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Research Paper

Cluster illumination differentially affects growth of fruits along their ontogeny in highbush blueberry (*Vaccinium corymbosum* L.)

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ABSTRACT

Shading highbush blueberry plants generally leads to a delayed fruit development. Experiments have been performed to study the effects of light on fruit growth independently from the rest of the canopy. Clusters were shaded during different fruit growth periods. The equatorial diameter of the fruits as a function of days after full bloom followed a double-sigmoidal growth pattern, being fitted using a Gompertz II nonlinear mixed model, and absolute growth rates were obtained from each fitted model. Both whole-cycle shaded and second-stage shaded fruits showed a delayed peak in absolute growth curves with respect to both first-stage shaded and whole-cycle unshaded controls. Our results suggest that deficiency of light during the last stage of highbush blueberry fruits may lead to a substantial delay (of about 10–16 days) in harvest as compared with well-illuminated fruits.

In order to estimate the contribution of intrinsic fruit photosynthesis to its own growth at different stages, clusters were subjected to girdling on their peduncles at different times. Girdling just before the second-stage resulted in fruits gaining between 35 and 40% of dry weight in comparison with the controls. This suggests that fruit photosynthesis may play a relevant role in fruit growth during the second sigmoidal stage, which in turn may contribute to explain the delayed growth observed in shaded fruits.

1. Introduction

The light environment in orchards is critical for crop production and quality. Low irradiance has a negative impact on many physiological processes such as fruit set, fruit ripening and final quality (Campbell and Marini, 1992; Marini et al., 1991). While these effects of light have traditionally received most attention, evidence gathered on several species indicates that light availability also modulates fruit growth after fruit set. Shading canopies generally leads to a delayed fruit development on several species (Keller et al., 1998; Marini et al., 1991; Smart et al., 1988). This effect has also been observed in highbush blueberry – *Vaccinium corymbosum* L. (Hicklenton et al., 2004; Lobos et al., 2009) and its magnitude may be sufficient to be considered as a promising tool to delay harvest for commercial purposes (Rodríguez Beraud and Morales Ulloa, 2015).

Yáñez et al. (2009) have measured light distribution in the canopy of rabbitteye blueberry bushes (*Vaccinium ashei* Reade) and found that fruits located 60 cm below the top of the canopy received 17–37% of full sun irradiance. Besides affecting the current photosynthesis, long term exposure of leaves at low irradiances appears to negatively affect their photosynthetic performance due to an acclimatory response. Kim et al. (2011) found that highbush blueberry leaves exposed for a long term at 40–61% of full sun irradiances reached their maximum net CO_2 assimilation rate at about 700–800 μmol m^{-2} s^{-1} PPFD, while leaves that only received 17–27% of full sun irradiance exhibited their maximum at about 500 μmol m^{-2} s^{-1} PPFD.

Many agronomical practices may modify irradiance at the whole plants or individual fruits levels. Shading may be increased by practices that promote foliage production, such as irrigation and fertilization, especially with nitrogen, and lack or insufficient pruning among others. Also, covering orchards with nets for different purposes (protection against birds or hail) reduce radiation reaching crops underneath (Stamps, 2009). Lobos et al. (2013) examined the productivity and development of northern highbush blueberry under photo-selective nets. They found that red and white nets at intermediate shade levels delayed fruit harvest without detrimental effects on return bloom, yield or fruit quality.

It is not clear if the light effects on fruit growth is the result of decreased photoassimilate availability (insufficient carbon export from leaves to sustain fruit growth), or a direct effect on fruits (due to either

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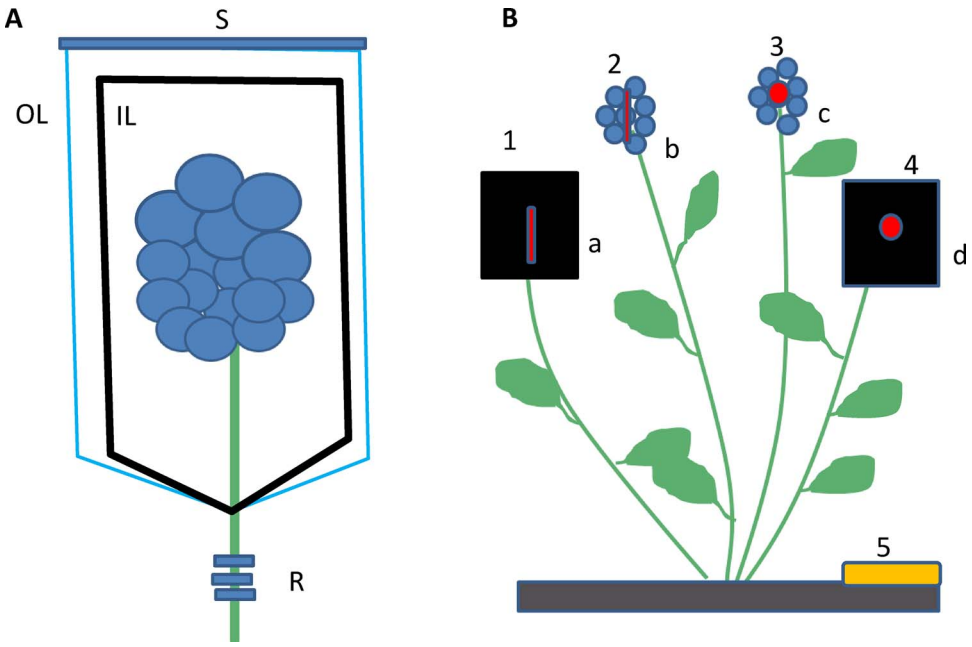

Fig. 1. A. Fruit cluster-shield: OL- outer polypropylene layer, IL-inner aluminum layer, R- twist tie ribbon, S- top seal. B- Treatments (at the first stage of sigmoidal fruit growth): a- SH, b- control, c- SH-2, d- SH-1; sensors: 1- inside thermocouple sensor, 2- outside thermocouple sensor 3- outside PAR sensor, 4- inside PAR sensor, 5- datalogger.

reduced fruit photosynthesis or some other light-mediated influence on fruit development). Attempts to separate the effects of light at the foliage level from those directly exerted on fruits, trials have been performed on grape berries – *Vitis vinifera* L. – (Morrison and Noble, 1990; Rojas-Lara and Morrison, 1989). It was found that the rates of berry growth were slower in fruits from vines with shaded leaves but no effects on berry growth were observed when only clusters were shaded. However, more recently, Chorti et al. (2010) observed a slight delay in berry development by shading clusters from fruit set to veraison.

Sour cherries – *Prunus cerasus* L.- (Flore and Layne, 1999) and rabbiteye blueberries (Birkhold et al., 1992) exhibited a positive net photosynthesis in the beginning of fruit development. Maximum net photosynthesis rate per unit of fresh weight of rabbiteye blueberry fruits is achieved immediately following petal fall (Birkhold et al., 1992). While photosynthetic rate per unit fruit area, or biomass, may be at its maximum early during fruit development, total fruit photosynthesis is also affected by its increase in size along development.

To analyze the contribution of fruit photosynthesis it is necessary to block the translocation of carbon from the rest of the plant. The removal of a bark strip around a tree's outer circumference is often used to study carbon relationships between different parts of the plant (De Schepper and Steppe, 2011). In particular, cluster peduncle girdling has been used to elucidate the effects of xylem flux during the grape berry growth (Creasy et al., 1993). However, no attempts to investigate carbon allocation to fruits by cluster girdling have been performed up to now in blueberry.

The objective of this study were 1) to determine the effect of cluster shading at different stages on the evolution of highbush blueberry fruit growth, and 2) to evaluate the relative contribution of photoassimilates from the cluster to sustain fruit growth during each stage of the double sigmoid growth curve.

2. Materials and methods

2.1. Experimental site and plant material

To evaluate the effect of shading on fruit growth, two experiments (from now on, Experiments 1 and 2) were carried out in two consecutive years in a commercial orchard of Southern highbush blueberry (*Vaccinium corymbosum*) cv OzarkBlue, located in Balcarce (37°49′S 58°12′W), South-East Pampas region, Argentina. At the time of the first experiment, plants were four years old. A further experiment (Experiment 3) was conducted in the same experimental site to evaluate the contribution of fruit photosynthesis to fruit growth. Germplasm of *Vaccinium corymbosum* cv. OzarkBlue is introgressed with *V. darrowi*; being considered as a northern (USA)-adapted blueberry (Ehlenfeldt and Martin, 2002; Manjula Carter and Clark, 2002). In the South-East Pampas region, the ocean proximity buffers summer maximum temperatures and provides an appropriate environment for this cultivar. Daily maximum and minimum temperatures, and daily radiation integral for both experiments at the experimental site are shown in the appendix (Fig. A1). Soil was a Typic Argiudoll, with pH 6.5 at the upper horizon. Previously to plantation, soil was amended with pine bark and rice hulls. Plants were set on raised beds, with a black plastic mulch and pine needles around the bushes during the first three years, later the plastic mulch was replaced with wheat straw, The orchard alley had a sod cover. Bushes were fertigated during spring with ammonium sulphate, twice a week. Soil water content at saturation was 38.5% (v/v). Drip irrigation was daily applied in the summer according to tensiometers placed at 20 and 30 cm depth. Soil moisture tension was kept within between 15–25 kPa. To correct alkalinity (pH = 7.5) of irrigation water, sulfuric acid was added to drop pH of water to 5.0–5.5. Commercial production started at the 3rd year after planting. Thereafter, plants were annually pruned during winter in order to achieve an open center. Due to commercial reasons, pruning intensity was minimum in the second winter resulting in a higher fruit load.

2.2. Effect of fruit shading during different developmental stages

A fruit cluster-shield was developed to allow full shading while minimizing its effect on temperature. The device consisted in a double layer cover, the inner one being consisting of aluminum foil (10 μ) and the outer one consisting of a 24 μ Crystal type polypropylene film, both of them with 5 mm perforations each 20 mm in order to maintain air flow and thus minimize any effect on air humidity or variation in gas concentrations within the shield respect of the open air. The shield, which had a twisted ribbon at the bottom, was also large enough to allow normal fruit growth (Fig. 1A). The outer polypropylene film was used to counteract the effect of shading on fruit temperature. Temperature measurement was performed with thermocouple sensors placed in sampled clusters, connected to a datalogger (Cavadevices, Buenos Aires, Argentina). The accuracy of this device to keep

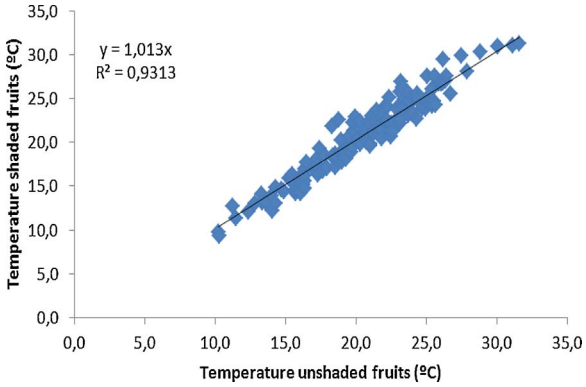

Fig. 2. Regression analysis between daily mean temperature of shaded vs. unshaded fruit clusters. Data from both experiments are included.

temperature of shaded fruits close to the unshaded ones was tested by regression analysis. A close fit (R^2 = 0,93) between readings of unshaded and shaded fruits was obtained, being the slope of regression line not significantly different from a 1:1 relationship, and y-intercept close to zero (Fig. 2). The following treatments were applied on Experiments 1 and 2 (Fig. 1B).

- Clusters shaded during the whole fruit growth period (SH);
- Clusters shaded during the first stage, and unshaded during the second sigmoid stage of fruit growth (SH – 1);
- Clusters unshaded during the first stage, and shaded during the second stage of fruit growth (SH – 2);
- Clusters unshaded during the whole fruit growth period (controls, C).

Each plant was considered as an experimental unit. Four clusters per plant (one for each treatment) were randomly selected and labeled at their bottom with colored ribbons. In order to lower the degree of development asynchrony among fruits within cluster (Coombe, 1976), only the five largest fruits were used for growth determination, independently of their position within the cluster (Diggle, 1995). Each cluster was measured periodically throughout the season, as proposed by Coombe (1976), since this procedure allows for a much smaller variance, relative to that obtained in independent, destructive samplings (De Silva et al., 1997). Six plants (replications) were randomly selected each year from the orchard. Different plants were used in each experiment. The first stage of the double – sigmoidal growth (i.e., first sigmoid curve) was considered to end when fruit diameter data turned constant in two consecutive measurements. This occurred at about 40 days after full bloom (DAFB) in experiment 1 for both shaded and unshaded clusters, and about 47 days after full bloom in the experiment 2, again irrespectively of shading treatment. At these times, shields were moved away from SH – 1 clusters and placed on SH – 2 ones

Fruit skin color was used as criteria for assessing fruit ripening. Color of 5 fruits within each cluster was visually monitored during ripening, and the time (DAFB) at which each fruit reached full dark blue coloration, corresponding to harvest, was recorded. Harvest dates were analyzed by ANOVA.

2.3. Fruit growth modeling

Godoy et al. (2008) developed a double-sigmoid mathematical model that enables a clear-cut separation of blueberry fruit growth phases, and to perform experiments in which treatments may be applied during a single phase (either first or second sigmoid) of fruit development.

The equatorial diameter of the fruits as a function of days after full bloom was fitted using a Gompertz II nonlinear mixed model:

$$\text{Fruit diameter} = (A + u)\,(1–\exp\,(-\exp(p^3(d))) + e \quad (1)$$

where parameter A corresponds to the upper asymptote (maximum expected value of the fruit diameter); u is the plant random effect; e is the random error (fruit effect); d is the number of days after full bloom (divided by 100) and $p^3(d)$ is a third degree polynomial in d (DAFB) with coefficients B, C, D and E. Plant random effects (u) are assumed as independent Gaussian variables with mean equal to zero and unknown variance σ_u^2. Within-plant errors, e, are also assumed independent Gaussian variables with mean equal to zero and unknown variance σ_e^2 and independent of the random effects u. Random effects of plants in the model have been introduced to capture the variation among plants (Pinheiro and Bates, 2000) and to induce intraclass correlation. Model fitting was performed using the nlme function of nlme package (Pinheiro et al., 2013) from computing environmental R (R Core Team, 2013), taking into account possible dependence between observations in the same plant. Starting values for A, B, C, D and E were required to assure fitting convergence in iteration of the nlme function. The values for B, C, D and E were obtained fitting data to generalized linear models for each treatment and season, without taking into account possible dependence between repeated observations, through function glm (R Core Team, 2013). Fruit diameter data were assumed to arise from a normal distribution. Mean fruit diameter, μ_{DIAM} was modeled through a link function (g), which was joined to the linear predictor $p_3(d)$, i.e. $g(\mu_{DIAM}) = p_3(d)$. The link function according to this case is log–log complement function, i.e. $g(\mu_{DIAM}) = \ln(-\ln(1-\mu_{DIAM}/A))$. Because the values of asymptotes (A) were unknown, each model was fitted using a value of A larger than the upper diameter observed by treatment (Lindsey, 1997).

The coefficient of determination was calculated for each model according to the following equation:

$$R^2 = 1–SS_{res}/SS_{tot}, \text{ where} \quad (2)$$

SS_{res} = Σ(individual fruit diameter − estimated fruit diameter)^2 = residual sum of squares

SS_{tot} = Σ(individual fruit diameter − mean fruit diameter)^2 = total sum of squares

The Wald's test was applied to evaluate the differences between parameters corresponding to different models with zero.

First derivatives or absolute growth rate (AGR) (Erickson, 1976) were obtained from each fitted model by numerical computation through the Graphmatica 1.60d software (Ksoft, 2004).

2.4. Cluster girdling on different fruit development timings

In Experiment 3, thirty clusters (replications) were chosen at random, fifteen ones were subjected to girdling on their peduncles, near the cluster. Girdling of 1 cm width was achieved by a grafting knife on two timings. First girdling was achieved at fruit set, and all fruits of the cluster were harvested at mid-lag stage of fruit growth. Last girdling was performed at mid-lag stage and all fruits of the cluster were harvested at veraison. Percentage of fruit set failure by cluster, fruit fresh weight and fruit dry weight were assessed. Weight was recorded using a digital scale with an accuracy of 0.01 g. Fruits were oven-dried at 60 °C for 10 days, and dry weight was determined. At each stage of fruit growth, between girdling and harvest, the contribution of fruit photosynthesis (CF) to its own growth, in terms of fresh and dry weight, was estimated as follows:

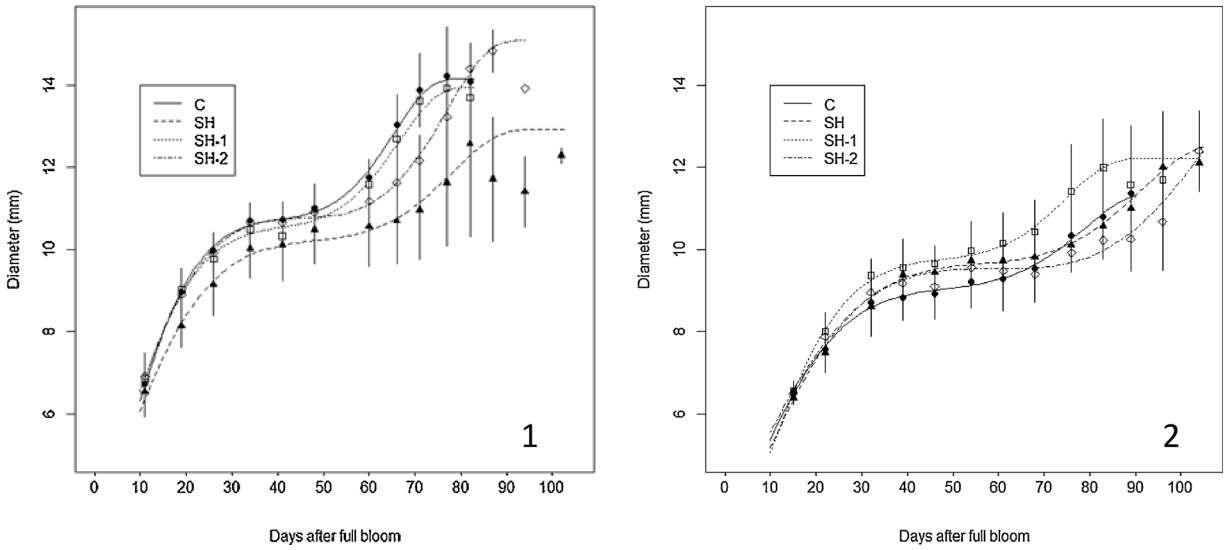

Fig. 3. A and B: Gompertz II double-sigmoidal models, Experiments 1 and 2, respectively.

CF (%) = 100 * (girdled final fruit weight − initial fruit weight)/(control final fruit weight − initial fruit weight). (3)

3. Results

3.1. Effect of fruit shading during different developmental stages

3.1.1. Growth and development

In both experiments, the evolution of fruit diameter followed a double-sigmoidal growth pattern. In all cases, a mixed Gompertz II model fitted satisfactorily to empirical data (Fig. 3), being all five parameters highly significant ($p < 0.0001$) (Tables A1, A2). In general, absolute growth rate values during Experiment 1 were higher than in Experiment 2, and accordingly, higher parameter A values were obtained for the former one. Fruit shading treatments affected growth dynamics, being fruit growth markedly delayed when shaded during the second sigmoidal growth period (Fig. 3). Unshaded controls, like fruits shaded solely during the first stage (SH-1) appeared to develop faster during the second sigmoidal growth stage than fruits that were shaded during the later stage (SH and SH-2). This can be most clearly seen in modeled absolute growth rate curves (Figs. 4 and 5). In Experiment 1, maximum modeled AGR values of C and SH-1 fruits occurred at about 65 DAFB, while in both SH and SH-2 maximums took place at around 78 DAFB. In Experiment 2, maximum modeled AGR values of SH-1 and C fruits were observed at 77 and 79 DAFB, respectively, while those of whole-time shaded fruits peaked at about 92 DAFB. Furthermore, during the second sigmoidal stage, AGR of SH-2 fruits appeared to peak at higher values than in SH fruits, resulting in a trend to higher parameter A (i.e., final diameter of fruits) values, although differences were not statistically significant ($p > 0.05$). In fact, fruit diameter at the end of each stage was not consistently affected by shading, and resulted in non-significant differences for parameter A values among treatments in either experiment. The lack of upper asymptote observed in SH-2 treatment at the end of the second season may be related to an asynchronous fruit ripening within the cluster and to rapid shedding of fruits upon reaching ripeness. As expected, variability in fruit growth curves tended to increase with time (Fig. 3), due to fruit inherent differences in growth rates or profiles (Lindsey, 1997).

The differential effects of shading between stages 1 and 2 were also apparent by the comparison of the parameters of the models (Table A4). Regarding parameters C, D and E, which describe growth rate as a function of time (Amorim et al., 1993), no significant differences were found ($p > 0.05$) between fruits shaded during the first stage and control ones. On the other hand, significant differences were found ($p < 0.05$) between parameters C, D and E of the models of the remaining shading treatments versus control. These relationships were maintained in the second experiment (Table A4). Regarding parameter B, which is related to the initial diameter of the fruit (Amorim et al., 1993), no significant differences ($p > 0.05$) were found between shading treatments and controls for either experiment indicating, as expected, no differences in initial fruit diameter among treatments.

ANOVA indicated that there were no significant differences among treatments ($p > 0.05$) during the first stage of growth. On the second stage there were no significant differences ($p > 0.05$) between C and SH-1 treatments, instead there were significant differences ($p < 0.05$) among these treatments and SH in experiment 1 (Table A3).

3.1.2. Harvest date

In both experiments, fruits under C and SH-1 treatments, i.e., those that were not shaded during the second sigmoid growth phase, ripened in parallel and were harvested almost at the same time (Table 1). SH-2 and SH treatments were harvested between 10–16 days after the control.

3.2. Effect of cluster girdling on different fruit development timings

Cluster girdling at fruit set led to a significantly higher ($p < 0.001$) fruit set failure than control clusters. As the control had 13% of fruit abortion, girdling had 56% of fruit set failure (Table 2). Fruits from clusters girdling at fruit set have lower fresh weight ($p < 0.001$) and dry weight ($p < 0.001$) than control at harvest at mid-lag stage harvest than control fruits. From these data, it could be estimated that the contribution of fruit photosynthesis to its own growth, either in fresh weight or dry weight terms, it was about 10% (Table 2).

On the other hand, cluster girdling at mid-lag stage had not effect on fruit abortion. The estimated contribution of fruit photosynthesis to its own growth in fresh weight was about 60%, while for dry weight was between 35–40% (Table 2).

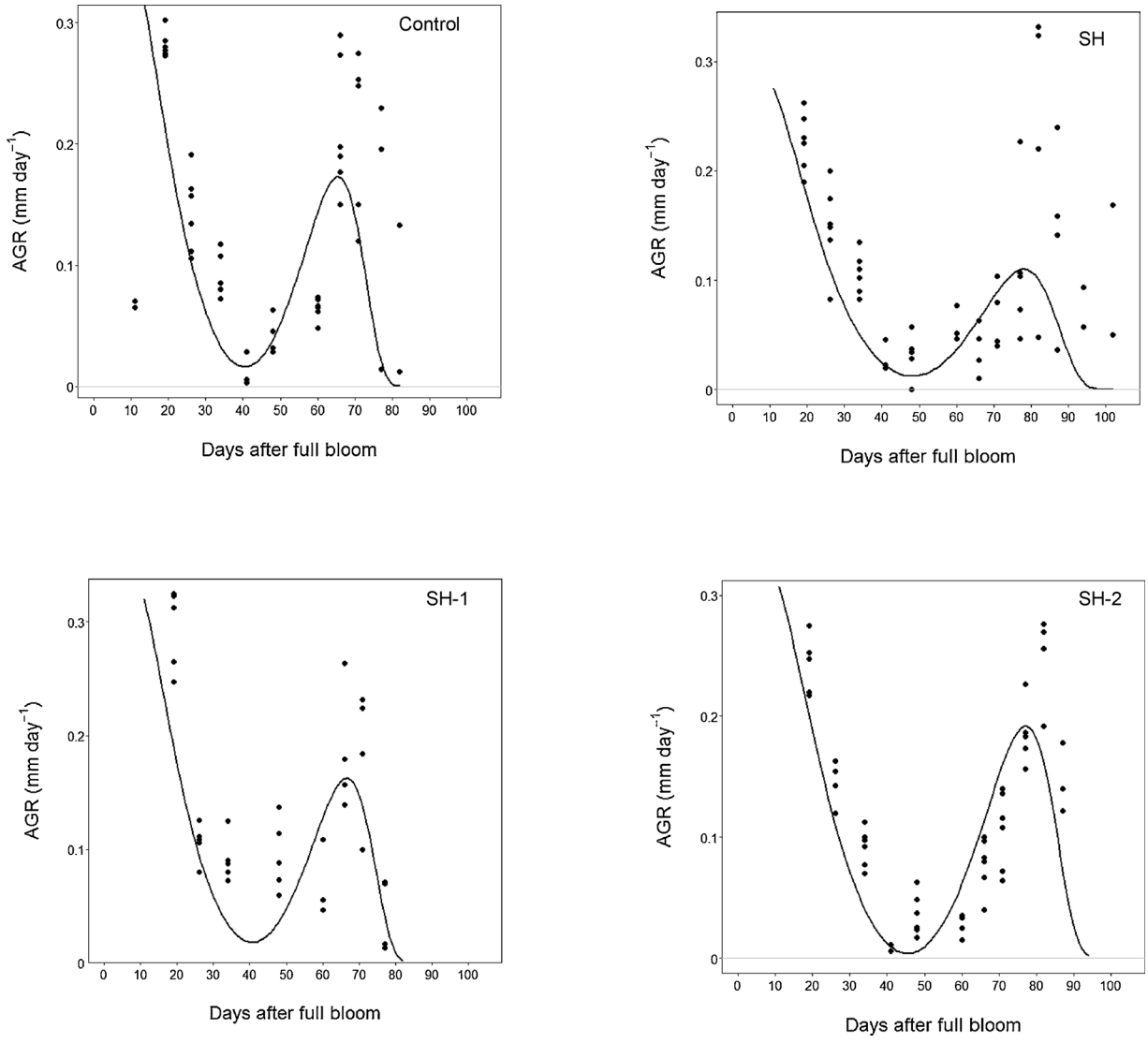

Fig. 4. Absolute growth rates at Experiment 1.

4. Discussion

Fruit crops production strongly depends on light environment, being both canopy and direct fruit illumination important factors affecting fruit growth and quality. Up to now, there is no information available on the effect of light incident on blueberry clusters upon fruit development.and its sensitivity in the two stages of growth. In this work, by shading fruits during just one half of the whole period of highbush blueberry fruit development (i.e., during either the first or second sigmoidal growth phases) it was possible to evaluate the impact of light environment on both growth stages separately. Because growth is very sensitive to temperature and fruit temperature is affected by incident light, we developed a simple device that allows virtually complete shading without modifying fruit temperature (Fig. 1). By this means, it was found that only the second phase of double sigmoid fruit growth was sensitive to shading. Both whole-cycle shaded and second-stage shaded fruits showed a delayed peak in absolute growth curves with respect to both first-stage shaded and whole-cycle unshaded controls (Figs. 4 and 5). This was also reflected by significant differences in parameters C, D and E, which contribute to define the double sigmoid growth curve shape (Amorim et al., 1993). Both experiments rendered essentially similar results regarding the effects of light on the position of growth curve peaks during both sigmoid phases. Smaller fruits were however obtained at harvest in Experiment 2 rather than in the first one, which was reflected in differences in parameter A values and also in maximum AGR values of modeled curves (Table A4, Figs. 4 and 5). These differences are attributable to a lower pruning intensity during the winter before the second experiment, which led to a heavy fruit load and a low leaf area: fruit number ratio, which is known to result in reduced fruit size (Maust et al., 1999). On the other hand, no clear effect on final fruit size was found by shading, and in some cases shaded fruits reached even larger final size than control ones (such as SH-2). This may be associated with the longer duration of growth in fruits that were shaded during the second sigmoid phase which consequently led to a delay in harvest date (Table 1). This is attributable to growth compensation through assimilates imported from the rest of the plant, being canopy photosynthetic rates at their maximum during this stage (Darnell et al., 1992).

Literature about light effects on fruit growth along its ontogeny is very scarce and some conflicting results can be found. Working with

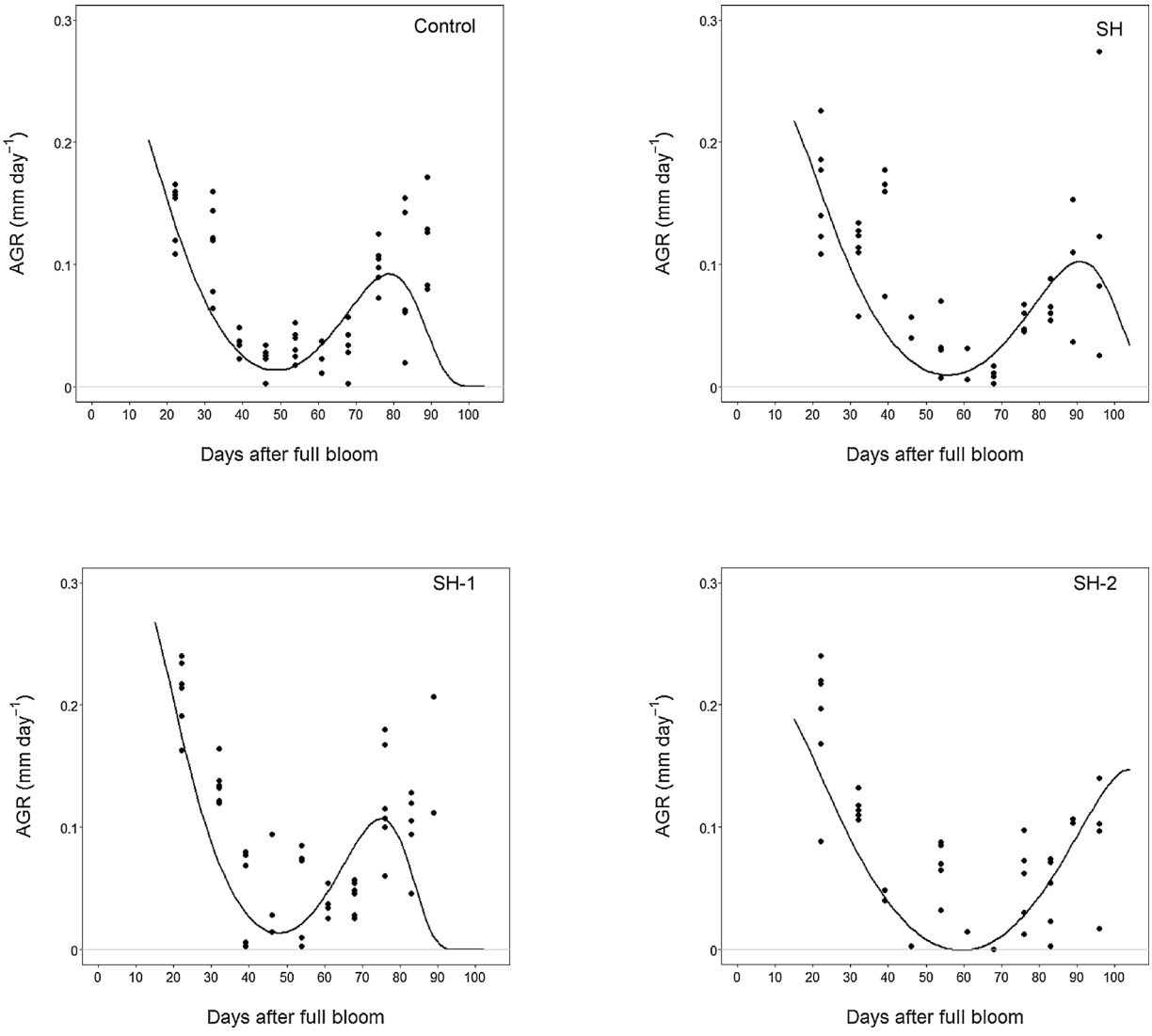

Fig. 5. Absolute growth rates at Experiment 2.

Table 1
Mean harvest date in days after full bloom and degree-days of blueberry fruits under different shading treatments. For degree-days calculation, 7 °C and 23 °C were assumed as base and maximum temperatures, respectively. Degree-days were accumulated from sept., 1st, in both experiments.

Experiment	Treatment				
		C	SH − 1	SH − 2	SH
1	DAFB	72 ± 5	76 ± 3	82 ± 5	88 ± 10
	Degree-days	594	644	695	760
2	DAFB	86 ± 3	85 ± 5	102 ± 5	96 ± 3
	Degree-days	655	641	747	691

Table 2
Estimates of the contribution of fruit photosynthesis to its own growth (CF), in terms of fresh (FW), dry weight (DW), and fruit set failure (SF) of girdled fruits vs. control fruits.

Girdling time	Treatment	FW(mg)	CF FW (%)	DW (mg)	CF DW (%)	SF (%)
Fruit set[a]	Control	336 ± 67	8–10	46 ± 9	9–11	13
	Girdling	107 ± 22		19 ± 3		56
Mid- lag stage[b]	Control	445 ± 107	55–64	62 ± 14	34–41	0
	Girdling	401 ± 66		52 ± 8		1

[a] harvest was performed at mid-lag stage.
[b] harvest was performed at veraison.

Cabernet Sauvignon and Pinot Noir grapes (*Vitis vinifera* L.), Dokoozlian and Kliewer (1996) studied the effect of shading berries with aluminum-coated paper bags in three chronologically defined stages. In their work, in which no double sigmoidal growth pattern was observed, the authors found that lack of radiation had its greatest impact on fruit growth between fruit set and the beginning of berry softening. These results contrasted with earlier reports about light exposed fruits growing less than covered ones, which was attributed by Dokoozlian and Kliewer (1996) to a possible high-temperature growth inhibition in exposed fruits. On the other hand, Downey et al. (2004) when studying the effect of shading clusters with opaque boxes all along Shiraz grape fruit development, observed in one of the seasons that growth of shaded fruits closely tracked that of illuminated ones during the first sigmoidal phase, but it was substantially reduced during the second phase. This effect however was not clearly observed when the experiment was

repeated in subsequent years. Berry temperature was not measured neither in Dokoozlian and Kliewer (1996) nor in Downey et al. (2004) works. It is well known that light exposure is strongly associated with fruit temperature (Bergqvist et al., 2001; Kliewer and Lider, 1968; Yamane et al., 2006), and shading is indeed recommended for ameliorating high temperature stress in grapes (Caravia et al., 2016). It is possible that shaded fruits may have experienced lower temperatures than illuminated ones, especially in Dokoozlian and Kliewer (1996) experiments in which a highly reflective surface (aluminum) was used to shade the fruits. Therefore we suggest that the use of shading devices that ensure lack of temperature side-effects, such as the one used in the present work, is crucial to study the effects of illumination on fruit development.

Our results indicate that radiation on the fruit is able to modify differentially the rate of fruit growth between first and second growth phases of the double sigmoid curve. Possible explanatory alternatives are differential effect of light on fruit photosynthesis, and/or on a direct effect of light on cell growth, between growth stages. Regarding fruit photosynthesis, it has been proposed that in small fruits such as blueberries, contribution of fruit carbon fixation to its own growth may only be relevant during the first sigmoid phase (Birkhold et al., 1992). During the first phase the comparatively high surface area: volume ratio, together with a relatively high stomatal density, may favor CO_2 exchange, in comparison to the second sigmoid phase, in which stomatal density steadily decreases (Blanke and Lenz, 1989; Knoche et al., 2001). However, in our work, only significant effects of shading during the second stage were observed, which suggests that if fruit photosynthesis is the factor limiting growth, its importance during late fruit growth stages has been underestimated. Although fruit photosynthesis per unit area was high early in the development of small fruits, as reported by Birkhold et al. (1992) and Flore and Layne (1999), total fruit photosynthesis may be higher in more developed, larger fruits, since it has been reported that inner fruit tissues may be photosynthetically active (Hetherington et al., 1998). Parenchymatic cells in the flesh of young fruits present chloroplasts, even when the number of grana and chlorophyll content are substantially lower than in leaf chloroplasts (Blanke and Lenz, 1989; Lawes, 1989). However, Laval-Martin et al. (1977) reported that fixation of $^{14}CO_2$ by tissue slices of the pericarp of mature green cherry tomato (*Lycopersicum esculentum* Mill.) occurred at higher rates than in the leaves when expressed on a chlorophyll basis. On the other hand, high internal CO_2 concentrations in fruits of C3 species (Czarnowski and Starzecki, 1992) are associated with low photorespiratory rates, in contrast with the leaves of the same plants (Hetherington et al., 1998; Smillie et al., 1999). Smillie et al. (1999) have proposed that photosynthetically generated electrons in tomato fruits may be used to facilitate reduction of oxaloacetate to malate, thereby promoting additional CO_2 fixation via PEPC (phosphoenolpyruvate carboxylase) in an analogous manner to C4 fixation pathway (Blanke and Lenz, 1989). Some PEPC isoforms have been found to be specific in tomato fruits (Guillet et al., 2002), which display particularly high PEPC activity (Laval-Martin et al., 1977). Carrara et al. (2001) found that the activity of PEPC in tomato fruit was higher than the Rubisco activity during the first phases of fruit development. Guillet et al. (2002) proposed that the main function of fruit PEPC is to synthesize organic acids as counter-ions that accumulate in the vacuole, providing necessary turgor pressure to allow rapid fruit cell expansion.

The experiment of cluster girdling was aimed to roughly estimate the contribution of intrinsic fruit photosynthesis to its own growth, by interrupting phloem transport and thus translocation of carbohydrates from leaves. The restriction imposed by peduncle girdling at the time of fruit set prevented the development of nearly half of the fruits of the cluster, while the remaining fruits only gained about 10% of the dry weight of control ones, thus revealing that almost all carbon needed to sustain fruit growth is imported from leaves. On the other hand, girdling just before the second sigmoidal growth phase resulted in fruits gaining between 35–40% of dry weight of controls. This suggests that fruit photosynthesis may play a relevant role in fruit growth during the second sigmoidal stage, which in turn may contribute to explain the delayed growth observed in shaded fruits. These results are somewhat different from those obtained by Birkhold et al. (1992) for rabbiteye blueberry cv. Bonita in which analysis of CO_2 exchange led the authors to estimate that fruit photosynthesis contributed by 12%, 24% and 13% of fruit C supply during the stages I, II and III of the double-sigmoid pattern, respectively. The lower value of fruit photosynthesis contribution during stage III obtained by Birkhold et al. (1992) in comparison with the second sigmoid phase at the present study may in part be due to differences among genotypes, but mainly to the different experimental approach. These authors measured fruit photosynthesis by quantifying the decrease in CO_2 concentration in a closed system. Carbon fixation in fruits has typically been measured using gas exchange or ^{14}C-uptake technique, but both methods may underestimate photosynthetic rates. Changes in external CO_2 may not adequately reflect CO_2 uptake in photosynthesis and will in particular neglect the amount of internal generated and photosynthetically refixed CO_2 (Aschan and Pfanz, 2003).

The involvement of fruit photosynthesis in its own growth does not rule out the possibility that a direct (non-photosynthetic) effect of light on fruit growth may also take place in intact plants. Likewise in *Ribes* and *Rubus* fruits cell division cease at anthesis (Westood, 1978), the majority of cells present in blueberry mesocarp at ripening being formed preanthesis (Cano-Medrano and Darnell, 1997), therefore any effect of light on fruit growth during the second sigmoidal growth stage must entirely rely on changes in cell expansion. Direct effects of light on cell expansion have been reported for leaves of several dycotiledon species (Stiles and Van Volkenburgh, 2002, 2004; Van Volkenburgh and Cleland, 1990). Putative receptors of this direct effect of light on cell expansion are phytochrome (red light) and cryptochrome (blue light) (Franklin and Whitelam, 2005). However light signaling components have been known to modulate fruit ripening in several species such as grape and tomato (Alba et al., 2000; Giliberto et al., 2005; Llorente et al., 2016; Smart et al., 1988; Weller et al., 2001) and these effects may not necessarily include growth. Up to our knowledge there are still no reports about non-photosynthetic effects of light on fruit growth, and this possibility deserves more research.

5. Conclusion

Our work shows that deficiency of light inciding directly on clusters during the last stage of fruit growth may lead to a substantial delay (of about 10–16 days) in harvest as compared with well-exposed fruits, while no effects were found when shading occurred during the first stage of fruit growth.

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Appendix A

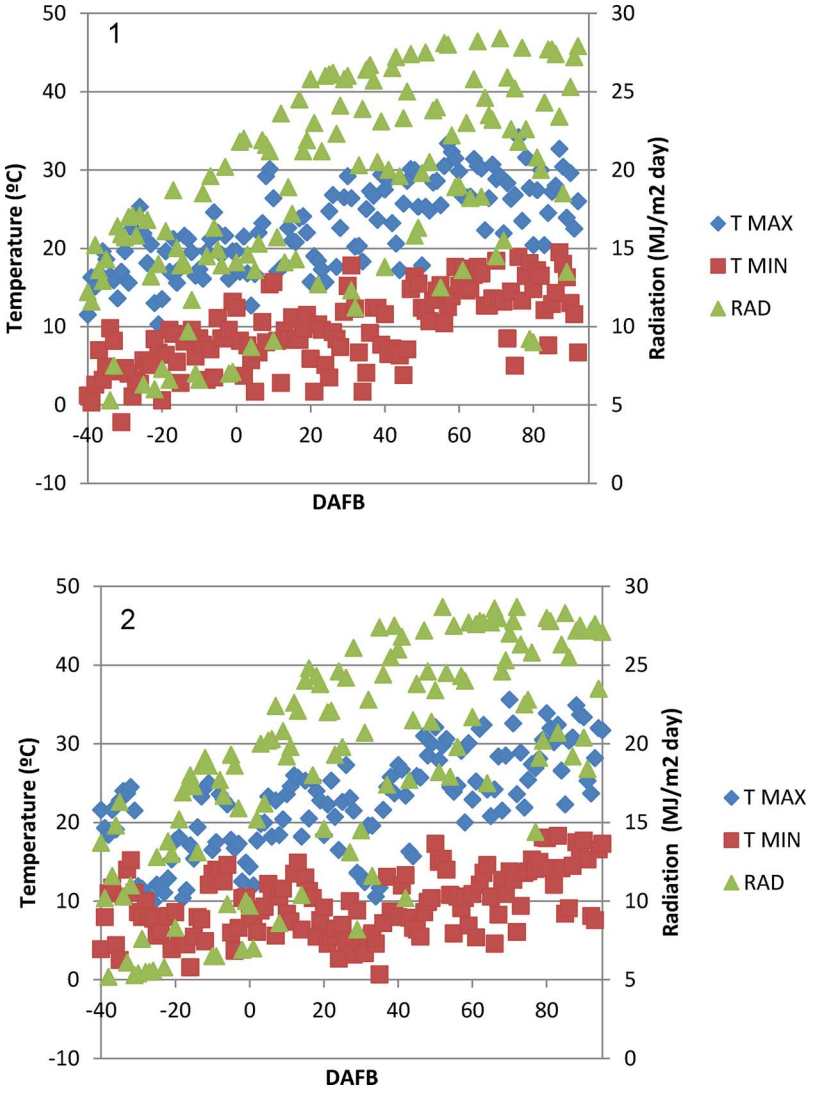

Fig. A1. Daily maximum and minimum temperatures (°C) and daily radiation integral on top of the canopy (MJ/m^2 day). 1: season corresponding with Experiment 1 (DAFB: 14/10); season corresponding with Experiment 2 (DAFB: 11/10).

Table A1
Estimated parameter values of double-sigmoid mixed Gompert II curves fitted to diameter data of blueberry fruits subjected to different shading treatments for Experiment 1. R^2: coefficient of determination of each model.

Treatment	Parameter		R^2
Control	A	14.17^{***}	0.87
	B	-1.60^{***}	
	C. 10^{-2}	13.81^{***}	
	D. 10^{-4}	-33.35^{***}	
	E. 10^{-6}	27.50^{***}	
SH-1	A	13.96^{***}	0.89
	B	-1.41^{***}	
	C.10^{-2}	12.20^{***}	
	D. 10^{-4}	-29.12^{***}	
	E. 10^{-6}	23.91^{***}	
SH-2.	A	15.11^{***}	0.85
	B	-1.41^{***}	
	C. 10^{-2}	10.63^{***}	
	D. 10^{-4}	-23.22^{***}	
	E. 10^{-6}	17.02^{***}	
SH	A	12.94^{***}	0.64
	B	-1.28^{***}	
	C. 10^{-2}	10.21^{***}	
	D. 10^{-4}	-20.62^{***}	
	E. 10^{-6}	14.28^{***}	

*** $p < 0.0001$.

Table A2
Estimated parameter values of double-sigmoid mixed Gompert II curves fitted to diameter data of blueberry fruits subjected to different shading treatments for Experiment 2. R^2: coefficient of determination of each model.

Treatment	Parameter		R^2
Control	A	11.43***	0.70
	B	−1.25***	
	C. 10^{-2}	9.78***	
	D. 10^{-4}	−19.33***	
	E. 10^{-6}	13.23***	
SH-1	A	12.22***	0.74
	B	−1.66***	
	C.10^{-2}	12.72***	
	D. 10^{-4}	−25.91***	
	E. 10^{-6}	18.08***	
SH-2.	A	13.99*	0.60
	B	−1.28***	
	C. 10^{-2}	7.19***	
	D. 10^{-4}	−12.14***	
	E. 10^{-6}	6.82***	
SH	A	12.54***	0.83
	B	−1.40***	
	C. 10^{-2}	9.14***	
	D. 10^{-4}	−15.99***	
	E. 10^{-6}	9.56***	

*** $p < 0.0001$.

Table A3
ANOVA among treatments by DAFB. Values correspond to means of treatments by DAFB.

Experiment	Stage of fruit growth	DAFB	Treatment			
			Control	SH-1	SH-2	SH
1	I	11	6.71 a	6.77 a	6.92 a	6.49 a
		19	8.97 a	9.02 a	8.90 a	8.30 a
		26	9.98 a	9.76 a	9.92 a	9.34 a
		34	10.70 a	10.49 a	10.66 a	10.19 a
	II	41	10.73 a	10.32 a	10.65 a	10.25 a
		48	10.99 a	10.98 a	10.90 a	10.48 a
		60	11.76 ab	11.59 ab	11.16 a	10.56 b
		66	13.04 c	12.70 ac	11.63 ab	10.69 b
		71	13.89 c	13.62 ac	12.16 ab	10.96 b
		77	14.24 b	13.88 b	13.15 ab	11.62 a
2	I	22	7.57 a	8.00 a	7.82 a	7.47 a
		32	8.72 a	9.37 a	8.98 a	8.60 a
		39	8.83 a	9.57 a	9.10 a	9.38 a
		46	8.92 a	9.65 a	8.97 a	9.44 a
	II	54	9.22 a	9.97 a	9.38 a	9.72 a
		61	9.28 a	10.15 a	9.24 a	9.72 a
		68	9.52 ab	10.43 b	9.14 a	9.80 ab
		76	10.33 ab	11.42 b	9.58 a	10.10 ab
		83	10.80 ab	12.00 b	9.92 a	10.57 ab
		89	11.36 a	11.57 a	10.08 a	11.00 a

Values in the same row followed by same letters are not significantly different ($p > 0.05$).

Table A4
Comparison of parameters from models corresponding to fruit shading and control (unshaded) treatments (F tests). In Experiment 1, estimation of the differences in parameters between shading and control treatments. In Experiment 2, estimation of the differences in parameters with respect to the differences between shading and control treatments in the Experiment 1. 1. SH-1, first stage shading; SH-2, second stage shading; SH, whole cycle shading; C, control (unshaded).

	Exp. 1			Exp. 2		
	SH-1	SH-2	SH	SH-1	SH-2	SH
Diff. A	−0.21 n.s	0.97 n.s.	−1.25*	0.99 n.s.	1.43 n.s.	2.43**
Diff. B	0.19 n.s.	0.19 n.s.	0.31 n.s.	−0.60 n.s.	−0.21 n.s.	−0.45 n.s.
Diff. C. 10^{-2}	−1.58 n.s.	−3.19*	−3.56*	4.58 n.s.	0.69 n.s.	2.82 n.s.
Diff. D. 10^{-4}	4.13 n.s.	10.15*	12.62**	−10.87 n.s.	−3.10 n.s.	−9.07 n.s.
Diff. E. 10^{-6}	−3.49 n.s.	−10.49**	−13.11**	8.47 n.s.	4.15 n.s.	9.30 n.s.

n.s. not significant ($p > 0.05$).
* $p < 0.05$.
** $p < 0.01$.

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