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Functional growth analysis of 'Sonata' strawberry plants grown under controlled temperature and daylength conditions

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ABSTRACT

In order to investigate the relationship between environmental conditions and vegetative growth and reproductive development in the strawberry, freshly rooted runner plants of the cultivar 'Sonata' were grown in a phytotron at temperatures of 12, 18 and 24 °C and photoperiods of 10 h short day (SD) and 20 h long day (LD) for 31 d and harvested at 10 d intervals. Plant dry weight and leaf area increases were exponential versus time, giving a linear regression with the natural log (ln). This rendered the relative growth rate (RGR) constant over time at each environmental condition. Over the entire 31 d growth period, the RGR increased linearly with increasing temperature across the range of temperatures with a further 10-13% enhancement by LD. A maximum RGR value of 0.077 g/g/d was determined in LD at 24 °C. Increases in the RGR was driven by a combined increase in net assimilation rate (NAR) and leaf area ratio (LAR) and was associated with an increased allocation of dry matter production into leaves and less into crowns and roots. Because of this, the shoot/root ratio increased consistently with increasing temperature and photoperiod, which was also associated with a significant increase in the tissue C/N concentration ratio. Low temperature promoted starch accumulation markedly in all parts of the plants, with a further enhancement by LD conditions, while the concentrations of soluble sugars were less affected by the climatic environment. Forcing of plants exposed to the various growth conditions for 31 d showed that all plants at 12 and 18 °C and 80% of those at 24 °C had initiated flowers in SD, whereas none had initiated flowers in LD regardless of temperature conditions. All these results demonstrate an opposite environmental relationship between vegetative growth and reproductive development in the strawberry.

Keywords: Carbohydrates, Growth rate, Photoperiod, Shoot/root ratio, Strawberry, Temperature

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1. Introduction

Growth and development of the strawberry plant are regulated by a complex set of interacting environmental factors, of which temperature, daylength and light intensity predominate (Darrow, 1936; Guttridge, 1985; Larson, 1994; Heide et al., 2013). Because of the economic importance of the crop, strawberry physiology and genetics have been extensively researched, and the literature in the field has been reviewed several times (e.g. Guttridge, 1985; Larson, 1994; Heide et al., 2013). However, while the environmental regulation of flower formation and the transition from vegetative to reproductive development have been subject to extensive research, the environmental regulation of vegetative growth of the strawberry plant has received less attention.

Growth analysis is commonly used to investigate the way in which environmental factors affect plant growth (Evans, 1972). The measure of growth used is the *relative growth rate* (RGR), which is a concept introduced by Blackman (1919) to describe the exponential phase of growth of annual crop plants. The concept assumes that new growth is simply related to existing biomass and represents the rate of increase in plant weight per unit of existing weight over a given period. It is the product of *net assimilation rate* (NAR), which is the increase in plant weight per unit of leaf area per unit time, and *leaf area ratio* (LAR) which is the ratio of leaf area to total plant weight:

RGR = NAR x LAR

Estimation of these parameters is very useful for investigation of the way in which environmental factors influence plant growth. For example, the equation illustrates that if the rate of photosynthesis is reduced for some reason, the plant can only maintain a constant RGR by increasing its leaf area, a response that is commonly observed (Fitter and Hay, 1987).

In the cultivated strawberry, growth analyses have been conducted on field-grown plants to investigate the effects of genotype, cultivation systems, and seasonal changes in the environment (Olsen et al., 1985; Strik and Proctor, 1988a, b; Fernandez et al., 2001). However, under field conditions, reliable data for root growth are difficult to obtain or not recovered at all, and hence, the analyses are either inadequate or limited to the aboveground parts of the plant. Furthermore, in the natural environment, changes in important climatic factors such as photoperiod, temperature and solar radiation change simultaneously and in parallel, thus causing covariations that make it difficult to disentangle and assess the specific effect(s) of each factor. To our knowledge, growth analysis has not been performed with container-grown strawberry plants

maintained under controlled environment conditions where important climatic factors can be controlled and varied systematically. It should also be noted that the growth of strawberry plants is influenced also by ontogenetic factors (Olsen et al., 1985). As the young plant grows, an increasing proportion of the plant tissues enter a state of negative carbon balance due to mutual shading and reduced photosynthesis of older leaves. In addition, when the plant enters the reproductive phase, production and allocation of photosynthates become strongly influenced by the strong sink effects of developing flowers and fruits.

This prompted us to undertake a classical growth analysis of young strawberry plants grown in a phytotron under controlled temperature and daylength conditions. The objective of the investigation was to quantify the impact of temperature and daylength on production and allocation of dry matter in young strawberry plants in order to facilitate our understanding of the processes by which the climatic environment control growth and development of young (vegetative) strawberry plants. Since starch content is known to greatly affect cold storage success and transplanting performance and growth vigor of strawberry plants (Bringhurst et al., 1960; López et al., 2002), the content and partitioning of nonstructural carbohydrates were also determined in plants from the various growth conditions. Furthermore, the parallel environmental effect on flower induction was also included in the investigation.

2. Materials and Methods

2.1. Plant material and handling

The seasonal-flowering (June-bearing) cultivar 'Sonata' was used for the experiment. The cultivar, originating from a cross between 'Elsanta' and 'Polka' at Plant Research International, Wageningen, NL in 1998, has constantly expanded its acreage in Northern Europe where it is now dominating the fresh consumption strawberry market (Fragaria Holland, 2008). Young runner plants were harvested in late April from stock plants grown in a greenhouse maintained at a minimum temperature of 20 °C and a photoperiod of 20 h established by extension of the natural daylight with low-intensity incandescent light (c. 15 µmol m^{-2} s^{-1} PPF). The runners were rooted directly in 9 cm plastic pots in a water-saturated atmosphere at 25 °C and 20 h photoperiod. After 14 days (on 11 May), when the plants were uniformly rooted, they were moved into day-light compartments of the phytotron at the Norwegian University of Life

Sciences at Ås (59°40’ N, 10°40’ E) and exposed to constant temperatures of 12, 18 and 24 °C and photoperiods of 10 and 20 h.

In the phytotron, the plants received natural daylight for 10 h per day (08.00-18.00 h). Whenever the photosynthetic photon flux (PPF) in the daylight compartments fell below approximately 150 µmol m^{-2} s^{-1} (as on cloudy days), an additional 125 µmol quanta m^{-2} s^{-1} were automatically added by high-pressure metal halid lamps (400 W Philips HPI-T). Daylength extension to 20 h long day (LD) was provided by low intensity light from 70 W incandescent lamps (c. 7 µmol m^{-2} s^{-1} PPF) in such a way that the 4 h dark period was centered around midnight (22.00 h to 02.00 h). Plants receiving short day (SD) treatment were in the dark from 18.00 h to 08.00 h. The daylength extension light amounted to less than 2% of the total daily light radiation, the plants thus receiving nearly the same daily light integral in both photoperiods. The plant trolleys were randomly positioned in the daylight rooms as a result of the every-day movements to and from the adjacent photoperiodic treatments rooms. Temperatures were controlled to ±1.0 °C and a water vapour pressure deficit of 530 Pa was maintained at all temperatures. In order to reduce the bias of runner formation and growth on total dry matter accumulation and partitioning (Pritts and Worden, 1988), new runners were removed in all treatments as soon as they appeared throughout the experimental period.

The growth medium used was a 1:1 (v:v) mixture of finely sifted peat-based potting compost and granulated vermiculite. Throughout the experimental period, the plants were irrigated daily to drip-off with a complete fertilizer solution consisting of a 2:3 (w:w) mixture of Superba™ Rød (9-5-25-4% NPKMg + micronutrients) and Calcinit™ (15.5-19% NCa) (Yara International, Oslo, Norway) with electric conductivity (EC) of 1.0 mS cm^{-1}. Plants were harvested for growth analysis after 10, 21, and 31 days of cultivation at the respective conditions. In order to reduce diurnal metabolic changes to a minimum during the day of harvest, all plants to be harvested on a given day were placed in the dark in a 5 °C cold rom from 08.00 h until harvested. At harvest, the plants were partitioned into three components: green leaves (lamina and petiole), crowns, and roots. The plants were removed from the pots and the roots washed clean of soil material and, after blotting on tissue paper, fresh weight was determined for each component. Total leaf area of each sample was measured with a LI-COR Inc. Model LI-3000 area meter. Plant material was then placed loosely in open paper bags and dried in a forced-air drying oven at 100 °C for 60 min, and then further dried to constant weight at 70 °C. The initial heat treatment at 100 °C was

used to inactivate carbohydrate-degrading enzymes (Acuña-Maldonado and Pritts, 2013). The dried tissues were ground in a mill (Thomas Wiley® Mini-Mill, A. H. Thomas Co., Scientific Apparatus, Phila., PA, USA) to pass through a 0.50 mm sieve and stored *in vacuo* at 4 °C until analysed. Based on the harvest data, relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR), were calculated as outlined by Evans (1972), using the curve-fitting computer program of Hunt et al. (2002). The relative leaf area growth rate (RLAGR) was calculated in the same way as the RGR, except that leaf area data instead of weight data were used as inputs.

2.2 Chemical analysis

Soluble sugars. We weighed approx. 100 mg dried plant material into an Eppendorf tube and extracted soluble carbohydrates with 80% ethanol using an ultrasonic bath (Model USC 200 TH, VWR, Leuven, Belgium) at 60 °C for 30 min with two repeated extractions with 2 ml each time. For each extraction, extracts were centrifuged at 15000 rpm/min for 3 min. The supernatants from the two repeated extractions were combined. The ethanol was completely evaporated from the supernatant at 60 °C by using a vacuum desiccator (Eppendorf AG 22331, Hamburg, Germany). Afterwards, we added 2 ml water to the extract and used the ultrasonic bath for 30 min at 60 °C. The extract was centrifuged at 15000 rpm/min for 3 min and the supernatant filtered through a 0.45 µm GHP membrane filter (Millipore) before chromatography.

The extracts were ran on a High Performance Liquid Chromatograph (Agilent 1200 series of HPLC, Agilent Technologies, Waldbronn, Germany) with a Refractive Index Detector to separate and identify soluble sugars. Sugars were separated using a column specialized for separating carbohydrates (Agilent Hi-Plex Ca USP L19, 4,0 * 250 nm, 8 µm; p/n PL1570-5810). For the mobile phase, 100% water was used as solvent. The flow rate was 0.3 ml min^{-1} and the column temperature was 80 °C. The amount of sugars was determined by comparison with standards of pure sugars.

Starch. Approx. 200 mg dried plant material was weight into a 15 ml Sarstedt plastic centrifuge tube. Soluble sugars were extracted as described above and discarded with the supernatant. Starch in the precipitate were solubilized by adding 2 ml dimethyl sulfoxide and placing the tube on a boiling water bath for 5 min. Immediately, 2.9 ml MOPS buffer (pH 7) and 0.1 ml thermostable α-amylase (*B. licheniformis*, Megazyme) was added and the tube was

incubated 6 min on a boiling water bath. The tube was then placed on a 50 °C water bath and 4 ml sodium acetate buffer (pH 4.5) and 0.1 ml (20 units) amyloglucosidase (*A. niger*, Megazyme) was added and the tube was incubated for 30 min at 50 °C. The glucose content after hydrolysis of starch was analyzed by HPLC as described above. The amount of starch was estimated from standards of pure starch hydrolyzed together with the plant samples.

Carbon/Nitrogen (C/N) ratio. Total C and N were determined with an Elemental Analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) in plant tissue samples prepared as described above.

Chlorophyll concentration was estimated with a Minolta SPAD-502 handheld leaf chlorophyll meter (Markwell et al., 1995). SPAD values were determined on three leaves of each harvested plant.

2.3. Assessment of flowering status

An extra set of plants (3 replicates with 5 plants each) were grown for 31 d at the respective treatment conditions and then forced for 60 d in a greenhouse under LD conditions at a minimum temperature of 20 °C for assessment of floral initiation status. Time to anthesis (first open flower) was recorded by second-daily observations, and the number of inflorescences and the total number of flowers were recorded in each plant at the end of the forcing period.

2.4 Experimental design and statistical analysis

The experiment was factorial with a split-plot design with temperatures as main plots and photoperiods as sub-plots. Each treatment had 3 replicates consisting of 62 plants each at start of the experiment, positioned on 2 trolleys (one trolley after the second harvest). In each replicate, 11, 8, 6, and 4 plants, respectively, were harvested on days 0, 10, 21, and 31. In addition, 5 plants remained in each replicate for 31 d for assessment of floral initiation status.

Experimental data were subjected to analysis of variance (ANOVA) by standard procedure using Mini-Tab® Statistical Software program package (Release 15, Minitab Inc., State College, PA, USA). Percentage values were always subjected to an arc sin transformation before performance of the ANOVA.

3. Results

3.1. Growth analysis

The results in Fig. 1 demonstrate that total plant dry weight and leaf area increased with increasing temperature and photoperiod, but usually only after some time lag in the case of photoperiod. Weight and area increases were exponential versus time, giving a linear time regression with the natural log (ln), thus rendering the relative growth rate (RGR) constant over time at each growth condition. (For primary growth data, see Fig. S1). However, due to adjustment to the new growth conditions, the RGR underwent transitional changes at 12 and 18 °C during the first ten-day growth period (Table 1). For the entire 31-day growth period, there was a linear increase in the RGR across the 12–24 °C temperature range with a consistent 10 to 13% enhancement by LD at all temperatures (Fig. 2). This was associated with a significant increase in the NAR with increasing temperature and photoperiod, whereas the LAR was enhanced by increasing temperature only.

The plants partitioned the greatest share of their production into leaves, and least into crowns. With increased temperature and extended photoperiod, the plants also allocated a greater share of their dry matter production into leaves and less into crowns and roots (Table 2). Because of this, the final shoot to root ratio increased markedly with increasing temperature and photoperiod (Table 1). This LD enhancement effect increased slightly with increasing temperature. Since the experiment was started with freshly rooted cuttings with small roots, the shoot/root ratio fell off sharply as root formation and growth continued in the successive harvests. The proportional sizes of shoots and roots after 31 d of growth under the various environmental conditions are illustrated in Fig. 3.

3.2 Plant carbon/nitrogen (C/N) ratio

The results in Table 3 show that the C/N ratio of the plant tissues increased significantly with increasing temperature and photoperiod in all plant parts. Generally, the ratio was lowest in the roots and highest in crowns. These effects were brought about by changes in both C and N concentrations. While C concentrations were higher in LD than in SD, it was not significantly affected by temperature. On the other hand, N concentrations decreased significantly by increases in both temperature and photoperiod. Tissue concentrations also varied significantly between the various plant parts; C concentrations were higher in leaves and crowns than in roots, while the opposite situation was found for N concentrations.

3.3. Nonstructural carbohydrate composition

The starch concentration increased with decreasing temperatures in all parts of the plants and was further enhanced by LD conditions (Table 4). The three-way ANOVA also revealed highly significant two- and three-factor interactions of temperature, photoperiod and plant part on the starch content. Sucrose concentration was also highest at low temperatures in leaves and increased in plants grown under LD conditions, especially in crowns and roots. Glucose concentrations likewise increased with increasing temperature and LD conditions, while there were no significant effects of photoperiod or temperature on the fructose levels. At low and intermediate temperature, starch concentration was higher in crowns and roots than in leaves, but this was reversed at the highest temperature, whereas the concentrations of each of the sugars were always highest in the leaves (Table 4). While the concentration of starch and sucrose decreased with temperature in all plant parts, the concentration of the hexoses increased somewhat. The sum of sugars was enhanced by LD conditions.

3.4. Chlorophyll concentration

Chlorophyll concentration as assayed by the SPAD chlorophyll meter, increased strongly with the progress of growth and development, and at the same time, the effects of temperature and photoperiod became clearly visible (Fig. 4). At the final harvest, chlorophyll concentration was consistently higher in SD than in LD, the differences decreasing with decreasing temperature. Under SD conditions, chlorophyll concentration was highest at 18 °C, whereas in LD the concentration decreased steadily with increasing temperature across the entire temperature range.

3.5. Flowering

Forcing of plants exposed to the various growing conditions for 31 d showed that all plants at 12 and 18 °C and 80% of those at 24 °C had initiated flowers in SD, whereas none had initiated flowers in LD regardless of temperature conditions (Table 5). The plants from 12 and 18 °C started flowering simultaneously after 5 weeks of forcing, while those from 24 °C were delayed for an additional 14 d. While the number of inflorescences per plant was little affected by temperature, the number of flowers per inflorescence increased consistently across the range of

temperatures, resulting in an increasing number of flowers per plant. Flowering was associated with increased crown branching under SD conditions, with an optimum at 18 °C. The number of runners formed during the forcing period increased significantly with increasing temperature in the plants raised under SD conditions, while there was no such after-effect of temperature in plants grown under LD conditions. Because of this interaction of temperature and photoperiod, there was no significant main after-effect of photoperiod on runner formation (Table 5).

4. Discussion

The demonstrated increases in both total dry matter production and leaf area of young strawberry plants with increasing temperature and photoperiod (Fig. 1) are in accordance with the results obtained under field conditions by Olsen et al. (1985). An exponential increase in dry matter accumulation, as revealed by a linear increase in the natural log (ln) of dry matter versus time, rendered the RGR constant over time at each growth condition (Table 1). The same growth parameter relations were found by Olsen et al. (1985) in the genotype they used, and in three out of seven genotypes used by Pritts and Proctor (1988a), even though these results referred to the first fruiting year. Genotype differences in growth rhythm and /or fruiting and runnering characteristics might have precluded the same response in the other genotypes.

The maximum RGR of 0.077 g/g/day obtained in LD at 24 °C is comparable with the maximum RGR of 0.044 g/g/day obtained in the establishment year under field conditions at midsummer by Olsen et al. (1985). The use of small plants with minimal leaf shading is probably the main reason for the superior growth rate in the present experiment. A temperature optimum of 24 °C for dry matter production in strawberry is higher than the 18 °C optimum previously reported by Heide (1977). The reason for this discrepancy is probably the superior light conditions during spring and early summer in the present experiment compared with autumn conditions in the former. The results confirm the profound effect of low temperature and SD on the reduction of growth rate as an early event in the sequence of processes (the autumn syndrome) associated with autumn preparation in strawberry plants.

The growth analysis demonstrated that enhancement of the RGR at high temperature was driven by a combined increase in the net assimilation rate (NAR) and the leaf area ratio (LAR), whereas the LD enhancement was driven by an increase only in the NAR (Fig. 2). Nevertheless, the relative leaf area growth rate (RLAGR) was enhanced by LD, indicating a specific leaf area

284 growth effect also of photoperiod. Possibly, the adjustment changes following transition to the
285 new conditions may, to some extent, have masked the over-all effect for the entire growth period.
286 Whatever the explanation, it is clear that the driving forces of growth enhancement by
287 temperature and daylength differs greatly in strawberry plants compared with temperate perennial
288 grasses. In the latter, a remarkable increase in LAR was the main driving force of the large
289 increase in RGR and dry matter production under LD conditions observed in the grasses without
290 simultaneous changes in the daily light energy (Hay and Heide, 1983; Heide et al., 1985; Hay,
291 1990; Solhaug, 1991). Despite the different driving force mechanisms involved, the shoot/root
292 ratio increased strongly under high temperature and long day conditions in both plant types.
293 The results confirmed the prominent effect of low temperature on the accumulation of starch
294 previously reported for strawberry roots and crowns (Bringhurst et al., 1960; López et al., 2002).
295 It is well documented that such an accumulation of starch in autumn is an important step in the
296 natural winter preparation of strawberry plants and a prerequisite for successful overwintering or
297 artificial cold storage and good transplant results (Lieten et al., 1995; López et al., 2002). It was
298 therefore, rather surprising that accumulation of starch at low temperature was enhanced by LD
299 conditions (Table 4). A possible explanation could be that the 31 d experimental period might not
300 have been long enough to fully establish the daylength effects. Predominant accumulation of all
301 individual sugars in leaves, and an increasing concentrations of glucose under high temperature
302 and LD conditions (and of sucrose in LD), is consistent with an increased sugar availability under
303 growth-promoting conditions. On the other hand, low temperature accumulation of starch in roots
304 and crowns is compatible with an alternative sugar utilization for storage when growth is reduced
305 at low temperature. Starch accumulation appears to be an important component of the autumn
306 syndrome in strawberry plants (Guttridge, 1985). The elevated leaf chlorophyll concentrations
307 detected under low temperature and SD conditions (Fig. 4), where dry matter production was
308 least, demonstrate that other factors than leaf chlorophyll concentration were limiting
309 photosynthesis and dry matter accumulation in strawberry plants.
310 The observed marked effects of climate on growth and chemical composition of the
311 strawberry plant have important practical implications for commercial strawberry production.
312 The results demonstrate that for the early establishment and raising period, long photoperiods and
313 temperatures of about 24 °C are optimal for growth and dry matter accumulation of strawberry
314 plants. However, because such conditions result in plants with a high shoot/root ratio and low

315 root starch content, conditions must later be changed to low temperature (<10 °C) and SD
316 conditions to ensure floral induction and development of plants with large and strong roots with
317 high starch content. This has proved vital for cold storage and/or transplanting successes of
318 strawberry planting material (Bringhurst et al., 1960, López et al., 2002). In Mediterranean
319 climates with high summer temperatures, high elevation nurseries are, therefore, commonly used
320 for raising of such ideal "waiting-bed" plants (López et al., 2002). In North America, strawberry
321 plants raised under diurnally fluctuating temperatures with cool nights, were similarly shown to
322 exhibit so-called Northern vigour (Tanino et al., 2006; Tanino and Wang, 2008).
323 The flowering data in Table 5 show that the strawberry cultivar 'Sonata' actually has an
324 obligatory SD requirement for initiation of flower primordia. While most June-bearing cultivars,
325 especially those of high-latitude origin, are facultative SD plants that initiate flowers also under
326 LD conditions if the temperature is low (Guttridge, 1985; Heide, 1977), it has been demonstrated
327 that some cultivars such as 'Senga Sengana', 'Elsanta', and 'Korona' lack this characteristic
328 (Heide et al. 2013). The present results demonstrate that also 'Sonata' belongs to this group of
329 obligatory SD plants. The elevated tissue C/N ratio observed under non- inductive LD and high
330 temperature conditions (Table 3) is in direct contrast with the old notion by Kraus and Kraybill
331 (1918), that a high tissue C/N ratio should be generally conducive to floral initiation. While sugar
332 accumulation in the apical bud has been associated with floral transition in photoperiodic plants
333 in general (Bernier et al., 1993), and in strawberry specifically (Eshghi et al., 2007), the present
334 sugar analyses did not indicate any specific mediation of sugars in photoperiod and temperature
335 induction of flowering in the strawberry.
336
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343

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Table 1

Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio, (LAR), and Shoot/Root ratio of young strawberry plants as affected by photoperiod and temperature during successive growth periods.

Period (days)	Temperature (°C)	Photoperiod (h)	RGR (g/g/day)	NAR ($mg/cm^2/day$)	LAR ($cm^2\ mg^{-1}$)	Shoot/Root ratio
0-10	12	10	0.069	0.65	0.109	12.4
		20	0.061	0.57	0.110	13.7
0-21		10	0.046	0.48	0.101	6.3
		20	0.046	0.45	0.109	5.7
0-31		10	0.046	0.56	0.090	2.8
		20	0.051	0.62	0.090	3.9
0-10	18	10	0.071	0.63	0.115	9.4
		20	0.080	0.72	0.114	10.5
0-21		10	0.060	0.59	0.107	4.8
		20	0.069	0.73	0.101	5.7
0-31		10	0.057	0.64	0.100	3.7
		20	0.066	0.70	0.102	4.7
0-10	24	10	0.060	0.45	0.136	10.6
		20	0.067	0.56	0.116	13.2
0-21		10	0.067	0.56	0.122	6.7
		20	0.071	0.64	0.116	8.8
0-31		10	0.069	0.68	0.107	4.5
		20	0.077	0.78	0.105	6.5
Probability level of significance (ANOVA)*						
Source of variation						
Temperature (*A*)			<0.001	<0.001	0.04	0.001
Photoperiod (*B*)			<0.001	<0.001	n.s.	0.003
A x B			n.s.	n.s.	n.s.	n.s.

Data are the means of three biological replicates.

* ANOVA for the period 0-31 days

n.s. - not significant.

Table 2

Effects of temperature and photoperiod on partitioning of dry matter production in young strawberry plants.

Temperature	Photoperiod	% dry matter		
(°C)	(h)	Leaves	Crowns	Roots
12	10	61.5	12.0	26.6
	20	68.8	10.6	20.6
Mean		*65.2*	*11.3*	*23.6*
18	10	70.6	8.0	21.4
	20	73.9	8.1	18.0
Mean		*72.3*	*8.1*	*19.7*
24	10	75.1	6.7	18.2
	20	80.3	6.1	13.5
Mean		*77.7*	*6.4*	*15.9*
Probability level of significance (ANOVA)				
Source of variation				
Temperature (*A*)		0.001	<0.001	0.004
Photoperiod (*B*)		<0.001	n.s.	<0.001
A x B		n.s.	n.s.	n.s.

Data are for the final harvest and represent the means of three biological replicates.

n.s. - not significant.

Table 3

Effects of temperature and photoperiod on tissue concentrations (mg g^{-2} DW) of carbon (C) and nitrogen (N), and the C/N ratio in young strawberry plants after 31 days of cultivation at the respective conditions.

Temperature (°C)	Photoperiod (h)	Plant part	C	N	C/N-ratio
12	10	Leaves	44.6	2.5	17.6
		Crown	43.7	2.3	19.1
		Root	41.4	3.3	12.7
	Mean		*43.3*	*2.7*	*16.4*
	20	Leaves	45.3	2.3	19.9
		Crown	44.6	2.3	19.2
		Root	42.3	3.4	12.7
	Mean		*44.1*	*2.7*	*17.3*
18	10	Leaves	43.9	2.8	15.5
		Crown	44.1	2.1	20.5
		Root	41.3	3.5	11.7
	Mean		*43.1*	*2.8*	*15.9*
	20	Leaves	45.0	2.3	19.2
		Crown	44.1	2.0	22.2
		Root	41.7	3.7	11.3
	Mean		*43.6*	*2.7*	*17.6*
24	10	Leaves	44.3	2.8	15.9
		Crown	42.5	2.1	20.0
		Root	42.4	3.1	13.9
	Mean		*43.1*	*2.7*	*16.6*
	20	Leaves	44.6	2.1	21.1
		Crown	43.0	1.9	22.8
		Root	42.6	3.0	14.3
	Mean		*43.4*	*2.3*	*19.4*
Probability level of significance (ANOVA)					
Source of variation					
Temperature (*A*)			n.s.	0.003	0.01
Photoperiod (*B*)			0.03	0.047	0.005
A x B			n.s.	n.s.	n.s.
Plant part (*C*)			<0.001	<0.001	<0.001
A x C			<0.001	<0.001	<0.001
B x C			n.s.	<0.001	<0.001
A x B x C			n.s.	n.s.	n.s.

Data are the means of three biological replicates, each with four plants per treatment.
n.s. - not significant.

Table 4

Effects of temperature and photoperiod on tissue concentrations (mg g^{-2} DW) of non-structural carbohydrates (starch and sugars) in young strawberry plants after 31 days of cultivation at the respective conditions.

Temperature (°C)	Photoperiod (h)	Plant part	Starch	Sucrose	Glucose	Fructose	Total sugars
12	10	Leaves	5.9	4.5	4.9	4.4	13.7
		Crown	10.1	2.6	1.9	2.2	6.6
		Root	11.2	1.9	2.1	2.3	6.3
	Mean		*9.1*	*3.0*	*2.9*	*3.0*	*8.9*
	20	Leaves	11.7	5.6	5.7	4.5	15.9
		Crown	13.1	2.9	2.5	2.7	8.1
		Root	11.5	2.6	2.5	2.3	7.4
	Mean		*12.1*	*3.7*	*3.6*	*3.2*	*10.5*
18	10	Leaves	4.7	3.6	5.1	4.4	13.1
		Crown	5.4	2.2	3.2	3.1	8.5
		Root	8.1	1.6	2.2	2.5	6.3
	Mean		*6.1*	*2.5*	*3.5*	*3.3*	*9.3*
	20	Leaves	6.7	3.6	6.5	5.6	15.7
		Crown	7.3	4.1	3.8	2.7	10.6
		Root	7.5	2.4	2.3	2.3	7.0
	Mean		*7.1*	*3.4*	*4.2*	*3.5*	*11.1*
24	10	Leaves	4.5	3.0	6.2	5.3	14.5
		Crown	2.4	1.5	2.9	2.8	7.2
		Root	3.5	2.1	2.8	2.5	7.4
	Mean		*3.5*	*2.2*	*4.0*	*3.6*	*9.7*
	20	Leaves	4.6	2.2	7.3	5.8	15.3
		Crown	4.0	4.0	3.0	2.9	9.9
		Root	3.2	2.3	3.3	2.6	8.1
	Mean		*3.9*	*2.8*	*4.5*	*3.8*	*11.1*

Probability level of significance (ANOVA)					
Source of variation					
Temperature (*A*)	0.001	0.05	0.03	n.s.	n.s.
Photoperiod (*B*)	0.002	0.008	0.01	n.s.	0.004
A x B	0.03	n.s.	n.s.	n.s.	n.s.
Plant part (*C*)	<0.001	<0.001	<0.001	<0.001	<0.001
A x C	<0.001	0.004	n.s.	n.s.	n.s.
B x C	<0.001	0.03	n.s.	n.s.	n.s.
A x B x C	<0.001	0.05	n.s.	n.s.	n.s.

Data are the means of three biological replicates, each with four plants per treatment.

n.s. - not significant.

Table 5

Effects of temperature and photoperiod on flowering in 'Sonata' strawberry plants. The plants were exposed to the respective conditions for 31 days and then immediately forced in 20 h LD at 20°C for 60 days.

Photoperiod (h)	Temp-erature (°C)	Flowering plants (%)	Days to anthesis	No. of infloresc. plant^{-1}	No. of flowers plant^{-1}	Flowers infloresc.$^{-1}$	No. of crowns plant^{-1}	No. of runners plant^{-1}
10	12	100	30.3	1.5	17.4	11.9	3.1	3.8
	18	100	28.3	2.3	27.0	12.5	3.7	6.2
	24	80	45.8	1.5	31.3	22.9	3.1	9.7
Mean		*93*	*34.1*	*1.8*	*25.4*	*15.3*	*3.3*	*6.3*
20	12	0	-	0	0	-	2.7	6.5
	18	0	-	0	0	-	1.2	6.5
	24	0	-	0	0	-	1.1	6.0
Mean		*0*	-	*0*	*0*	-	*1.7*	*6.3*
Probability level of significance (ANOVA)								
Source of variation								
Temperature (*A*)		<0.001	<0.001	0.05	0.01	0.006	n.s.	0.001
Photoperiod (*B*)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.s.
A x B		<0.001	<0.001	0.05	n.s.	0.001	0.003	0.001

Data are the means of three replicates, each with five plants in each treatment.

n.s. - not significant.

FIGURE CAPTIONS

Fig. 1. Linear regressions for the natural log (ln) of total dry weight and leaf area increases versus time as influenced by temperature and photoperiod in young 'Sonata' strawberry plants. The data represent the results of an experiment with three biological replicates, each with four plants per treatment.

Fig. 2. Growth analysis parameters for young strawberry plants grown in 10 h (SD, filled symbols) and 20 h (LD, open symbols) photoperiods at temperatures of 12, 18 and 24 h. The results represent growth over the entire 31-day growth period. Values are means ±SE of three biological replicates, each with four plants per treatment.

Fig. 3. Appearance of young strawberry plants after 31 days of cultivation in 10 h SD and 20 h LD and temperatures of 12, 18 and 24 °C as indicated.

Fig. 4. Time course changes in chlorophyll concentrations in leaves of young strawberry plants estimated with the Minolta SPAD-502 chlorophyll meter. Values are means of three biological replicates and represent the means of measurements on three leaves of each harvested plant. The vertical bars represent ±SE of the means.

SUPPLEMENTARY MATERIAL

Fig. S1. Time courses of total leaf area and dry weight increments in young strawberry plants grown in 10 h (SD, filled symbols) and 20 h (LD, open symbols) photoperiods at temperatures of 12, 18 and 24 °C as indicated. Values are means ±SE of three biological replicates.

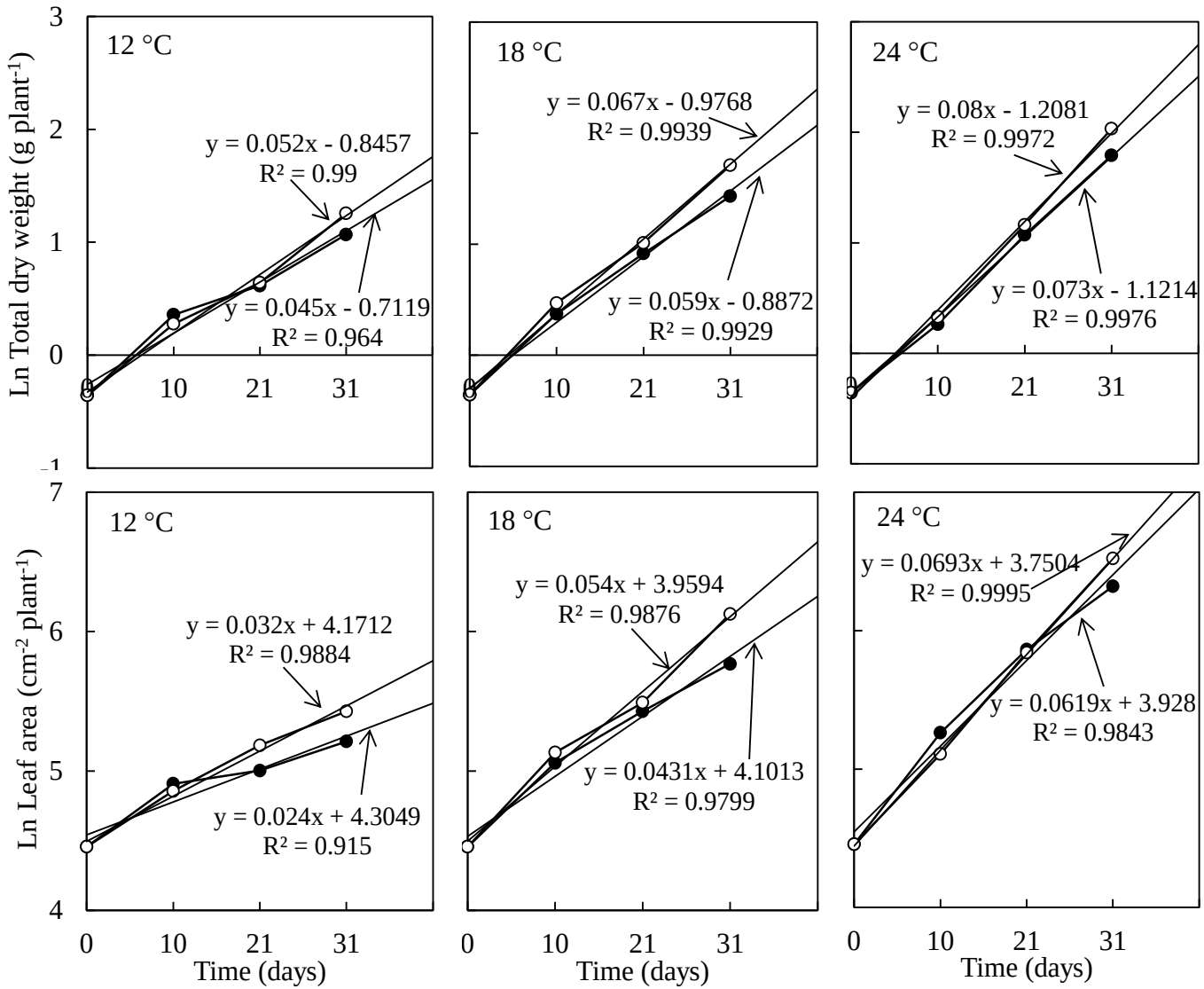
12 °C
y = 0.052x - 0.8457
R² = 0.99
y = 0.045x - 0.7119
R² = 0.964
18 °C
y = 0.067x - 0.9768
R² = 0.9939
y = 0.059x - 0.8872
R² = 0.9929
24 °C
y = 0.08x - 1.2081
R² = 0.9972
y = 0.073x - 1.1214
R² = 0.9976
Ln Total dry weight (g plant-1)
12 °C
y = 0.032x + 4.1712
R² = 0.9884
y = 0.024x + 4.3049
R² = 0.915
18 °C
y = 0.054x + 3.9594
R² = 0.9876
y = 0.0431x + 4.1013
R² = 0.9799
24 °C
y = 0.0693x + 3.7504
R² = 0.9995
y = 0.0619x + 3.928
R² = 0.9843
Ln Leaf area (cm-2 plant-1)
Time (days)
Time (days)
Time (days)

Fig. 1.

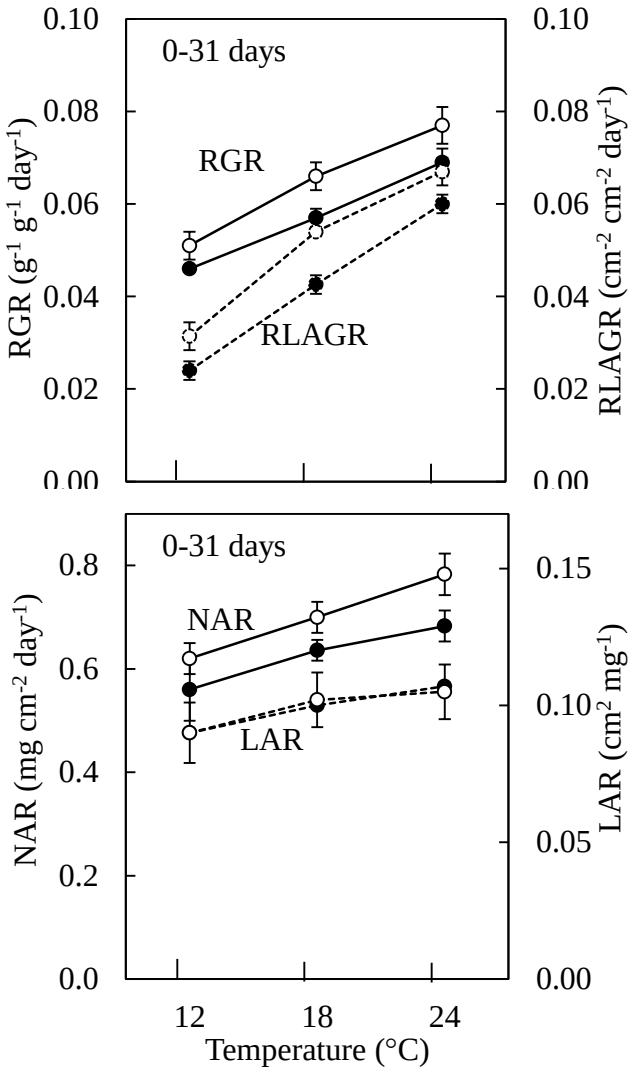
0-31 days
RGR
RLAGR
RGR (g-1 g-1 day-1)
RLAGR (cm-2 cm-2 day-1)
0.10
0.08
0.06
0.04
0.02
0.00
0-31 days
NAR
LAR
NAR (mg cm-2 day-1)
LAR (cm2 mg-1)
0.8
0.6
0.4
0.2
0.0
0.15
0.10
0.05
0.00
12
18
24
Temperature (°C)

Fig. 2.

Fig. 3.

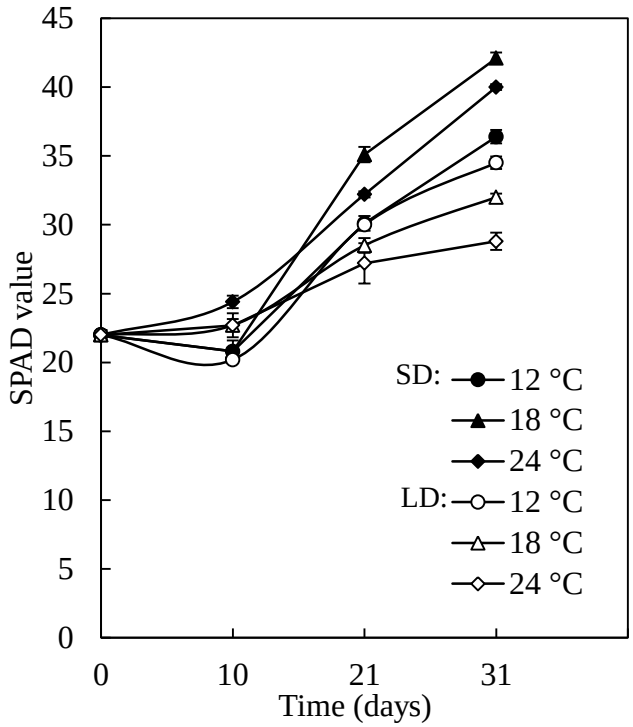
45
40
35
30
25
20
15
10
5
0
SPAD value
0
10
21
31
Time (days)
SD:
12 °C
18 °C
24 °C
LD:
12 °C
18 °C
24 °C

Fig. 4.

Supplementary material:

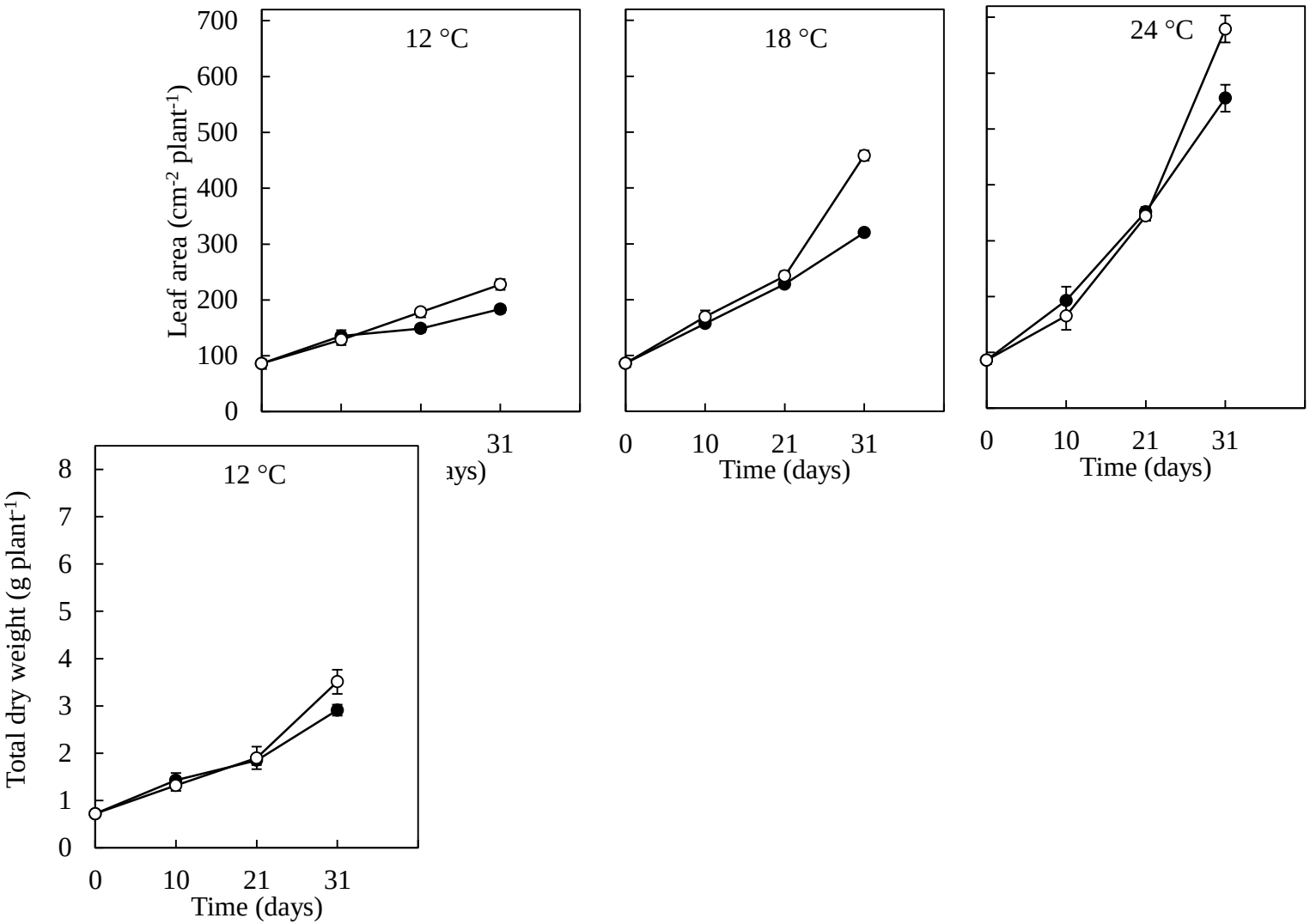

Fig. S1.