

1 Functional growth analysis of ‘Sonata’ strawberry plants grown under controlled
2 temperature and daylength conditions

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9

10 ABSTRACT

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

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

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37 1. Introduction

38 Growth and development of the strawberry plant are regulated by a complex set of interacting
39 environmental factors, of which temperature, daylength and light intensity predominate (Darrow,
40 1936; Guttridge, 1985; Larson, 1994; Heide et al., 2013). Because of the economic importance of
41 the crop, strawberry physiology and genetics have been extensively researched, and the literature
42 in the field has been reviewed several times (e.g. Guttridge, 1985; Larson, 1994; Heide et al.,
43 2013). **However, while the environmental regulation of flower formation and the transition from**
44 **vegetative to reproductive development have been subject to extensive research, the**
45 **environmental regulation of vegetative growth of the strawberry plant has received less attention.**

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

$$54 \text{RGR} = \text{NAR} \times \text{LAR}$$

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

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

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

75 This prompted us to undertake a classical growth analysis of young strawberry plants grown in
76 a phytotron under controlled temperature and daylength conditions. The objective of the
77 investigation was to quantify the impact of temperature and daylength on production and
78 allocation of dry matter in young strawberry plants in order to facilitate our understanding of the
79 **processes** by which the climatic environment control growth and development of young
80 (vegetative) strawberry plants. Since starch content is known to greatly affect cold storage
81 success and transplanting performance and growth vigor of strawberry plants (Bringhurst et al.,
82 1960; López et al., 2002), the content and partitioning of nonstructural carbohydrates were also
83 determined in plants from the various growth conditions. Furthermore, the parallel environmental
84 effect on flower induction was also included in the investigation.

85

86 **2. Materials and Methods**

87 *2.1. Plant material and handling*

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

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

100 In the phytotron, the plants received natural daylight for 10 h per day (08.00-18.00 h).
101 Whenever the photosynthetic photon flux (PPF) in the daylight compartments fell below
102 approximately 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (as on cloudy days), an additional 125 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ were
103 automatically added by high-pressure metal halid lamps (400 W Philips HPI-T). Daylength
104 extension to 20 h long day (LD) was provided by low intensity light from 70 W incandescent
105 lamps (c. 7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) in such a way that the 4 h dark period was centered around
106 midnight (22.00 h to 02.00 h). Plants receiving short day (SD) treatment were in the dark from
107 18.00 h to 08.00 h. The daylength extension light amounted to less than 2% of the total daily light
108 radiation, the plants thus receiving nearly the same daily light integral in both photoperiods. The
109 plant trolleys were randomly positioned in the daylight rooms as a result of the every-day
110 movements to and from the adjacent photoperiodic treatments rooms. Temperatures were
111 controlled to ± 1.0 °C and a water vapour pressure deficit of 530 Pa was maintained at all
112 temperatures. In order to reduce the bias of runner formation and growth on total dry matter
113 accumulation and partitioning (Pritts and Worden, 1988), new runners were removed in all
114 treatments as soon as they appeared throughout the experimental period.

115 The growth medium used was a 1:1 (v:v) mixture of finely sifted peat-based potting compost
116 and granulated vermiculite. Throughout the experimental period, the plants were irrigated daily to
117 drip-off with a complete fertilizer solution consisting of a 2:3 (w:w) mixture of Superba™ Rød
118 (9-5-25-4% NPKMg + micronutrients) and Calcinit™ (15.5-19% NCa) (Yara International, Oslo,
119 Norway) with electric conductivity (EC) of 1.0 mS cm^{-1} . Plants were harvested for growth
120 analysis after 10, 21, and 31 days of cultivation at the respective conditions. In order to reduce
121 diurnal metabolic changes to a minimum during the day of harvest, all plants to be harvested on a
122 given day were placed in the dark in a 5 °C cold rom from 08.00 h until harvested. At harvest, the
123 plants were partitioned into three components: green leaves (lamina and petiole), crowns, and
124 roots. The plants were removed from the pots and the roots washed clean of soil material and,
125 after blotting on tissue paper, fresh weight was determined for each component. Total leaf area of
126 each sample was measured with a LI-COR Inc. Model LI-3000 area meter. Plant material was
127 then placed loosely in open paper bags and dried in a forced-air drying oven at 100 °C for 60
128 min, and then further dried to constant weight at 70 °C. The initial heat treatment at 100 °C was

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

137

138 2.2 Chemical analysis

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

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

155 Starch. Approx. 200 mg dried plant material was weight into a 15 ml Sarstedt plastic
156 centrifuge tube. Soluble sugars were extracted as described above and discarded with the
157 supernatant. Starch in the precipitate were solubilized by adding 2 ml dimethyl sulfoxide and
158 placing the tube on a boiling water bath for 5 min. Immediately, 2.9 ml MOPS buffer (pH 7) and
159 0.1 ml thermostable α -amylase (*B. licheniformis*, Megazyme) was added and the tube was

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

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

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

171

172 *2.3. Assessment of flowering status*

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

178

179 *2.4 Experimental design and statistical analysis*

180 The experiment was factorial with a split-plot design with temperatures as main plots and
181 photoperiods as sub-plots. Each treatment had 3 replicates consisting of 62 plants each at start of
182 the experiment, positioned on 2 trolleys (one trolley after the second harvest). In each replicate,
183 11, 8, 6, and 4 plants, respectively, were harvested on days 0, 10, 21, and 31. In addition, 5 plants
184 remained in each replicate for 31 d for assessment of floral initiation status.

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

189

190 **3. Results**

191 *3.1. Growth analysis*

192 The results in Fig. 1 demonstrate that total plant dry weight and leaf area increased with
193 increasing temperature and photoperiod, but usually only after some time lag in the case of
194 photoperiod. Weight and area increases were exponential versus time, giving a linear time
195 regression with the natural log (ln), thus rendering the relative growth rate (RGR) constant over
196 time **at each growth condition. (For primary growth data, see Fig. S1)**. However, due to
197 adjustment to the new growth conditions, the RGR underwent transitional changes at 12 and 18
198 °C during the first ten-day growth period (Table 1). For the entire 31-day growth period, there
199 was a linear increase in the RGR across the 12–24 °C temperature range with a consistent 10 to
200 13% enhancement by LD at all temperatures (Fig. 2). This was associated with a significant
201 increase in the NAR with increasing temperature and photoperiod, whereas the LAR was
202 enhanced by increasing temperature only.

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

212

213 *3.2 Plant carbon/nitrogen (C/N) ratio*

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

222

223 *3.3. Nonstructural carbohydrate composition*

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

236

237 *3.4. Chlorophyll concentration*

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

245

246 *3.5. Flowering*

247 Forcing of plants exposed to the various growing conditions for 31 d showed that all plants at
248 12 and 18 °C and 80% of those at 24 °C had initiated flowers in SD, whereas none had initiated
249 flowers in LD regardless of temperature conditions (Table 5). The plants from 12 and 18 °C
250 started flowering simultaneously after 5 weeks of forcing, while those from 24 °C were delayed
251 for an additional 14 d. While the number of inflorescences per plant was little affected by
252 temperature, the number of flowers per inflorescence increased consistently across the range of

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

259

260 4. Discussion

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

270 The maximum RGR of 0.077 g/g/day **obtained** in LD at 24 °C is comparable with the
271 maximum RGR of 0.044 g/g/day obtained in the establishment year under field conditions at
272 midsummer by Olsen et al. (1985). The use of small plants with minimal leaf shading is probably
273 the main reason for the superior growth rate in the present experiment. A temperature optimum of
274 24 °C for dry matter production in strawberry is higher than the 18 °C optimum previously
275 reported by Heide (1977). The reason for this discrepancy is probably the superior light
276 conditions during spring and early summer in the present experiment compared with autumn
277 conditions in the former. The results confirm the profound effect of low temperature and SD **on**
278 **the reduction of growth rate** as an **early event in the sequence of processes (the autumn**
279 **syndrome) associated with autumn preparation** in strawberry plants.

280 The **growth analysis demonstrated that enhancement of the** RGR at high temperature was
281 driven by a combined increase in the net assimilation rate (NAR) and the leaf area ratio (LAR),
282 whereas the LD enhancement was driven by an increase only in the NAR (Fig. 2). Nevertheless,
283 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 (°C)	Photoperiod (h)	% dry matter		
		Leaves	Crowns	Roots
12	10	61.5	12.0	26.6
	20	68.8	10.6	20.6
<i>Mean</i>		<i>65.2</i>	<i>11.3</i>	<i>23.6</i>
18	10	70.6	8.0	21.4
	20	73.9	8.1	18.0
<i>Mean</i>		<i>72.3</i>	<i>8.1</i>	<i>19.7</i>
24	10	75.1	6.7	18.2
	20	80.3	6.1	13.5
<i>Mean</i>		<i>77.7</i>	<i>6.4</i>	<i>15.9</i>

Probability level of significance (ANOVA)

Source of variation

Temperature (A)	0.001	<0.001	0.004
Photoperiod (B)	<0.001	n.s.	<0.001
<i>A x B</i>	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	
		<i>Mean</i>		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	
		<i>Mean</i>		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	
		<i>Mean</i>		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	
		<i>Mean</i>		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	
		<i>Mean</i>		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	
		<i>Mean</i>		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 ($^{\circ}\text{C}$)	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	
		<i>Mean</i>		<i>9.1</i>	<i>3.0</i>	<i>2.9</i>	<i>3.0</i>	<i>8.9</i>
	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	
	<i>Mean</i>		<i>12.1</i>	<i>3.7</i>	<i>3.6</i>	<i>3.2</i>	<i>10.5</i>	
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	
		<i>Mean</i>		<i>6.1</i>	<i>2.5</i>	<i>3.5</i>	<i>3.3</i>	<i>9.3</i>
	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	
	<i>Mean</i>		<i>7.1</i>	<i>3.4</i>	<i>4.2</i>	<i>3.5</i>	<i>11.1</i>	
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	
		<i>Mean</i>		<i>3.5</i>	<i>2.2</i>	<i>4.0</i>	<i>3.6</i>	<i>9.7</i>
	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	
	<i>Mean</i>		<i>3.9</i>	<i>2.8</i>	<i>4.5</i>	<i>3.8</i>	<i>11.1</i>	

Probability level of significance (ANOVA)

Source of variation

Temperature (<i>A</i>)	0.001	0.05	0.03	n.s.	n.s.
Photoperiod (<i>B</i>)	0.002	0.008	0.01	n.s.	0.004
<i>A x B</i>	0.03	n.s.	n.s.	n.s.	n.s.
Plant part (<i>C</i>)	<0.001	<0.001	<0.001	<0.001	<0.001
<i>A x C</i>	<0.001	0.004	n.s.	n.s.	n.s.
<i>B x C</i>	<0.001	0.03	n.s.	n.s.	n.s.
<i>A x B x C</i>	<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
<i>Mean</i>		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
<i>Mean</i>		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.
<i>A x B</i>	<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 \pm 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 \pm 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 \pm SE of three biological replicates.

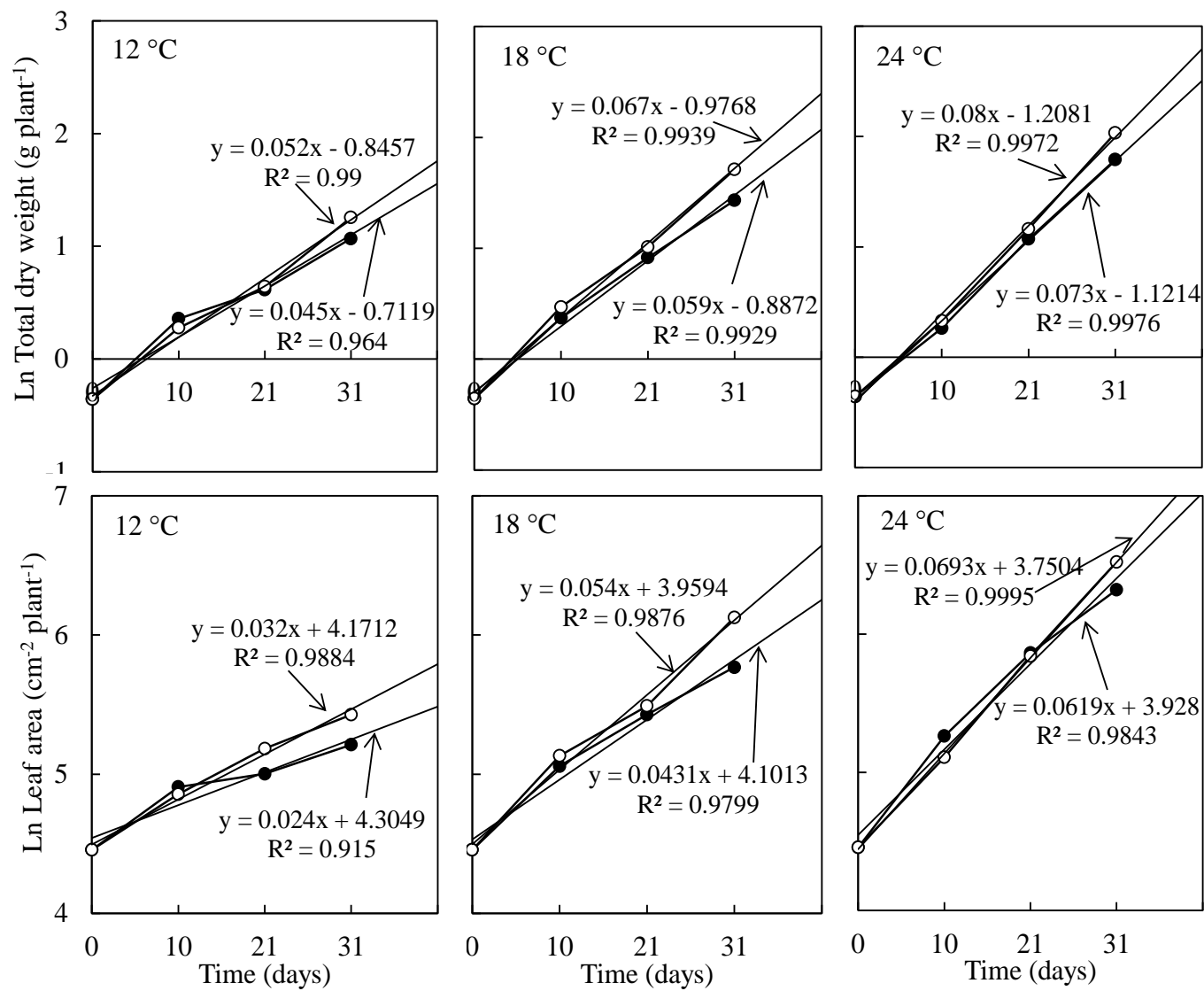


Fig. 1.

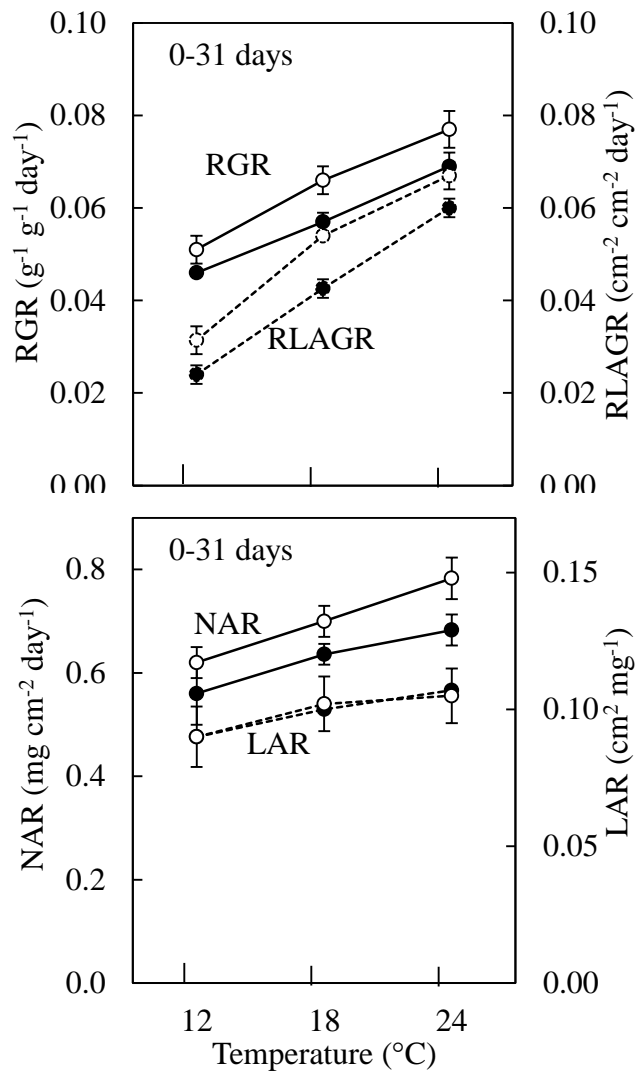


Fig. 2.



Fig. 3.

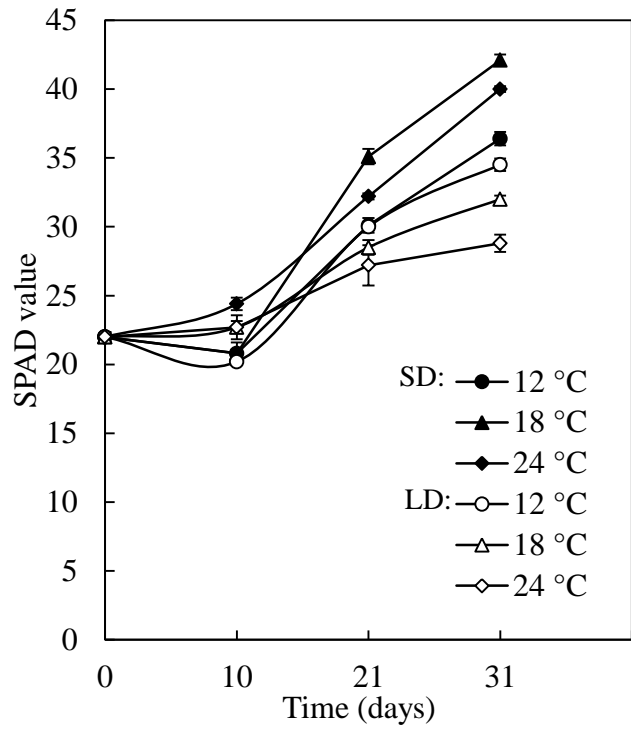


Fig. 4.

Supplementary material:

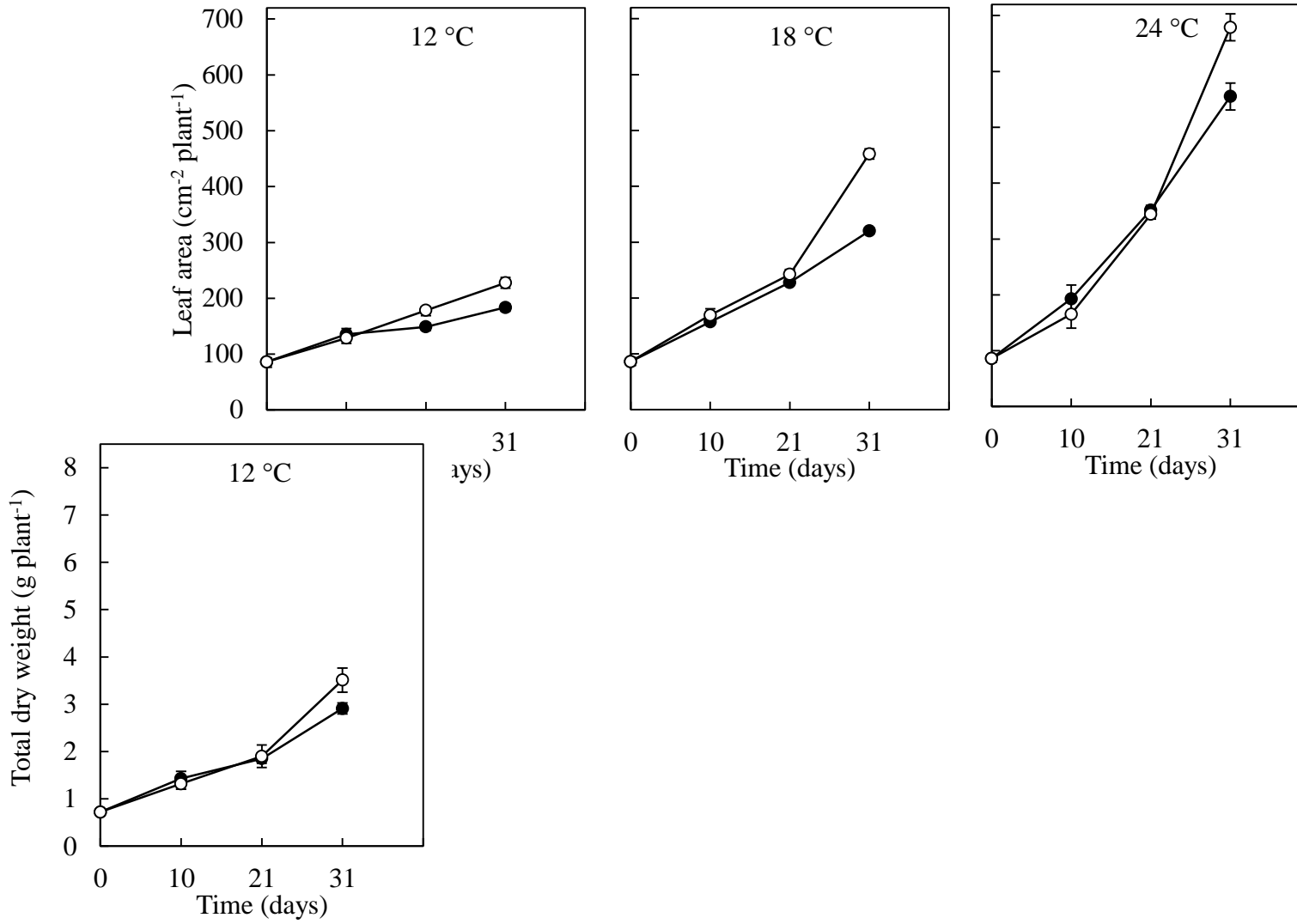


Fig. S1.