol⁻¹
- Chamber 2-TA +5°C and 720 µmol CO₂ mol⁻¹
- Chamber 3-TA and 360 µmol CO₂ mol⁻¹
- Chamber 4-TA and 720 µmol CO₂ mol⁻¹

Fifty milliliter of 0.01% NH₄NO₃ solution was applied twice a week per plant at the beginning of 1 December 2010 and lasted for nearly 6 months. A fixed day length of 10 h from 7:00 AM to 17:00 PM, which corresponds to the day length of early spring in Zhejiang, was used.

The strawberry cultivar used in this study was Toyonoka (Fragaria × ananassa Duch. cv. Toyonoka) a short-day cultivar which need short-day and low temperature (chilling) treatments to accelerate flower bud initiation [63–64], and now is widely planted in Zhejiang. Strawberry seedlings were planted in 25 cm × 18 cm pots using field soil (red soil, total nitrogen content 0.96 g/kg dry soil). Prior to the treatments in chambers, plants grew under the ambient autumn temperatures of Jinhua, Zhejiang, in an unheated greenhouse from November to December for one month (chilling and short-day treatments), and the mean daily temperature in November was about 13.2°C. All plants were watered daily and fertilized weekly with 150 ml per plant of Peters fertilizer (20:20:20, N/P/K). Plants with similar height and crown diameter were moved to chambers and 8 pots were placed in each chamber and four pots per treatment. The plants in each chamber were rotated inside chambers per week and between chambers per month to reduce the microclimate effects of different chambers. Blossoms were self-pollinated by hand using a small brush. As daily routine, the ripeness of fruit was determined by color, and firm red-ripe fruits free from defects or decay were harvested from each growth chamber during the fruiting stage. Fruit dry weight, fruit number, total achenes and total aborted achenes were determined. All of berries were graded in three size classes (grade 1<0.4 g; grade 2, 0.4–0.7 g; grade 3>0.7 g) according to FDW. The berries of each plant were cut into small slices, mixed, and frozen at −24°C for analyzing until the end of the harvest season.

Fruit Sample Preparation
To prepare the fruit samples, four 100 g samples of berries from four replicates of each treatment were homogenized for 2 min in a rotating blade homogenizer (Midea, JP351, China). Solution of homogenate extract (2 g) in methanol (23 ml) was used for determination of total flavonoid, total phenolic, DPPH and ABTS. Solution of homogenate extract (2 g) in distilled water (25 ml) was used for determination of anthocyanin content. All compounds mentioned above in each sample from each plant were measured in triplicate and four samples of each treatment were determined.

Determination of Antioxidant Compounds Content
The amount of all the antioxidant compounds was determined according to Zheng et al. and Lu et al. [65–66]. The total...
flavonoid content was determined by a colorimetric assay with modifications. Briefly, 0.3 mL extract solution was separately mixed with 1.5 mL of methanol, 0.1 mL of 2% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.0 mL of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using a UV-vis spectrophotometer (Jinghua, JH752, China). The total flavonoid content was expressed as rutin equivalents in milligrams per gram dry weight of strawberry.

The total phenolic content was determined colorimetrically using Folin–Ciocalteu reagent, with modifications. The total phenolic assay was conducted by mixing 5.25 mL of deionized water, 0.5 mL of extract, 0.75 mL of 20% Na2CO3, and 0.5 mL of Folin–Ciocalteu reagent. After 40 min of reaction in a water bath at 40°C, the absorbance at 575 nm was measured using a spectrophotometer. Results were expressed as gallic acid equivalents milligrams per gram of dry weight of strawberry.

The total anthocyanins content was determined with a modified pH differential method, using two buffer systems: potassium chloride 0.025 M at pH 1.0 and sodium acetate 0.4 M at pH 4.5. Briefly, 1 mL of sample was transferred to a 10 mL volumetric flask and made up with each buffer. The absorbance of each equilibrated solution was then measured at 510 and 700 nm, using a UV-vis spectrophotometer. Quartz cuvettes of 1 cm path length were used, and all measurements were carried out at room temperature (25°C). Absorbance readings were made against distilled water as a blank. The total anthocyanins content was calculated on the basis of cyanidin-3-glucoside with a molecular weight of 445.2 g/mol and an extinction coefficient of 29600 L/mol·cm⁻¹·m. Results were expressed as milligram cyanidin-3-glucoside equivalents per gram of dry weight of strawberry.

Determination of Total Antioxidant Capacity (DPPH and ABTS)

The DPPH free radical scavenging activity was evaluated according to the method of our previous study [65–66]. The extracts (0.1 mL) of strawberry in ethanol were reacted with 10 mL of 0.03 g/L DPPH ethanol solution at room temperature. The extract (0.1 mL) with 10 mL distilled water was used as control. The absorbance was measured at 517 nm after 30 min of reaction in the dark. DPPH radical scavenging capacity was expressed as Trolox equivalent antioxidant capacity (μmol of Trolox/1 g of dry strawberry fruits).

The ABTS assay was based on the method of Re et al. [69] with slight modification. ABTS⁺ reagent was produced by reacting 10 mL of 7 mM ABTS solution with 178 μL of 140 mM potassium persulfate aqueous in the dark at room temperature for 13 h before use. The ABTS⁺ solution was diluted with ethanol to appropriate absorbance. One-tenth of a milliliter of extract was added to 3.9 mL of diluted ABTS⁺ solution to react in the dark at room temperature for 6 min, and the absorbance at 732 nm was recorded. Trolox was used as standard with the final concentration ranging from 0 to 16.5 μM. Results were expressed as Trolox equivalent antioxidant capacity (μmol of Trolox/1 g of dry strawberry fruits).

Determination of Fruit Nitrogen Content

Fruit nitrogen content was determined by macro-Kjeldahl digestion method [70], with modifications. Briefly, 0.5 g dry fine powder of strawberry fruit was accurately weighed into macro-Kjeldahl flasks to which the catalyst mixture (0.3% TiO2, 0.3% CuSO4, and 10% K2SO4 on a weight basis) and concentrated sulfuric acid (10 mL) were added. The digests were heated for 1.5 h beyond the point when the solutions had cleared. They were then cooled and diluted to 50 mL with distilled water. After addition of 3 mL of 20 g/L H2BO3 solution in the inner chamber of a clean Conway dish, 4 mL diluted digest was added in the outer chamber. The covered Conway dishes were sealed and incubated at 40°C for 24 h. The absorbed ammonia in H2BO3 solution was titrated with 0.02 mol/L HCl solution. The results were expressed as milligram per gram of dry weight of strawberry. Each sample from each plant was measured in triplicate and four samples of each treatment were determined.

Analysis of Sugars Using HPLC

For analysis of sugars, 10 g of snap-frozen strawberry powder (wet) were stirred by a magnetic stirring apparatus in 100 mL of extraction solution containing 90 mL of distilled water, 5 mL of 1 mol L⁻¹ zinc acetate and 5 mL of 0.25 mol L⁻¹ potassium ferrocyanide for 90 min at room temperature. The solution was filtered through a membrane-filtered supernatant (0.2 µm). Glucose, fructose and sucrose were analyzed by injection of a 50 μl sample volume into a DuoFlow HPLC system (Bio-RAD, USA) using a Sepax Amethyst-Amino column, 250 mm x 4.6 mm diameter, 5 μm particle size (Sepax, USA; Part no. 3222905-4625). The column temperature of 20°C was controlled and an acetonitrile: pure water solution (80:20 v/v) was used as mobile phase (flow rate 0.8 ml min⁻¹). Carbohydrates were detected with a refractive index detector (RID-10A, Japan) and their concentrations were calculated by comparing sample peak area to standards using OriginPro 8.5 software. Each sample from each plant was measured in triplicate and four samples of each treatment were determined. The results were recalculated per dry mass.

The sweetness index was calculated by multiplying the sweetness coefficient of each individual sugar (glucose = 1, fructose = 2.3 and sucrose = 1.35), as described by Keutgen and Pawelzik [71].

Statistical Analyses

Data in this study were subjected to analysis of variance, and means were compared by least significant difference (LSD). Multivariate general linear model function (MGLM) was performed to analyze the main effects of CO2 concentration, air temperature and nitrogen input combined with their interactions on the quality of strawberry growing in chambers. Regression analysis was conducted to examine relationships between fruit dry weight and total achene number. In this study, all statistical analyses were conducted using SAS software (SAS Institute Inc., Cary, NC, USA).

Acknowledgments

We thank Ms YQ Liu and YW Zhang for their assistance in plant management.

Author Contributions

Conceived and designed the experiments: H. Lu PS. Performed the experiments: PS H. Lou YH DS YZ TD H. Lu. Analyzed the data: PS H. Lou YH DS YZ TD H. Lu. Contributed reagents/materials/analysis tools: PS H. Lou YH DS YZ TD. Wrote the paper: PS NM H. Lu.
References

1. IPCC (2007) Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press.

2. Xiong W, Lin E, Ju H, Xu Y (2007) Climate change and critical thresholds in China’s food security. Climatic Change 81: 205–221.

3. Santos BM, Chandler CK (2009) Influence of nitrogen fertilization rates on the performance of strawberry cultivars. Int J Fruit Sci 9: 126–135.

4. Konin M, Vojtás P, Polonien P (2001) Influence of photoperiod and duration of the short-day treatment on vegetative growth and flowering of strawberry (Fragaria × ananassa Duch.). J Hortic Sci Biotech 76: 77–82.

5. Kumakura H, Shishido Y (1995) Effects of temperature and light conditions on flower initiation and fruit development in strawberry. Jpn J Agric Res Q 29: 241–245.

6. Chen K, Hu GQ, Lenz F (1997) Effect of CO₂ concentration on strawberry. IV. Carbohydrate production and accumulation. J Appl Bot-Anget Bot 71: 183–188.

7. Malou T, Namuco OS, Ziska LH, Horie T (1997) Effects of high temperature and CO₂ concentration on spindle fertility in indica rice. Field Crop Res 51: 213–219.

8. Baker JT, Allen LH, Boote KJ, Jones J, Jones JW (1989) Response of soybean to air temperature and carbon dioxide concentration. Crop Sci 29: 98–105.

9. Prasad PTV, Boote KJ, Allen LH, Thomas JMG (2002) Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (Phaseolus vulgaris L.). Global Change Biol 8: 710–721.

10. Prasad PTV, Boote KJ, Allen LH, Thomas JMG (2003) Optimal-temperature conditions are detrimental to peanut (Arachis hypogaea L.) reproductive processes and yield under both ambient and elevated carbon dioxide. Global Change Biol 9: 1775–1787.

11. Ahmed FE, Hall AE, Madore MA (1993) Interactive effects of high temperature and elevated carbon dioxide concentration on cowpea [Vigna unguiculata (L.) Walp.]. Plant Cell Environ 16: 835–842.

12. Wheeler TR, Batts GR, Ellis RH, Hadley P, Morison JIL (1998) The growth and yield responses of dwarf soybean [Glycine max (L.) Merr.] induced by short periods of high temperature. J Am Soc Hortic Sci 69: 40–46.

13. Reddy KR, Hodges HF, Kimball BA (2000) Crop ecosystem responses to climate change: cotton. In: Reddy KR, Hodges HF, editors. Climate change and global crop productivity. Oxford: CAB International. 161–187.

14. Arrows R, Chapman III (2003) The micro-environment of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 33: 1–67.

15. Hikosaka K, Kinugasa T, Oikawa S, Onoeda Y, Hirose T (2011) Effects of elevated CO₂ concentration on seed production in C₃ annual plants. J Exp Bot 62: 1523–1530.

16. Li W, Han X, Zhang Y, Li Z (2007) Effects of elevated CO₂ concentration, irrigation and nitrogen fertilizer application on the growth and yield of spring wheat in semi-arid areas. Agr Water Manage 87: 106–114.

17. Xioa G, Zhang Q, Wang R, Xiong Y (2009) Effects of elevated CO₂ concentration, supplemental irrigation and nitrogen fertilizer application on rain-fed spring wheat yield. Acta Ecol Sin 29: 205–210.

18. Yoshida H, Horie T, Nakaai S, Ohno H, Nakagawa H (2011) Simulation of the effects of genotype and N availability on rice growth and yield response to an elevated atmospheric CO₂ concentration. Field Crop Res 124: 433–440.

19. Deng X, Woodward FI (1996) The growth and yield responses of spinach (Spinacia oleracea L.) to elevated CO₂ and N supply. Ann Bot-London 81: 67–71.

20. Prasad PTV, Boote KJ, Allen LH, Thomas JMG (2002) Effects of elevated CO₂ concentration on seed-set and yield of kidney bean (Phaseolus vulgaris L.). Global Change Biol 8: 710–721.

21. Wang SY, Bunce JA, Maas JL (2003) Elevated carbon dioxide increases contents of antioxidant compounds in field-grown strawberries. J Agric Food Chem 51: 4107–4112.

22. Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, et al. (2007) FT protein overexpression contributes to long-distance signaling in floral induction of Arabidopsis. Science 316: 1030–1033.

23. Nakagawa H, Horie T, Nakaai S, Ohno H, Nakagawa H (2011) Simulation of the effects of genotype and N availability on rice growth and yield response to an elevated atmospheric CO₂ concentration. Field Crop Res 124: 433–440.

24. Levine LH, Pareá PW (2009) Antioxidant capacity reduced in scallions grown under high CO₂ and N supply. Ann Bot-London 81: 67–71.

25. Dixon RA, Harrison MJ, Lamb CJ (1994) Early events in the activation of plant cell wall hydrolases in response to fungal elicitor or tobacco mosaic virus. Virology 197: 283–301.
58. Lin YL, Chao YY, Huang WD, Kao CH (2011) Effect of nitrogen deficiency on antioxidant status and Cd toxicity in rice seedlings. J Plant Growth Regul 64: 263–273.

59. Zhu C, Zhu J, Zeng Q, Lin G, Xie Z, et al. (2009) Elevated CO2 accelerates flag leaf senescence in wheat due to ear photosynthesis which causes greater ear nitrogen sink capacity and ear carbon sink limitation. Funct Plant Biol 36 (4): 291–299.

60. Franzaring J, Weller S, Schmid I, Fangmeier A (2011) Growth, senescence and water use efficiency of spring oilseed rape (Brassica napus L. cv. Mozart) grown in a factorial combination of nitrogen supply and elevated CO2. Environ Exp Bot 72: 294–296.

61. McConnaughay KDM, Bassow SL, Berntson GM, Bazzaz FA (1996) Leaf senescence and decline of end-of-season gas exchange in five temperate deciduous tree species grown in elevated CO2 concentrations. Global Change Biol 2: 25–33.

62. Baldocchi D, Wong S (2007) Accumulated winter chill is decreasing in the fruit growing regions of California. Climatic Change 87: 153–166.

63. Ledesma NA, Nakata M, Sugiyama N (2008) Effect of high temperature stress on the reproductive growth of strawberry cvs. ‘Nyoho’ and ‘Toyonoka’. Sci Hortic-Amsterdam 116: 186–193.

64. Morishita M, Mochizuki T, Yamakawa O (1993) Flower induction and selection on earliness of strawberry seedlings by short-day and low night temperature treatment. J Jpn Soc Hortic Sci 61: 857–864.

65. Zheng H, Jiang J, Lou H, Hu Y, Kong X, et al. (2011) Application of artificial neural network (ANN) and partial least-squares regression (PLSR) to predict the changes of anthocyanins, ascorbic acid, total phenols, flavonoids, and antioxidant activity during storage of red bayberry juice. J Agric Food Chem 59: 592–600.

66. Zheng H, Lu H, Zheng Y, Lou H, Chen C (2010) Automatic sorting of Chinese jujube (Ziziphus jujuba Mill. cv. ‘hongxing’) using chlorophyll fluorescence and support vector machine. J Food Eng 104: 402–408.

67. Lu H, Zheng H, Hu Y, Lou H, Kong X (2011) Bruise detection on red bayberry (Myrica rubra Sieb. & Zucc.) using fractal analysis and support vector machine. J Food Eng 104: 149–153.

68. Lu H, Zheng H, Lou H, Jiang L, Chen Y, et al. (2010) Using neural networks to estimate the losses of ascorbic acid, total phenols, flavonoid, and antioxidant activity in asparagus during thermal treatments. J Agric Food Chem 58: 2995–3001.

69. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 26: 1231–1237.

70. American Society for Testing Materials (2002) Standard test method for total Kjeldahl nitrogen in water. West Conshohocken: ASTM International.

71. Keutgen A, Pawelzik E (2007) Modifications of taste-relevant compounds in strawberry fruit under NaCl salinity. Food Chem 103: 1487–1494.