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Additional Information

1 **Relation of carbohydrate reserves with the forthcoming crop, flower**

2 **formation and photosynthetic rate, in the alternate bearing Salustiana**

3 **sweet orange (_Citrus sinensis_ L.).**

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**ABSTRACT**

The influence of crop load on photosynthetic $CO_2$ fixation and the accumulation and mobilization of carbohydrate reserves in leaves, twigs and roots, was determined in the alternate bearing Salustiana cultivar of sweet orange (*Citrus sinensis* [L.] Osbeck) in order to assess the significance of the carbohydrate reserves in relation to flower formation and fruit set, and the effect of carbohydrate use in fructification on $CO_2$ fixation. A heavy crop load failed to increase leaf photosynthesis as compared to non fruiting trees. In fruiting trees most of the fixed carbon accumulated in the mature fruit, and no accumulation of reserve carbohydrates occurred in the roots before harvest. In the non fruiting trees, part of the fixed carbon was transported to the root and utilized in growth processes and, after December, stored as reserves. Reserve carbohydrate accumulation in the leaves started by early December, and the levels in the leaves were, until bud sprouting, the same in **on** and **off** trees. The heavy flower formation which followed an **off** year caused the rapid mobilization of the stored reserves, which were exhausted at full bloom. We could not find evidence for carbon fixation regulation by fruit demand or by the carbohydrate levels in the leaves. The carbohydrate reserves played no role in fruit set, which relied on current photosynthesis.

**1. Introduction**

Under tropical climate conditions, *Citrus* trees accumulate carbohydrate reserves during the winter rest and mobilise them during the spring flush of growth (Goldschmidt and Koch, 1996). This behaviour is similar to that described in deciduous fruit trees, which accumulate carbohydrate reserves before leaf fall and utilise them during the dormant season and the spring growth (Schaffer et al.*,* 1999), except for some differences in the partitioning of the reserves, and their importance in plant growth regulation and survival. In deciduous trees, the root system is the major storage organ for carbohydrates (Loescher et al., 1990). In *Citrus,* the root system may still be the major storage organ for carbohydrates, but carbohydrates also accumulate in the leaves at a high concentration (Goldschmidt and Golomb, 1982). The importance of reserve carbohydrates in deciduous trees seems evident. Winter respiration and the beginning of both vegetative and, in some species, reproductive growth, occur in the absence of photosynthesing leaves, and must be totally dependent on reserves (Loescher et al., 1990). On the contrary, photosynthesis proceeds in *Citrus* during winter at a rate high enough to affect growth significantly (Syvertsen et al., 1997; Goldschmidt ,1999). Therefore, the reserves should not be as critical for winter and spring growth as in deciduous trees, yet a role for carbohydrate reserves in some aspects of development has been postulated.

The accumulation of reserves is inversely related to crop load (Goldschmidt and Golomb, 1982), and a depletion of them under heavy crop load has been related to tree collapse (Smith, 1976) and the triggering of an alternate bearing habit (Monselise and Goldschmidt, 1982; Guardiola ,1992; Syvertsen and Lloyd, 1994). Although flower formation could be correlated in some experiments with the accumulation of

carbohydrates (Smith, 1976; Goldschmidt and Golomb, 1982), carbohydrate levels are not the sole factor regulating flower formation (Goldschmidt, 1999; García-Luis and Guardiola, 2000). During flower formation and fruit set, part of the reserves are translocated to the reproductive organs (Akao et al., 1981), but the contribution of the reserves to these processes must vary widely as indicated by the rate of their depletion. This rate of depletion may vary among cultivars (Borrás et al., 1984; González-Ferrer et al., 1984), but differences in the rate of depletion within a cultivar have also been reported (García-Luis et al., 1988; Ruiz and Guardiola, 1994; Ruiz et al., 2001). The rate of depletion has been related to flower number (García-Luis et al., 1988).

There are some studies about the significance of reserves in alternate bearing citrus trees (Goldschmidt and Golomb, 1982; Borrás et al., 1984). However, the research has only been performed on one day during the flowering period. In the present report, we have studied the seasonal variation of carbohydrate reserves in the leaves, in the twigs (both in the bark and in the wood) and in the roots, as well as the rate of mobilization during the spring flush of growth, in relation to crop load.

It has been suggested that the carbohydrate accumulation may interfere with photosynthesis by way of product inhibition (Goldschmidt and Koch, 1996), a suggestion supported by the inhibition demonstrated in girdled branches in the absence of a sink outlet (Schaffer et al., 1986). However, this effect is controversial under regular cropping conditions (Goldschmidt and Koch, 1996).

The main objectives of the present work were: i) to assess the importance of reserves for the development of the forthcoming crop, and their role, if any, in the initiation of an alternate bearing cycle; ii) to analyse the regulatory role of carbohydrates in flowering and how the changes in carbohydrate reserves during the year reflect the variations in supply and demand on the diverse organs; and iii) to study the effect of crop load and

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leaf carbohydrate levels on $CO_2$ fixation rate. The measurements were performed in the

Salustiana cultivar of sweet orange. This cultivar has a strong alternate bearing

behaviour, which allowed us to study the above-mentioned parameters both in trees

without fruit and in trees with a heavy crop load.


**2. Materials and methods**

*2.1. Plant material*

The experiments were performed on 35-year-old trees of the "Salustiana" cultivar of

sweet orange (*Citrus sinensis* [L.] Osbeck) grafted onto Troyer citrange (*Citrus sinensis*

[L.] Osbeck x *Poncirus trifoliata* Raf) rootstock, grown at Museros, Valencia, Spain.

The trees of this parthenocarpic (seedless) cultivar presented an intense alternate

bearing habit. In the year of beginning our experiments (2001; year 1), some of the trees

(**on** trees), formed a huge number of flowers, resulting in a heavy crop load, which was

followed by a year (2002; year 2) with very few flowers formed or none at all. Some of

the trees (**off** trees) presented the same fruiting habit but shifted by one year. They

formed almost no flowers, and had a low yield, the year we started the experiment, but

formed many flowers the following year. Under the conditions of our experiments, bud

sprouting of the spring flush of growth occurred from mid February (fruitless trees) to

early-mid March (fruiting trees). Vegetative shoots (bearing only leaves), leafy

inflorescences (bearing flowers and leaves) and leafless inflorescences (bearing only

flowers) were formed. Flower opening started by April, 1. Two waves of fruit

abscission were produced from April to June. The fruit was harvested by early February

the following year.


*2.2. Experimental design*

123 Shortly before flower opening, **on** and **off** trees were chosen. For the sake of clarity, the

124 trees are named in the text as **on** or **off** according to the number of flowers they formed

125 in the first year of the experiment. In these two groups of trees we determined the

126 photosynthetic $CO_2$ fixation rate and carbohydrate accumulation in the leaves, the bark

127 and the wood of the vegetative sprouts formed during the spring flush of growth, and in

128 the roots. These changes were related to the use of dry matter (=carbohydrates) in

129 fructification, which was calculated for the **on** trees during the first year of the

130 experiment.

131

132 *2.3. The characteristics of the spring flush of growth and dry matter use in fructification*

133 A sufficient number of vegetative shoots formed during the preceding year and totalling

134 at least 1,000 axillary buds, were selected before flower opening in each one of six trees

135 of each group on year 1. The numbers of vegetative shoots, leafy inflorescences, and

136 leafless inflorescences, started during the spring flush of growth, as well as their

137 characteristics (number of leaves and of flowers), were measured. The number of

138 abscised flowers and fruits was measured periodically (at 10-12 days intervals)

139 collecting the abscised organs with a plastic net placed under the tree canopy. Adding to

140 this value the number of mature fruit at harvest, we obtained the total number of flowers

141 formed. From these figures, the number of mature fruit present on the tree at any time

142 from flower initiation until harvest, and the number of shoots and of leaves formed

143 during the spring flush of growth, were calculated. Values are averages of six trees.

144 The use of dry matter in fructification was calculated from the number of mature fruit at

145 the end of each one of the periods considered and the amount of dry matter accumulated

146 by the mature fruit in that period. The increase in weight of the fruit was calculated as

147 the difference in dry weight of the fruit at the end and the beginning of each time

148    interval. In the first two samplings, the values thus calculated were corrected for

149    respiratory losses (Bustan and Goldschmidt, 1998). The weights were calculated by

150    measuring the diameter of 200 ovaries/fruits selected at random. The relationship

151    between fruit diameter and weight was determined in random samplings performed on

152    each date on non-measuring trees of the same characteristics, in order to avoid any

153    alteration in fruit number.

154

155    *2.4. Carbohydrate contents in the leaves, twigs and roots*

156    Soluble sugars and starch in the leaves from vegetative shoots were determined from

157    June of year 1, to the end of June of year 2. From June to September of the first year,

158    three independent samples of 50 leaves each (two different trees per sample) were

159    sampled from the mid portion of vegetative sprouts, both from **off** and **on** trees. From

160    October onwards, and until late June on year 2, each sample consisted of the apical

161    portion (having the five most apical phytomers) of ten twigs (from two different trees

162    per sample). These twigs were separated into their component parts (leaves, bark and

163    wood), which were analysed separately. The sampling procedure, the handling of the

164    samples, and carbohydrate determinations were performed as described by Ruiz and

165    Guardiola (1994).

166    Roots were sampled at a depth of 10 to 25 cm from positions close to a drip emitter

167    (five positions per tree). On each tree, ten fibrous roots, with a thickness of 1.5 to 3 mm,

168    were sampled. After careful washing to remove the soil, the fibrous roots were

169    separated from the recently formed feeder roots. These two root samples were analysed

170    separately. The analytical procedure was the same as for the leaves.

171

172    *2.5. Determination of leaf gas exchange*

173    The rate of net $CO_2$ fixation ($A_N$) was measured from June to October of the year 1, and

174    from March to May during the year 2, in leaves of vegetative shoots of the spring flush

175    of growth formed the first year of the experiment. The measurements were performed in

176    attached leaves exposed to the sunlight in ambient conditions, with an LCi Portable

177    Photosynthesis System (ADC, Herst, UK). During the measurements, the leaves were

178    held perpendicularly to sunlight. On each day, three repeated measurements were

179    performed on ten leaves from different **on** trees, and ten leaves from **off** trees. In each of

180    the periods indicated, the measurements were performed on fifteen to twenty different

181    days, and the values were averaged. The measurements were performed from 10 a.m.

182    until 12 p.m., to avoid the midday drop in photosynthesis. The measurements from the

183    leaves of the two tree types were intercalated, to ensure the similarity of the

184    environmental conditions.

185

186    *2.6. Tree girdling*

187    To determine the influence of the transport to the root system on carbohydrate

188    distribution within the plant, **on** and **off** trees were girdled at the base of the scaffold

189    branches, cutting the bark with a single edge knife. This procedure removed a 2 mm

190    thick ring of bark. Carbohydrate accumulation in the leaves and in the bark from

191    vegetative sprouts, were determined 6-9 weeks after girdling. No girdled trees served as

192    controls. Each treatment consisted of three one-tree replicates. The experiment, with the

193    same lay-out, was performed on different trees in June, September, October and

194    November.

195

196    *2.7. Statistical analyses*

197    Results were subjected to an analysis of variance (SPSS for Windows version 12.0.1,

198    Illinois, USA). Mean comparisons were performed with Tukey's test.

199

200    **3. Results**

201    *3.1. The characteristics of the spring flush of growth*

202    The characteristics of the spring flush of growth are presented in Table 1. During the

203    first year, the percentage of bud sprouting was much higher ($P \leq 0.01$) in the **on** trees

204    (56.3 ± 2.7 %) than in the **off** trees (22.4 ± 0.29 %). This resulted in a much higher

205    number of inflorescences initiated (20.6 vs. 0.8 thousands per tree respectively; $P \leq$

206    0.01) and of flowers formed (75 vs. 2 thousands per tree; $P \leq 0.01$). On the contrary, the

207    number of vegetative sprouts initiated was 5-fold higher (8.2 vs. 1.6 thousands per tree)

208    in the **off** trees. The number of leaves initiated in spring was similar in both tree groups.

209    In the **off** trees, most of the 60 thousand new leaves were on vegetative sprouts; in the

210    **on** trees, about 80% of the 49 thousand leaves formed were located in leafy

211    inflorescences. Individual leaf area was higher ($P \leq 0.05$) in the **off** trees (30 ± 1 cm$^2$

212    per leaf) than in the **on** trees. In the **on** trees**,** the individual leaf area was similar in the

213    vegetative sprouts (26 ± 1 cm$^2$) and in the inflorescences (23 ± 1 cm$^2$).

214    The number of old leaves (up to 1-year-old at flowering time), could also be calculated

215    from our flower counts. As the **on** trees formed 75 thousand flowers, and the flower

216    count (in thousands of flowers per 100 nodes) was 0.17 ± 0.025 (average value of six

217    trees), the number of nodes (= old leaves) present was close to 44 thousand per tree

218    ([75/0.17] x 100). A similar calculation for the **off** trees yielded 33 thousand old leaves

219    (calculated from a flower count of 0.006 flowers per 100 nodes and a total flower

220    number of 2 thousand per tree).

During the second year of the experiment, the number of flowers formed was high in the **off** trees (which were in an on year), and low, although somewhat higher than expected, in the **on** trees (which were in an off year; Table 1). These figures were obtained from the counts of abscised flowers and fruits, and of mature fruit. The characteristics of the spring flush of growth were not measured.

*3.2. Use of dry matter in fructification*

In the **on** trees, fruit abscission occurred from anthesis until the end of June, with two distinct peak values: shortly after anthesis (during April), and by the end of May. The fruit dropped during the first wave of abscission had shown little growth (data not shown). During the second wave of abscission, the rate of accumulation of dry matter in the fruit increased gradually, reaching a value close to the maximum by mid June, shortly before abscission ceased (Fig. 1).

The rate of utilization of dry matter in flower formation and fruit growth was high from bud sprouting to anthesis, reflecting the cost of flower formation (Fig. 1). Then it fell to a low value, around a fifth of the previous one, coinciding with the first peak of abscission, to increase gradually to reach a highest value shortly before the end of abscission, as the rate of accumulation of dry matter in the mature fruit increased (Fig. 1). After the period of abscission, dry matter use in fruit formation paralleled individual fruit growth, decreasing gradually from September onwards.

During the first year of the experiment, the rate of dry matter utilization in the **off** trees was in the range 3-5 % of the values presented for the **on** trees during flower formation and fruit abscission and of 11% after fruit abscission (Table 2). The amount of dry matter recovered in the fruit at harvest ranged between 64% (**on** trees) and 74% (**off**

245  trees) of the total dry matter use. The rest was lost through flower and fruit abscission,

246  and respiratory losses.

247  During the second year, the **off** trees entered an **on** year and formed a huge number of

248  flowers (Table 1). This year, the rate of dry matter use from flower initiation to anthesis

249  was 256 g tree$^{-1}$ day$^{-1}$, rising at the end of June to 415 g tree$^{-1}$ day$^{-1}$. These values are

250  similar to the ones reported above for the **on** trees during the first year.

251

252  *3.3. Carbohydrate content in leaves, bark and wood*

253  The carbohydrate content in the leaves from the vegetative sprouts formed during the

254  spring flush of growth of the first year is presented in Fig. 2. During the first year, the

255  total carbohydrate content in the leaves during the final stages of fruit abscission (June)

256  was 2.5-fold higher in the **off** than in the **on** trees. Afterwards, there was a gradual loss

257  of carbohydrates from the leaves of the **off** trees, and from September to December the

258  carbohydrate content was lowest, and similar in both tree classes. Carbohydrate

259  accumulation in the leaves started by early December and proceeded at a similar rate in

260  both tree classes until the start of bud sprouting in the **off** trees by the end of February.

261  In these trees, which entered an **on** year, the level of carbohydrates fell to a low value at

262  anthesis (early April), recovered partially at the beginning of fruit abscission, to fell to a

263  lowest value close to 10% (on dry matter basis) by the end of abscission (end of June;

264  Fig. 2). In the **on** trees, which entered an **off** year, carbohydrate content in the leaves

265  was maximal during the period April to May, decreasing by the end of June (Fig. 2).

266  The changes in starch content accounted for most of the changes in total carbohydrates

267  in the leaves (Fig. 2), and the two parameters were closely related ($r^2 = 0.94$; n = 30, $P \leq$

268  0.001). The changes in soluble sugar content were much smaller, and in most of the

269  samples no differences were found between the two tree classes. At flowering, the sugar

concentration in the leaves was higher in the trees entering an **off** year than in those entering an **on** year (Fig. 2).

The pattern of the changes in carbohydrates in the bark and in the wood was similar to that described for the leaves (Fig. 3). Accumulation in the bark started after November, and no significant differences were found between the **on** and the **off** trees until sprouting. At this time, there was a drastic reduction in carbohydrates, both in the bark and in the wood, in those trees entering an **on** year. As for the leaves, the changes in starch contents accounted for most of these changes (evidence not presented). The $r^2$ value between these two parameters was 0.94 (bark) and 0.80 (wood).

### 3.4. The effect of girdling on leaf carbohydrates

Girdling the scaffold branches caused the accumulation of carbohydrates in the leaves of the **off** trees. This accumulation was much greater during June, October and November, above 30% of the content in the leaves of the ungirdled controls, than in September, when it was only 14% of the value of the controls (Fig. 4).

In the **on** trees, girdling during September and October did not affect the carbohydrate concentration in the leaves (Fig. 4). When performed in June or in November, girdling increased carbohydrate contents by 10% above the controls (Fig. 4).

Both in the **on** and the **off** trees, the effect of girdling (performed in October or in November) on carbohydrate contents in the bark of the twigs was similar to that described for the leaves.

### 3.5. Carbohydrate content in the roots

From October to early December, carbohydrate content in the roots was the same in the **off** and in the **on** trees (Fig. 5). At this date, a gradual accumulation of carbohydrates

295   started in the roots of the **off** trees**,** both in the fibrous and, in a lesser amount, in the

296   feeder roots (Fig. 5). This accumulation ceased at the time of bud sprouting, when a

297   significant part of the accumulated reserves were utilised in 20 days (Fig. 5). At this

298   time, there was a significant increase in the proportion of feeder roots (Fig. 6).

299   In the **on** trees, the accumulation of carbohydrates started after harvest, and ended by

300   bud sprouting (Fig. 5). The maximum concentration of carbohydrates in the roots of the

301   **on** trees was much smaller than in the **off** trees. After sprouting, the concentration of

302   carbohydrates in the roots was identical in the two tree classes.

303   Most of the changes in carbohydrate contents reflected the accumulation and the

304   mobilization of starch, whose concentration was closely related to the concentration of

305   total metabolizable carbohydrates ($r^2$ = 0.98 and 0.90 for the feeder and the fibrous

306   roots, respectively; n = 20; $P \leq 0.001$).

307

308   *3.6. The effect of fruit load on photosynthesis*

309   Leaf photosynthesis was largely determined by the environmental conditions, which

310   were the main factor in the variability of this parameter. During the periods with a

311   highest rate of dry matter utilization in the **on** tress, that is to say, by March-April when

312   flowering is going on, and June-July when the fruit growth rate is the highest (Fig. 2),

313   there were no significant differences in photosynthetic rates between the trees with a

314   low and a high crop (Table 3). It is important to point out that differences in

315   carbohydrates between tree groups are highest during these periods (Fig. 3).

316

317   **4. Discussion**

318   It is a well established fact that the fruit is a major and priority sink in *Citrus*, and that a

319   heavy fruit load reduces both the diversion of carbohydrates towards the root system

320     (García-Luis et al., 1995) and the accumulation of carbohydrate reserves (Goldschmidt

321     and Koch, 1996). The amount of the mid-winter carbohydrate reserves in an **off** year in

322     an adult tree of the alternate bearer Wilking mandarin has been estimated at ca. 24 Kg

323     (Goldschmidt and Golomb, 1982). These reserves are mobilized during the next **on** year

324     (Syvertsen and Lloyd, 1994; Goldschmidt and Koch, 1996), and it has been speculated

325     they could satisfy a considerable portion of the dry matter requirements of the following

326     crop (Goldschmidt and Golomb, 1982). The aim of the present study was to assess the

327     importance of these reserves for the development of the forthcoming crop, and their

328     role, if any, in the initiation of an alternate bearing cycle.

329

330     *4.1. Use of dry matter in fructification*

331     In our experiments, the concentration of reserve carbohydrates in the **off** trees, which

332     entered an **on** year, at the onset of bud sprouting was of the same order of magnitude as

333     reported for other alternate *Citrus* cultivars at the end of an **off** year (Goldschmidt and

334     Koch, 1996). These reserves were used-up during bud sprouting, and at the time of full

335     bloom (early April) the carbohydrate concentration in the leaves and in the twigs had

336     fallen to a minimum value (Fig. 2 and 3). During the period of fruit abscission (May and

337     June), in which competition for carbohydrates is considered to be a limiting factor for

338     fruit retention (Goldschmidt, 1999), fruit nutrition was supported by current

339     photosynthesis and the carbohydrates stored after anthesis (during April; Fig. 2 and 3).

340     The relatively high carbohydrate contents in the leaves and in the bark at full bloom,

341     around 10 % on a dry matter basis, may represent a non-utilizable fraction

342     (Goldschmidt and Koch, 1996; Ruiz et al., 2001).

343     As reported for other woody species (Loescher et al., 1990), the root system was the

344     main storage organ for carbohydrates. These reserves fell dramatically at the onset of

345    bud sprouting, in particular in the fibrous roots (Fig. 5), coinciding with the resumption

346    of root growth (Fig. 6). This fall in carbohydrate reserves was relatively smaller than in

347    the leaves and in the twigs, and the fibrous roots of the trees which enterd an **on** year,

348    had during anthesis a carbohydrate concentration twice the amount found in early

349    October. These reserves could potentially be utilized, but the net change in these

350    reserves during the period of fruit abscission was very small (Fig. 5), and the girdling

351    experiments demonstrated that at this time the root system competed with the fruit for

352    the available carbohydrates (Fig. 4). Although some transport of carbohydrates from the

353    root system to the shoot was demonstrated in labelling experiments (Kubota and

354    Motoyama, 1972; Goldschmidt and Koch, 1996), we found no evidence for the

355    contribution of the root reserves to fruit set. A similar conclusion was reached by

356    Loescher et al. (1990) in their review on root reserves in deciduous trees, as these

357    authors stated that evidence that the roots play a special role on fruiting behaviour is

358    unclear.

359    While the reserve carbohydrates may have supported the initial stages of vegetative

360    growth and reproductive development (Akao et al., 1981), they played no significant

361    role in fruit set. Most of the reserves stored during the **off** year may have gone in

362    surplus flower formation, whose cost in term of carbohydrates was about 60 % of the

363    total reserves (Table 2). This surplus flower formation had little or no effect on fruit set

364    (Becerra and Guardiola, 1984), but in some conditions may impair it (Becerra and

365    Guardiola, 1984; Guardiola et al., 1984).

366

367    *4.2. The role of carbohydrate reserves in alternate bearing habit*

368    It is generally accepted that the cycles of alternate bearing are caused by the inhibition

369    of flower formation after a heavy fruit load (Guardiola, 1992), and flower formation has

been related to carbohydrate levels (Goldschmidt and Golomb, 1982; García Luis et al.,

1988, 1995). This role of flower inhibition by the fruit was supported by our results

(Table 1), but we could not find evidence for a regulatory role of carbohydrates. Most

alternate *Citrus* cultivars are late maturing, and the presence of developing fruit

prevents the accumulation of carbohydrates in all tree organs during an **on** cycle

(Syvertsen and Lloyd, 1994). In the early maturing Salustiana orange used in this study,

the low fruit strength of the fruit after November (Fig. 1) allowed the accumulation of

reserves during winter in the leaves and the twigs of the **on** trees (Figures 2 and 3), and

during the period of flower induction and initiation (from early November until bud

sprouting) the carbohydrate levels in these organs were identical in the **off** and the **on**

trees. We may conclude therefore that reserve carbohydrates do not play a regulatory

role in flower formation, a role whose importance was questioned by Goldschmidt

(1999) and García-Luis and Guardiola (2000). As most of the flowers formed in the

axillary buds of the vegetative shoots initiated during the spring flush of growth of the

preceding year, the 5-fold higher number of these buds in the **off** trees than in the **on**

trees (8.2 *vs.* 1.6 thousand vegetative shoots per tree, respectively; Table 1) would

explain only in part the 20 to 35-fold differences in flower formation between the **off**

and the **on** trees (Table 1). A direct effect of the fruit on flower initiation in the buds

also seems evident.


*4.3. Source-sink effects on seasonal carbohydrate reserves*

The changes in carbohydrate reserves during the year reflect the variations in supply

and demand. The carbohydrate demand during the initial stages of vegetative growth

and reproductive development in trees which entered in an **on** year determined an initial

drop in the leaf and twig reserves which was followed by a transient recovery and a

395  further drop at the end of fruit abscission (Fig. 2 and 3). In trees which entered an **off**

396  year with a low carbohydrate demand for fructification, carbohydrate accumulation

397  continued until early April and remained high until the end of May (Figures 2 and 3).

398  The carbohydrate levels in the leaves during spring in trees with a low crop were twice

399  as much the levels in the high crop trees (Fig. 2). The differences in the pattern of the

400  changes in carbohydrate reserves reported by different authors (see references in

401  Introduction), which lead sometimes to high starch levels during May in the northern

402  hemisphere (Borrás et al. 1984), might be the result of differences in flower formation

403  rather than a varietal characteristic.

404  After fruit abscission, carbohydrate levels in the leaves declined and stayed low during

405  summer and autumn. As no effect of the fruit on photosynthesis (Table 3) or on leaf

406  carbohydrates (Fig. 2) was found during those months, most of the carbon fixed by the

407  **off** trees must have been transported to the root system, a conclusion supported by the

408  girdling experiments (Fig. 4). The smaller retention caused by girdling in the **off** trees

409  during September, may reflect competition from the summer flush of growth. This

410  higher transport of carbohydrates to the root system in the **off** trees did not increase the

411  root reserves (Fig. 5) nor root growth as assessed by the proportion of feeder roots to the

412  total root sample (Fig. 6), and was probably used in the thickening of the major roots.

413  Interestingly, Goldschmidt ad Golomb (1982) demonstrated in Wilking mandarin a 2-

414  fold higher weight of the major roots after an **off** year than after an **on** year.

415  Despite the low sink strength of the fruit from December onwards, it prevented the

416  accumulation of carbohydrate reserves in the root, which only occurred after harvest, an

417  effect previously observed in potted trees (García-Luis et al., 1995). This fruit effect,

418  whose regulation was not investigated, did not affect the accumulation of carbohydrates

419  in the leaves (Fig. 2). During this period of time, fruit load affected the carbohydrate

420 reserves in the roots but had no effect on their concentration in the leaves. As pointed

421 out by Goldschmidt and Golomb (1982), the leaves are not always the most sensitive

422 indicator organs. In this study we explained how, depending on the time of sampling,

423 the carbohydrate concentration in the leaves of the trees with a high crop was lower

424 (March-July) or the same (autumn and winter months) than in the trees with a low crop.

425

426 *4.4. Photosynthesis regulation*

427 The regulation of photosynthesis ($A_N$) by sink demand has been demonstrated in *Citrus*

428 either using potted trees (Syvertsen and Lloyd, 1994; Goldschmidt and Koch, 1996;

429 Iglesias et al. 2002), or altering the source-sink balance in the tree (Li et al., 2003), and

430 a regulatory role for the carbohydrate level in the leaves has been suggested (Iglesias et

431 al., 2002). However, to what extent sink demand controls photosynthetic rates in *Citrus*

432 under regular cropping conditions is not clear, nor is the mechanism of regulation

433 (Goldschmidt and Koch, 1996). We addressed this issue by measuring $A_N$ under orchard

434 conditions at developmental stages in which the differences in the sink strength of the

435 developing flowers and fruits between the high and low crop  trees were highest; the

436 flower formation period (March-April), and the main fruit growth stage (June-July). The

437 highest differences in leaf carbohydrate contents happened during these periods. In both

438 of them, the environmental conditions were the main determinant of $A_N$. No

439 enhancement of $A_N$ by a high sink strength related to fruiting was found in our

440 experiments. A delay of leaf senescence caused by fruiting, and an enhancement of

441 autumn photosynthesis, has been demonstrated in apple (Tartachynk and Blake, 2004),

442 but is doubtful whether a similar regulation may occur in *Citrus*, whose leaves show a

443 high photosynthetic efficiency during 2 years (Kubota and Motoyama, 1972). Our data

444 supported the view that photosynthesis in unmanipulated *Citrus* trees was source rather

than sink limited during most of the year. As shown previously for deciduous trees like

apple (Lakso et al., 1899) and cherry (Roper et al., 1988), fruit load had no significant

effect on photosynthesis as carbohydrates may be translocated to alternate sinks. Nor

could we find a depressing effect of leaf carbohydrates on $A_N$, as this parameter was

similar in trees with low and high crop when differences in carbohydrate content were

highest, and the 13 to 15-month-old leaves of the low crop trees (Table 3) had a very

high carbohydrate concentration (Fig. 2). Interestingly, the carbohydrate concentration

in these leaves (25 % on a dry matter basis) was higher than the concentration that

Iglesias et al. (2002) reported as inhibitory for manipulated Satsuma mandarin trees

(*Citrus unshiu*) (ca. 18% on dry matter basis). These conclusions may not extend to the

full year, as Syvertsen et al. (2003) found that during winter (late June in the southern

hemisphere, equivalent to late December in the northern hemisphere) defruited "Spring"

navel orange trees had a 40 % lower $A_N$ value than fruiting (and already harvested)

trees. Although defruiting may cause a transient change in $A_N$ (Gucci et al., 1991) and

the trees used by Syvertsen et al. (2003) were smaller in size (5 years old) than the ones

we used in our experiments, the possibility that tree behaviour during the winter months

may be different as described in this report cannot be dismissed.

In conclusion, the changes in carbohydrate reserves during the year reflected the

variations in supply and demand. Carbohydrates were stored in shoots and roots by

winter in non-fruiting trees, and mobilised during the spring flush of growth. Shoot

reserves were used-up during bud sprouting and flowering, and at the time of full bloom

carbohydrate concentration had fallen to a minimum value. We did not find evidence for

the contribution of the root reserves to fruit set. Thus, from the period of fruit

abscission, fruit nutrition was supported by current photosynthesis. Fruit load caused the

inhibition of flowering after heavy crop load in alternate bearing trees, but a regulatory

470 role of the carbohydrates could not be observed. Nor could be found an effect of leaf

471 carbohydrates or fruit load on photosynthesis. Thus, fixation rates were mainly

472 modulated by environmental conditions in Salustiana sweet orange.

473

481

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558

**Legends for figures**

560

561    **Fig. 1**. Number of surviving fruit (●), and rate of dry matter (DM) utilization in

562    fructification by the **on** trees.

563

564    **Fig. 2.** Changes in total metabolizable carbohydrates, starch and sugars in the leaves of

565    vegetative sprouts formed during the spring flush in **on** (●) and **off** (○) trees. The dotted

566    vertical lines indicate the time of harvest. The beginning of bud sprouting during the

567    second year is indicated by arrows in the axis (S). It occurred earlier in the **off** trees,

568    which entered an **on** year, than in the **on** trees, which entered an **off** year. Results

569    expressed as percentages on dry weight (DW) basis.

570

571    **Fig. 3.** Total reserve carbohydrates content in the bark (A) and wood (B) of vegetative

572    twigs formed during the spring flush in year 1, in **on** (●) and **off** (○) trees. On year 2, the

573    **off** trees formed 63 thousands of flowers whilst the **on** tree formed 3.5 thousands of

574    flowers. Results expressed as percentages on dry weight (DW) basis. Values are

575    averages of three independent samples ± SE. The dotted line indicates the time of

576    harvest; the arrows bud sprouting.

577

578    **Fig. 4.** The effect of girdling on the accumulation of carbohydrates in the leaves in **on**

579    (open bars) and **off** (hatched bars) trees. Results expressed as a percentage of the

580    carbohydrate contents in the leaves of non girdled (control) trees ± SE (n = 3). The

581    asterisks indicate statistically significant differences to the controls at $P \leq 0.05$ (*) and $P$

582    $\leq 0.01$ (**).

583

**Fig. 5.** Total reserve carbohydrates content in the thin (A) and the fibrous (B) roots in

**on** (●) and **off** (○) trees. On year 2, the **off** tree formed 63 thousand flowers whilst the

**on** tree formed 3.5 thousand flowers. Values are averages of three independent samples

± SE. The dotted line indicates the time of harvest; the arrows bud sprouting.

**Fig. 6.** Feeder roots weight, expressed as a percentage of the total weight of the sample,

in the root samples from **on** (●) and **off** (○) trees.

**Table 1**

Table 1. Flowering and yield parameters of the trees used in this study.

| Parameter and year | **On** trees | **Off** trees |
|---|---|---|
| First year | | |
| Bud sprouting (%) | 56.3 a | 22.4 b |
| Shoots initiated in spring (thousands tree$^{-1}$) | | |
| - Vegetative | 1.6 b | 8.23 a |
| - Leafy inflorescences | 6.4 a | 0.48 b |
| - Leafless inflorescences | 14.2 a | 0.34 b |
| Number of flowers (thousands tree$^{-1}$) | 75 a | 2.0 b |
| Number of leaves (thousands tree$^{-1}$) | 49 a | 60 a |
| Fruit set (units tree$^{-1}$) | 1,790 a | 124 b |
| Second year | | |
| Number of flowers (thousands tree$^{-1}$) | 3.5 b | 63 a |

Values within a line with different letters are statistically different ($P < 0,05$).

**Table 2**

Table 2. Dry matter used in fructification (Kg tree$^{-1}$).

| Developmental stage | **on** trees (75,000 flowers) | **off** trees (2,000 flowers) |
|---|---|---|
| Flower formation | 14.6 a | 0.41 b |
| From flowering to the end of drop | 15.0 a | 0.80 b |
| From the end of drop to harvest | 26.1 a | 3.1 b |
| Total | 55.7 a | 4.3 b |
| Dry weight in mature fruit at harvest | 35.4 a | 3.2 b |

Values within a line with different letters are statistically different ($P < 0,05$).

**Table 3**

Table 3. Photosynthetic rates at ambient conditions in the leaves of vegetative sprouts from the spring flush of growth in **on** and **off** trees. The values are average of 15 determinations (10 replicates each time) performed from June to July (4 to 5 month-old leaves), and 20 determinations (10 replicates each time) performed from March to April (13 to 14 month-old leaves).

| Tree characteristics | Leaf age and photosynthetic rate ($CO_2$ $m^{-2}$ $s^{-1}$) | |
| --- | --- | --- |
| | 4 to 5 months | 13 to 14 months |
| On trees | 7.0 a | 9.1 a |
| Off trees | 8.2 a | 7.9 a |

Values within a column with different letters are statistically different ($P < 0,05$).

**Figure 1**
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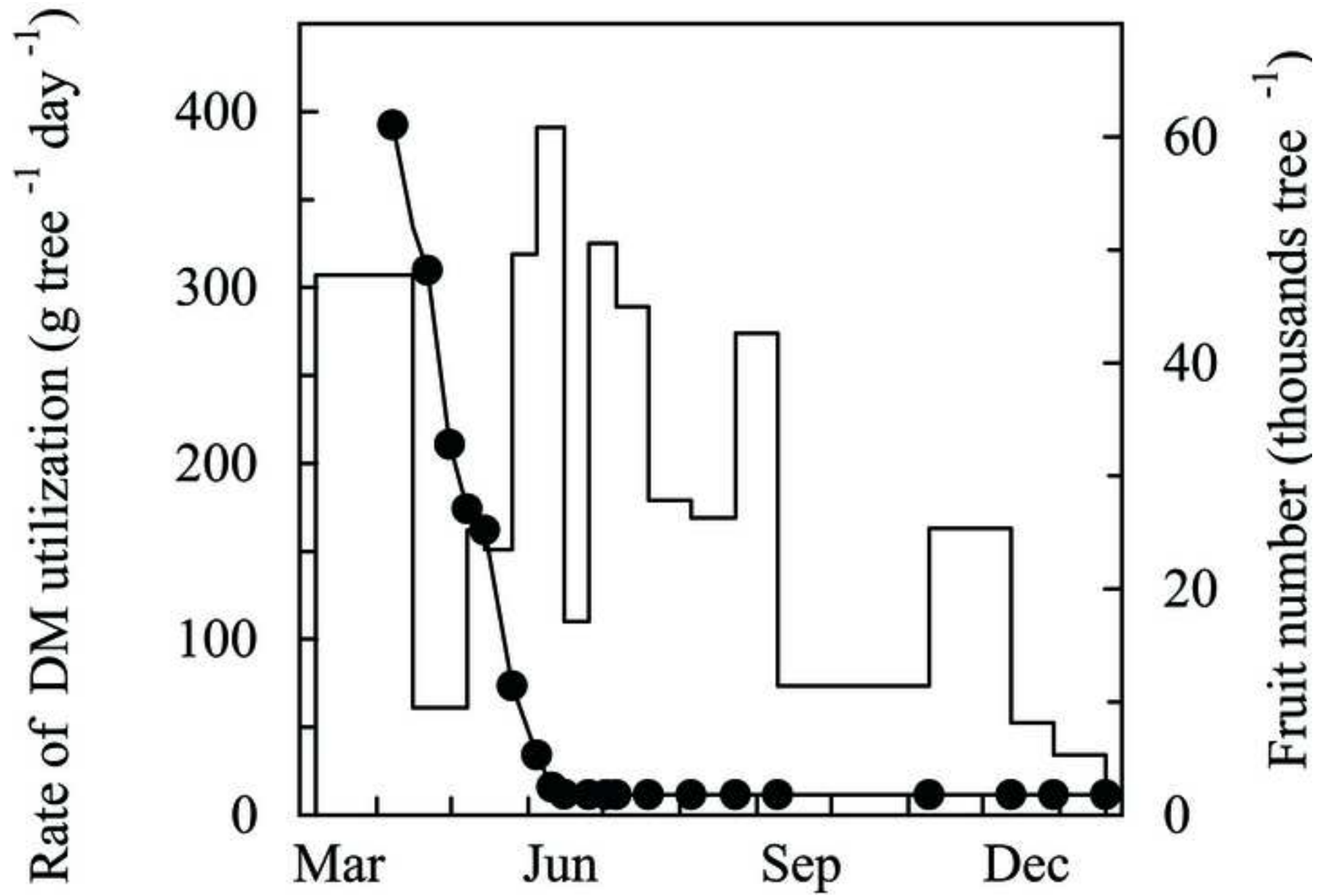
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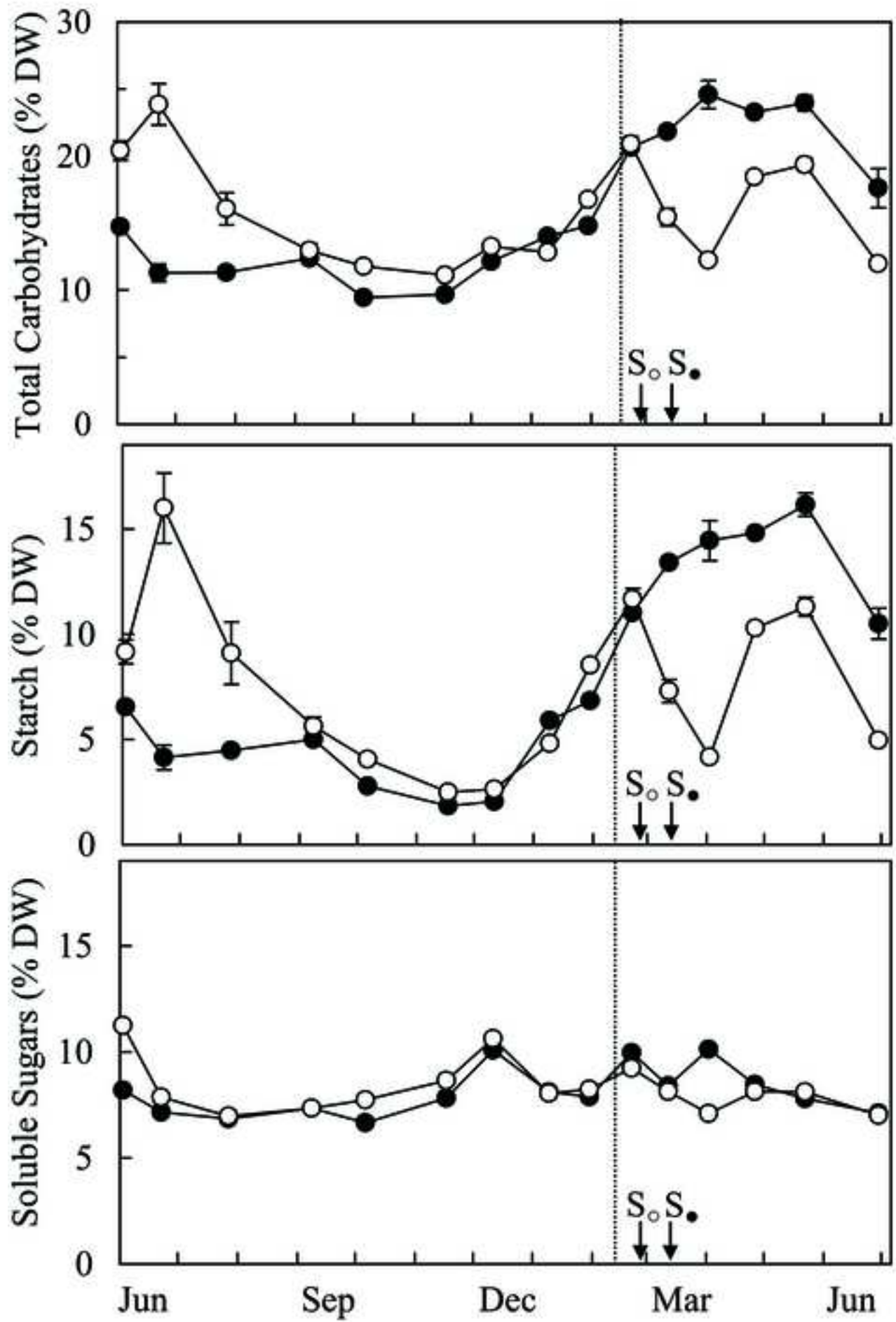**Click here to download high resolution image**

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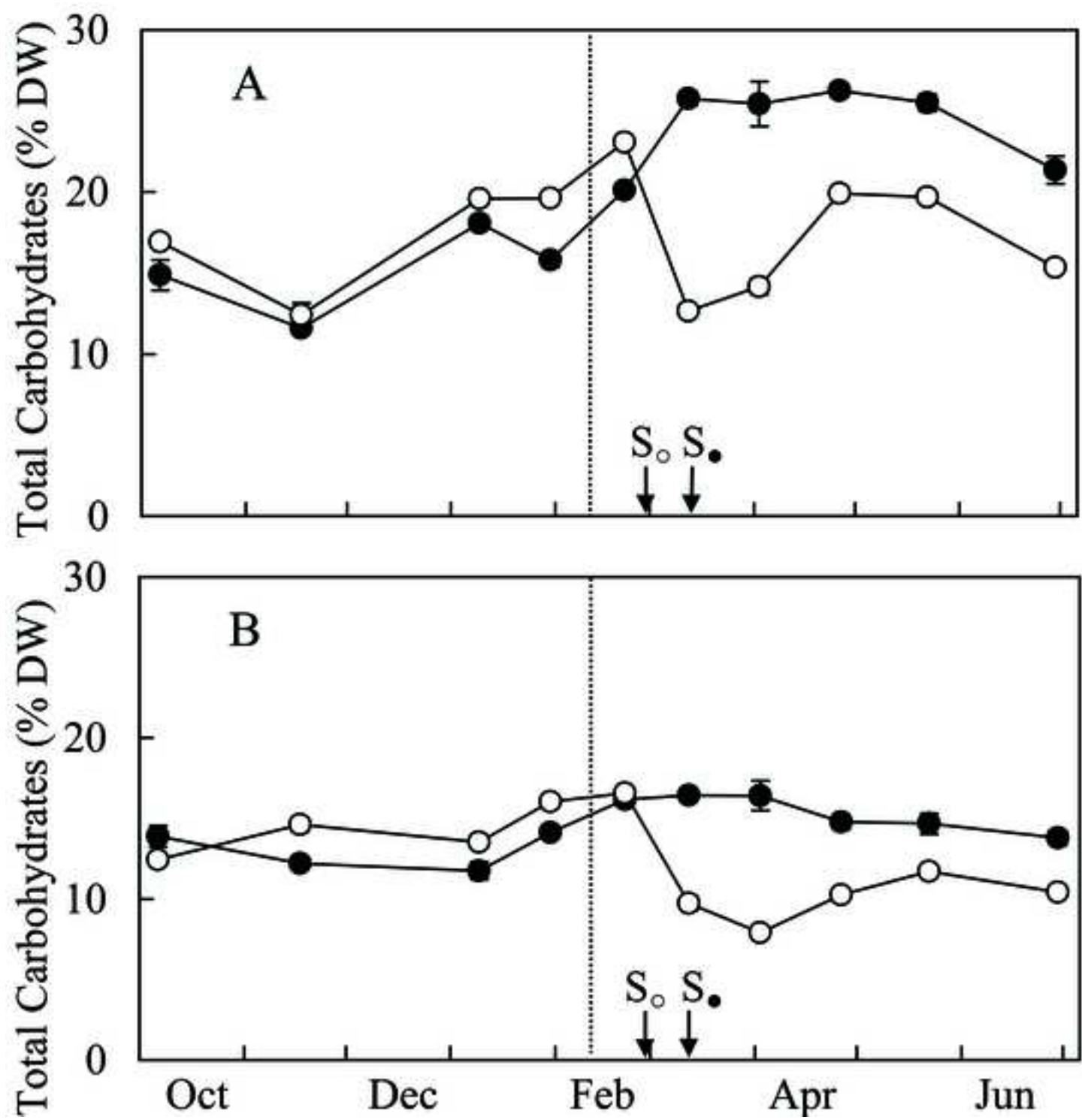**Click here to download high resolution image**

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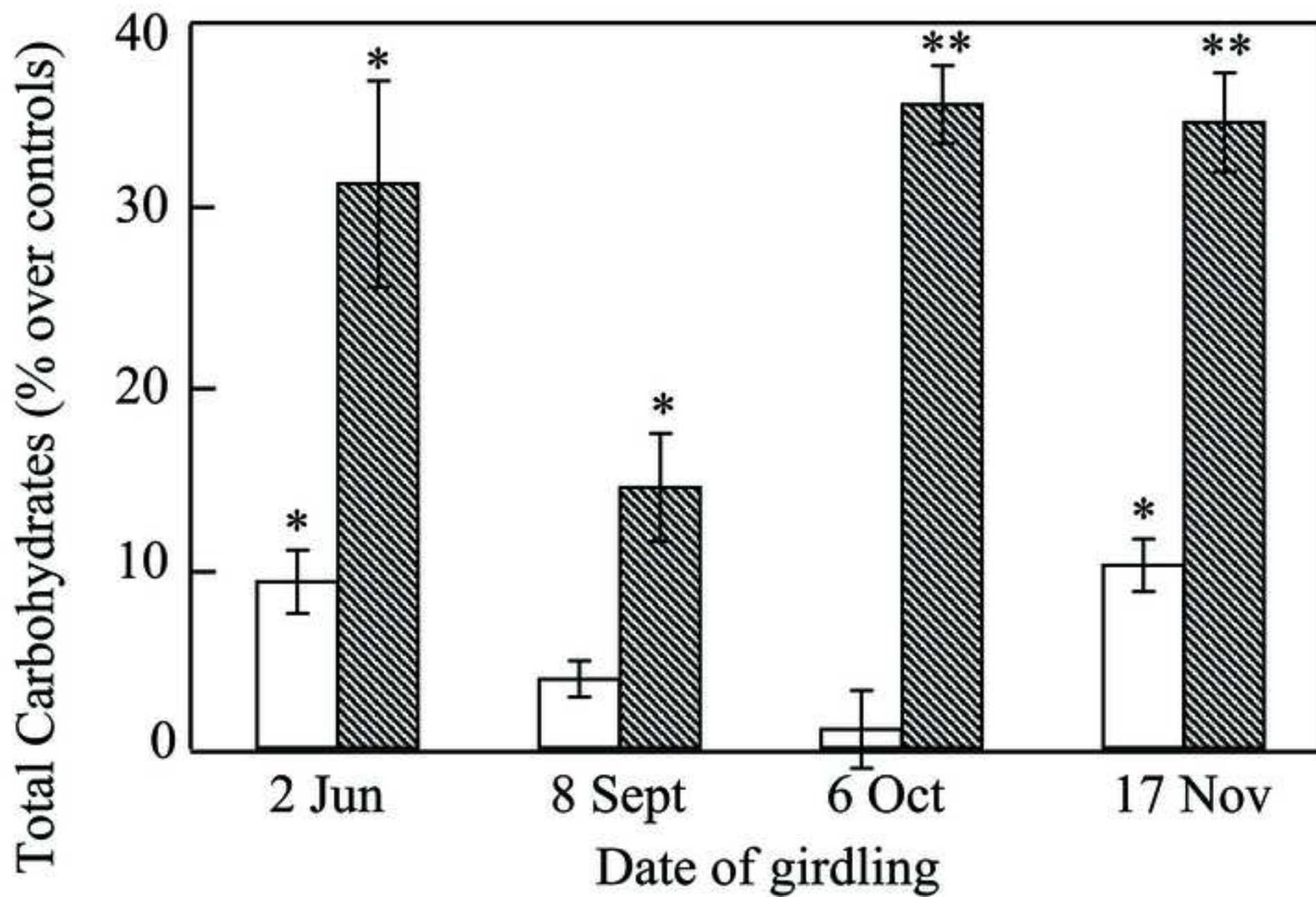**Click here to download high resolution image**
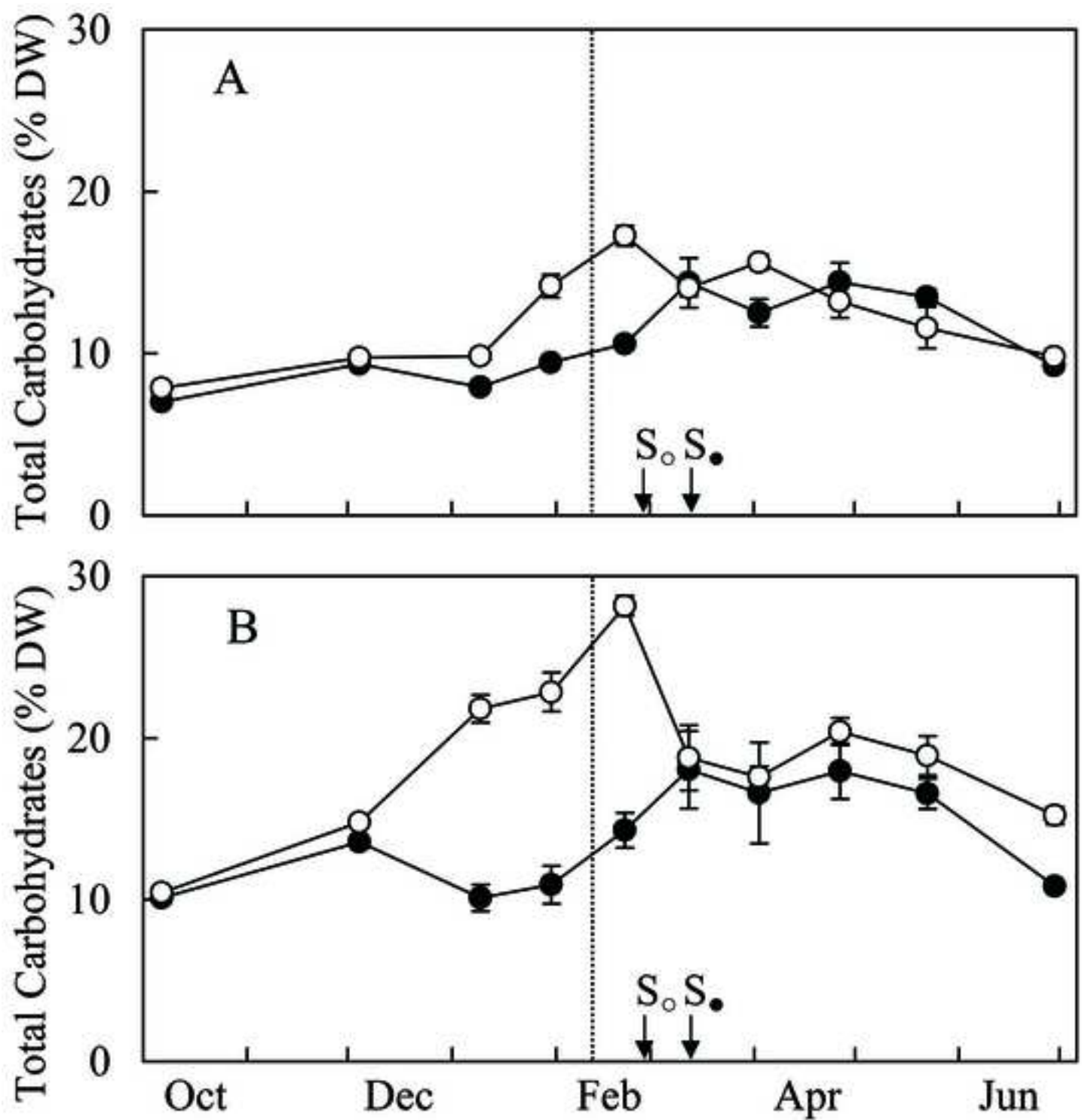
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