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Assessment of the seasonal pattern of burr and nut growth in a chestnut 'Marrone-type' cultivar

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Abstract: Seasonal patterns of burr and nut growth were measured in sweet chestnut trees (*C. sativa* Mill.) of the cultivar 'Marrone di Castel del Rio'. Burr and nut growth expressed as diameter, fresh and dry weight, and dry matter were assessed from mid of June to mid of October. Results evidenced that burr mainly grows in the initial period of the season, while the nut had a rapid growth in the last part of the season (*i.e.,* beginning of September). Dry matter of burrs started decreasing when its accumulation in the nuts was increasing. Burrs with the highest number of nuts (three), experienced a limited nut growth compared to burrs with two or one nuts per burr, but only at the beginning of nut filling phase. Lateral nut evidenced a higher growth in weight (fresh and dry) in the first part of the season with occasionally reduced dry matter compared to central nuts. The burr diameter had a strong positive relationship with the internal nut diameter and with the total nut weight.

Keywords: sweet chestnut, biometric measurements, burr development, nut development, nut quality

1. Introduction

Chestnut (*Castanea sativa* Mill.) is among the most cultivated nut tree-crop species in Italy (ca. 37.000 ha) (Istat, 2022). To intensify its cultivation, a good knowledge of the burr and nut seasonal growth pattern is required, but up to now very little information has been available, especially on *C. sativa* species. Results describing the nut quality of different *C. sativa* 'Marroni' ecotypes were performed but only at harvest (Bassi and Marangoni, 1984; Bassi and Sbaragli, 1984). Perulli et al. (2020) evaluated the *C. sativa* burr seasonal development but to our knowledge, literature is still lacking on nut seasonal growth data and on its relationship with burr development. These biometrical data would be very useful for a better understanding of sweet chestnut biological processes and for improving *C. sativa* management practices (*e.g.,* scheduling irrigation based on differential nut phenological sensitivity). The aim of the present preliminary investigation was to provide basic knowledge, for a *C. sativa* 'Marrone' ecotype, on the seasonal growth patterns of the burr main components (*i.e.*, the burr and nut) in terms of diameter, fresh and dry weight, dry matter and growing differences related to the nut position inside the burr, number and type of nuts per burr.

2. Material and Methods

2.1. Orchard location and chestnut ecotype characteristics

The study was conducted in 2019 in the Tuscan-Emilian Apennines in Monterenzio (Bologna, Italy), at 500 m elevation (44° 16' N and 11° 24' E), in a mature (150-200 years old) sweet chestnut orchard (*Castanea sativa* Mill.). Trees were, most likely, of the 'Castel del Rio' ecotype ('Marrone-type') grafted on seedling rootstocks. This ecotype is characterized by a medium tree vigour, an expanded canopy and medium productivity (Breviglieri, 1955; Bagnaresi et al., 1977; Mellano et al., 2012). Full bloom occurred in the middle of June (June 12th) while full burr fall occurred on October 20th, corresponding to 120 days after full bloom (DAFB).

2.2. Seasonal burr and nut data collection and management

The growth seasonal patterns of burr and nut were monitored from 26 DAFB (16th July) to 112 DAFB (10th October), every ten days along the whole season. Burrs were randomly collected and were chosen between 2 to 5 m above the ground and were representative of the burr average size on each of the 4 selected trees, similar in canopy size and vigour. From each burr were extracted the three internal nuts, distinguished as one central (C) and two lateral nuts (L). The 'Marrone di Castel del Rio' ecotype typically contains 3 nuts for each burr (Fideghelli et al., 2016) (Figure 1). Nuts were considered as the sum of shell, kernel, and pericarp.

Figure 1. Nuts ('Marrone di Castel del Rio' ecotype) enclosed in the burr (dehiscence time).

On each sampling date, on both burr and nuts, maximum diameter (mm), fresh and dry weight (g) and dry matter percentage (%) were calculated on 20 burrs and 60 healthy nuts (20 C and 40 L).

Burr and nut diameter were measured using a digital calliper (Calibit, HK-Horticultural Knowledge s.r.l., Bologna, Italy), while the fresh weight was determined with a high precision scale (Model PE3600, METTLER TOLEDO LLC, USA). Once weighted for the fresh weight (FW), nuts were put in an oven at 65 °C for seven days and weighted again to get the dry weight (DW). Dry matter percentage (%DM) was calculated with the following formula:

$$\%DM = \frac{DW}{FW} \times 100$$

Percentages of burr and nut dry weight on the whole burr (nut + burr) dry weight were calculated as the ratio between burr dry weight-whole burr dry weight and nut dry weight-whole burr dry weight, respectively. Using the nut dry matter content (g nut^{-1}), the specific rates of nut dry matter accumulation (gDM gFW^{-1} d^{-1}) were calculated for each sampling date.

The effect of the number of nuts inside each burr in terms of fresh and dry weight, diameter and dry matter was calculated from 63 to 112 DAFB. Only starting from 63 DAFB, it was possible to correctly distinguish the aborted nuts. Nuts were then named based on their number, position, and type (*e.g.,* aborted) inside each burr: three nuts (LCL), two nuts (LC, LL), one nut (L, C) and aborted nuts (A). Burrs with rotten nuts and with worm presence (*e.g., Cydia fagiglandana*, *Pammene fasciana*, *Cydia splendana*) were excluded by the measurements.

2.3. Statistical analysis

Nut diameter, fresh and dry weight, and dry matter were compared between lateral and central nuts using a one-way ANOVA analysis. Differences among LCL, LC, LL, L, C and A, in terms of fresh and dry weight, diameter and dry matter were assessed applying the Kruskal-Wallis test followed by the paired Wilcoxon test. Analyses were carried out using R software (www.rproject.org).

3. Results

3.1. Burr and nut seasonal growth

Burr and nut diameters grew almost linearly throughout the growing season (Figures 3a and 3b). Burr and nut diameters were positively and linearly correlated with an R^2 of 0.97 (Figure 2). No statistical differences, in terms of diameter, were found between central (C) and lateral (L) nuts (Figure 3b).

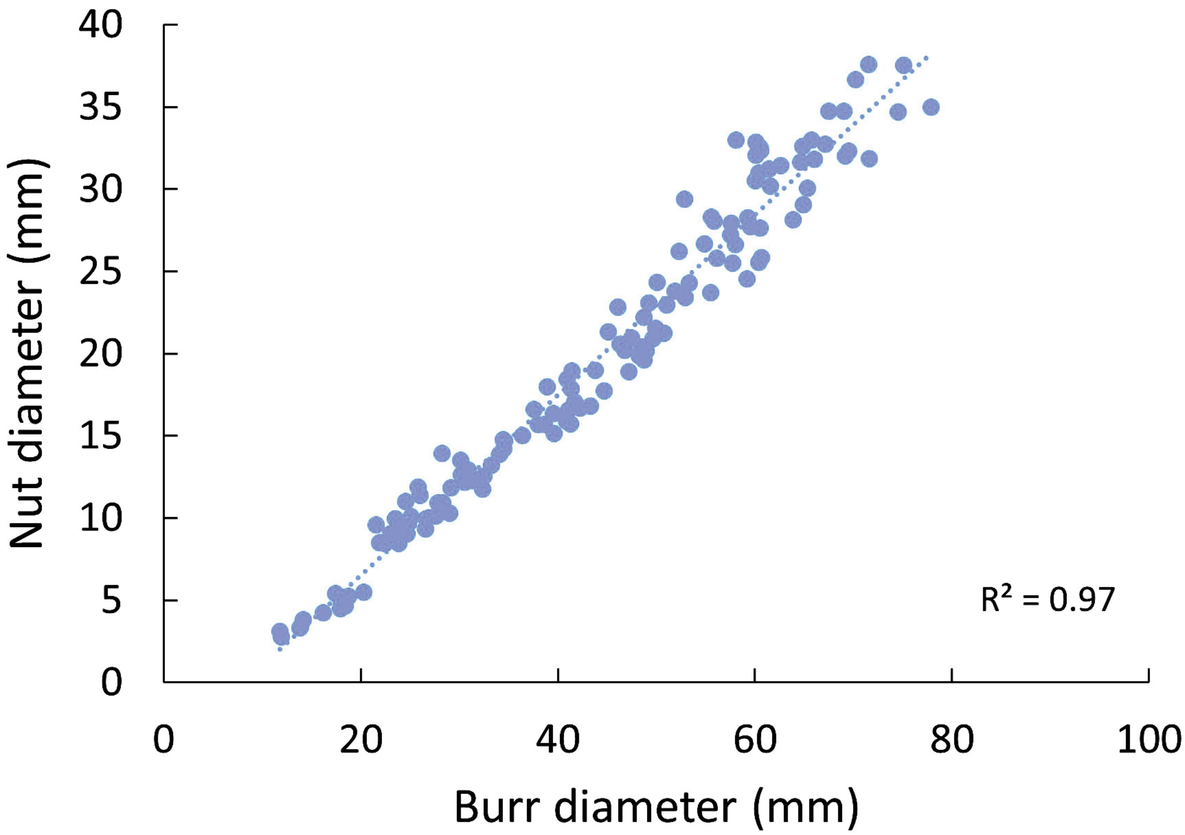

Figure 2. Relationship between burr diameter and nut maximum diameter during the growing season ($y = 0.5478x - 4.4362$, $R^2=0.97$).

Burr fresh and dry weight followed a similar seasonal pattern of burr diameter growth (Figure 3c). Nut growth in weight, expressed as either fresh and dry weight, was described by a thinned and constant curve till 74 DAFB (2nd September) when it rapidly increased to a maximum by 112 DAFB (10th October) (Figure 3d). Nut dry weights and fresh weights were linearly correlated ($R^2=0.99$, data not shown).

Both in fresh and dry weights were significantly higher lateral (L) than central (C) nuts, at 26 and 35 DAFB, respectively (Figure 3d).

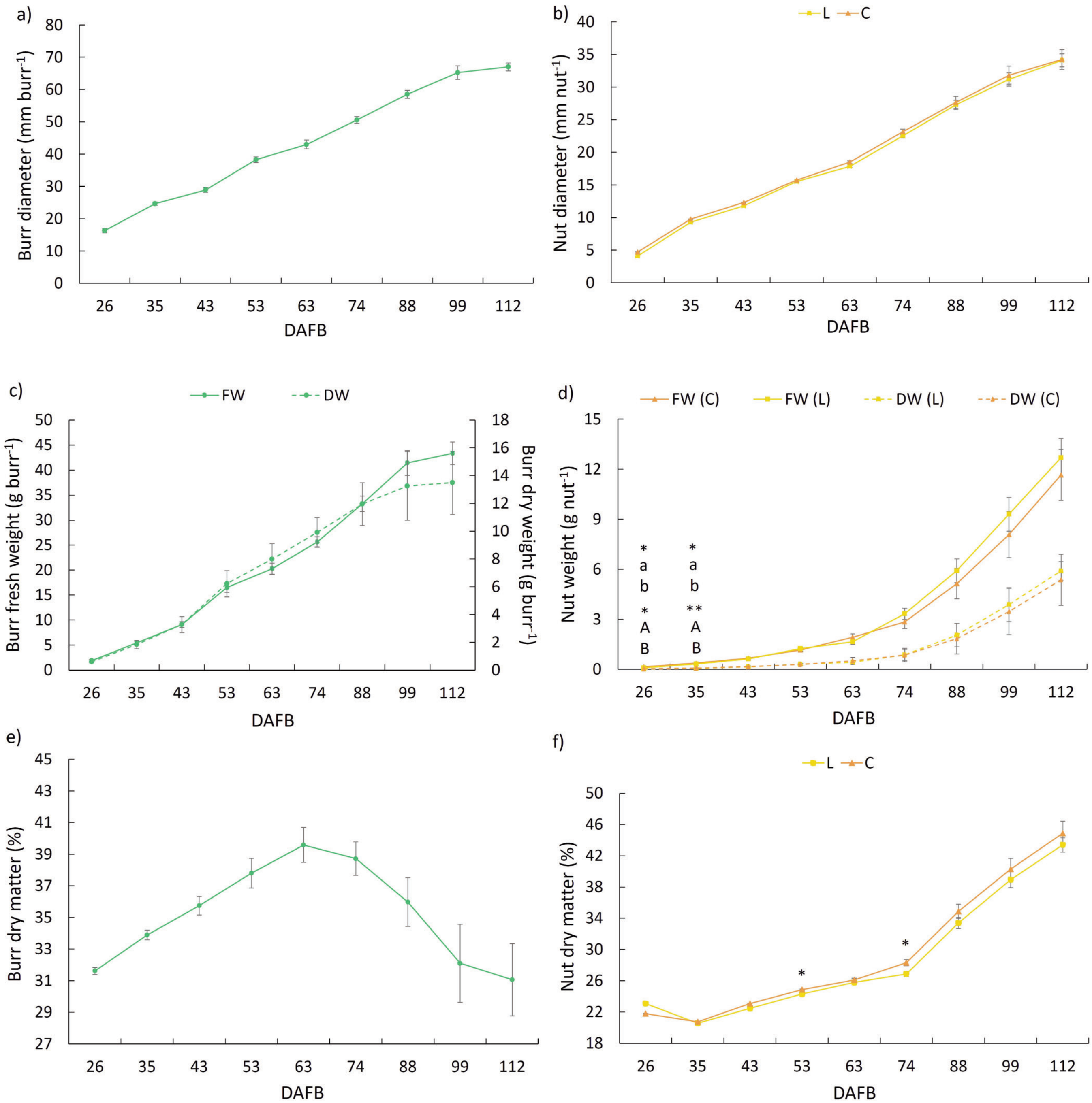

Figure 3. Seasonal patterns for (a) burr diameter, (b) central (C) and lateral (L) nut diameters, (c) burr fresh and dry weights, (d) central (C) and lateral (L) nut dry and fresh weights, (e) burr dry matter, (f) central (C) and lateral (L) nut dry matter. Values presented are the mean ± standard error. *, **: effect significant at P ≤ 0.05 and P ≤ 0.01, respectively.

The relationship between burr diameter and total nut fresh weight (sum of all the nuts per single burr) was best described by a fifth-degree polynomial function (R^2=0.95; Figure 4).

Nuts dry matter linearly and slowly grew until 74 DAFB, then it rapidly increased, reaching its maximum value at 112 DAFB (44.1%) (Figure 3f). Dry matter was higher for C nuts compared to L nuts at 53 and 74 DAFB. Burr, contrary to nuts, showed a bell-shaped dry matter accumulation pattern, with a peak at 63 DAFB (39.6%) after which dry matter started to rapidly decrease until 112 DAFB (Figure 3e).

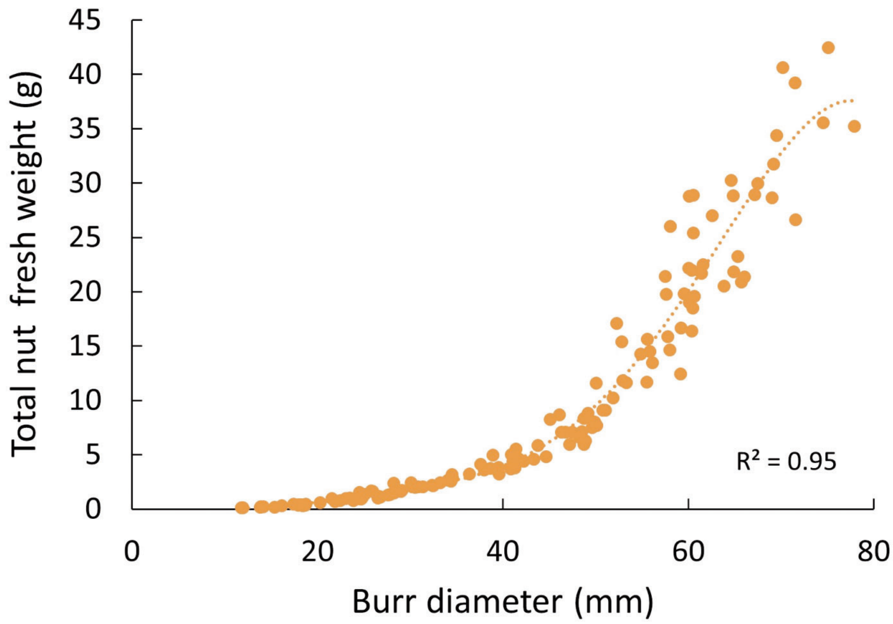

Figure 4. Relationship between burr diameter and total nut fresh weight (*i.e.,* sum of all the nut per burr) during the growing season ($y = -3E\text{-}07x^5 + 5E\text{-}05x^4 - 0.0035x^3 + 0.1064x^2 - 1.4153x + 6.7914$, $R^2 = 0.95$).

3.2. Burr and nut relationship

During the burr development, the percent incidence of burr dry weight progressively decreased from ca. 80-90% of the initial stages (26 to 74 DAFB) to ca. 50% towards the end of the season, whereas the nut dry weight clearly increased proportionally (Figure 5). Nut and burr dry weights reached almost equal values at 112 DAFB. Similar results were achieved for burr and nut fresh weights percentages (data not shown). The nut dry matter gain was between 0.0013 and 0.0026 gDW gFW^{-1} d^{-1} till 74 DAFB when it rapidly increased to 0.0047 gDW gFW^{-1} d^{-1} and 0.0050 gDW gFW^{-1} d^{-1}, respectively at 88 and 99 DAFB. At 112 DAFB, the dry matter gain decreased to 0.0035 gDW gFW^{-1} d^{-1} (Figure 5).

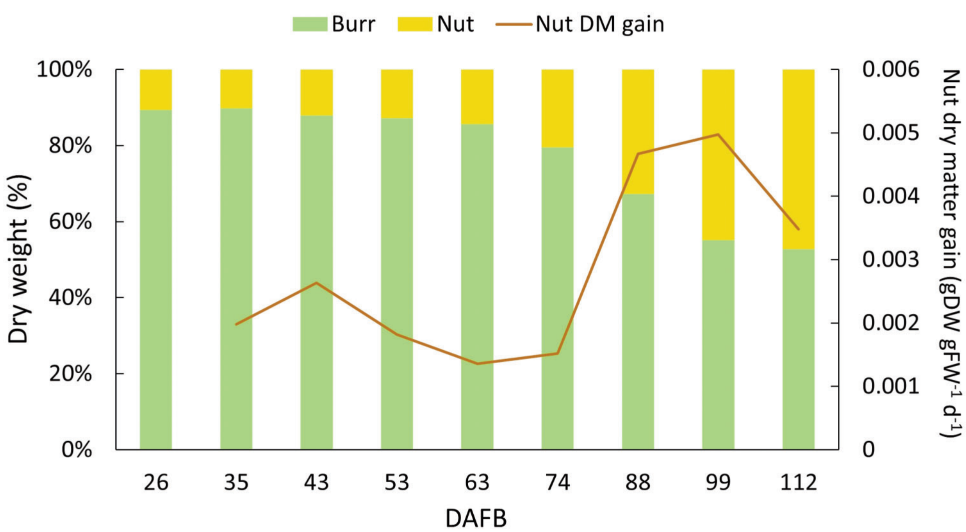

Figure 5. Percentages of burr (green histogram) and nut (orange histogram) dry weights, respectively, on the whole burr (nut + burr) dry weight. The claret line represents the nut daily dry matter (DM) gain.

3.3. Nut growth based on nut number and position per burr

Aborted nuts (A) showed for fresh and dry weight, and diameter, statistically lower values, throughout the growing season, compared to LC, LL, L and C nuts (Figure 6). LCL nuts, in terms of fresh and dry weight, and diameter, had comparable values to A nuts at 63 DAFB and intermediate values compared to LC, LL, L, C and A, respectively at 74 and 88 DAFB. Conversely, no statistical differences were observed for fresh and dry weight, diameter and dry matter, among LCL, LL, LC, L and C, in the last part of the season (Figure 6). Dry matter evidenced comparable values for all the nuts until 74 DAFB when the A nuts showed statistically lower values compared to LCL, LC, LL, L and C (Figure 6d).

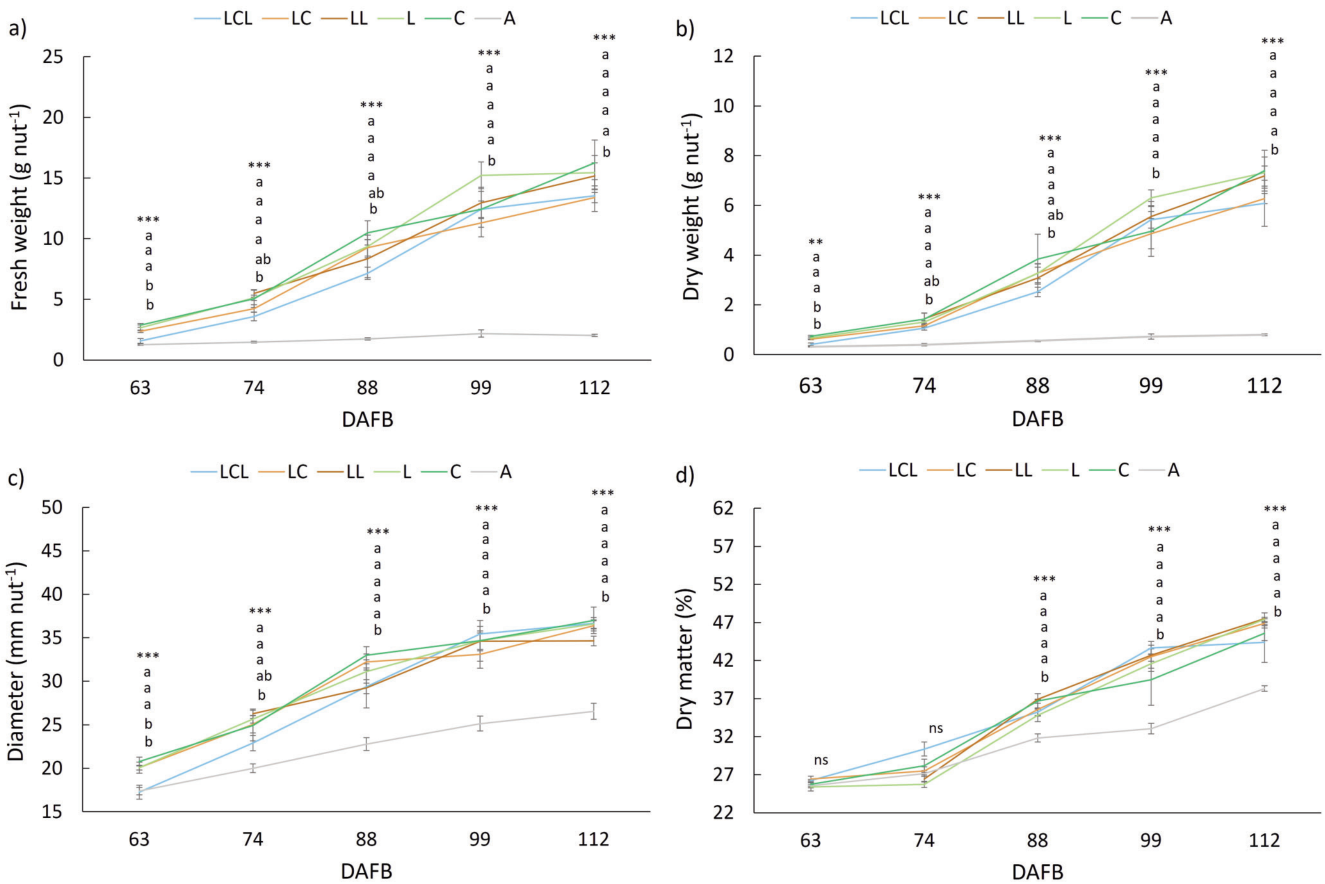

Figure 6. Nut growth pattern, in (a) fresh weight, (b) dry weight, (c) diameter and (d) dry matter for burrs with three nuts (LCL), two nuts (LC, LL), one nut (L, C) and aborted nuts (A), respectively. Values presented are the mean ± standard error. ns, ** and ***: effect not significant or significant at P ≤ 0.01 and P ≤ 0.001, respectively. Per each sampling date, means followed by the same letters are not statistically different.

4. Discussion

In the present study, nut experienced a limited growth in fresh and dry weight in the first part of the season (26-63 DAFB) while it raised to its maximum growth in the last part of the season (74-112 DAFB), gaining almost half of the whole burr dry weight in ca. one month of time (Figures 3 and 5). This transition in terms of growth represents a critical time in nut development and in 2019 it occurred ca. 74 days after flowering (beginning of September) in *C. sativa* 'Marrone di Castel del Rio'. At 74 DAFB, the endosperm (kernel) likely began to accumulate starch and dry mass. To the best of our knowledge, these results were still unknown for *C. sativa*, although similar results were confirmed by Chen et al. (2017) and Zhang et al. (2015) who reported that, in *C. mollissima* Blume (an early-ripening species), the increase in endosperm dry mass started at 60 DAFB. The nut increase in dry mass is primarily due to starch accumulation (Zhang et al., 2015). Starch is abundant in the nut, where it can account for 46~64% of total nut dry weight (Wang et al., 2008). Our results on nut dry weight and dry matter gain (Figure 5) seem to confirm that the starch accumulation occurs mainly in the second part of the season (74-112 DAFB), when percent incidence of dry matter of the burr started to decrease (Figure 3e). Burrs could be considered the main photosynthate sink organ from 26 to 63 DAFB when burrs start to shift their resources for filling the nuts. Taylor et al. (2015) found that, in the first phases of the fruit development period, most of the photosynthates were transported and accumulated in the burrs. At that time (26-63 DAFB), the burr itself represented more than the 80% of the total dry weight, preceding in growth the nut (Figure 5).

At the beginning of the resources shift from burrs to the nuts (63-74 DAFB), burrs with three nuts (LCL) were indeed gaining weight and size at a lower rate compared to burrs with only two or a single nut (Figures 6a, 6b and 6c). The number of nuts per burr negatively affects the nut weight (Famiani et al., 1999). These differences vanished while the season progressed as likely further sources (*e.g.,* leaves) started to feed the nuts. It is known that in *C. sativa*, 10 leaves per burr is the leaf surface area (ca. 630 cm^2) required for a normal fruit development (Famiani et al., 1999). On the other hand, aborted nuts (A), as still keeping growing in diameter and accumulating dry matter until the end of the season, represent a partial loss of assimilates that could be allocated to the other nuts growing in the same burr (Figure 6c and 6d).

Independently from the number of nuts in the burr, the L nuts experienced higher growth rates in weight (dry and fresh) at the beginning of the season likely because, being in the external positions, had the possibility to expand and grow more rapidly compared to the C nuts that were growing in between of the two adjacent nuts (physical space limitation) (Figures 3b and 3d). The C nuts, on the contrary, had higher dry matter values at 53 and 74 DAFB, than the L nuts (Figure 3f). That could be hypothesized by a likely stronger and direct proximity of C nut hilum to phloem vessels involved in carbohydrates translocation.

The seasonal pattern of growth, expressed as burr diameter, burr fresh and dry weight and nut diameter fitted a linear regression line, while nut fresh weight and dry weight fitted a third-degree polynomial curve (Figures 3a, 3b, 3c, and 3d). The adoption of units of mass rather than the size are more correct for express growth, especially in a species where the fruit is enclosed by the external burr (Bollard, 1970). However, there is an advantage to using diameters as a measure of growth: it is a non-destructive measurement that could be regularly recorded during the season and that might be used to predict the internal nut size. Although the burr diameter was fitting a linear regression curve, as also confirmed by Perulli et al. (2020), burr size was found to be positively correlated to the nut diameter (R^2=0.97) and to the total nut fresh weight (R^2=0.95), respectively (Figures 2 and 4). Knowing the external size of the burr could be useful for establishing precision approaches towards the goal of achieving real-time monitoring of the internal nut growth and to predict nut weight at full burr fall. These data coupled to the adoption of plant-based sensors for an accurate and continuous monitoring of the burr growth rate (*e.g.,* on daily basis) (Perulli et al., 2023) and together with the knowledge of nut phenological development, would permit the farmers to adjust and improve orchard management practices (*e.g.,* irrigation scheduling based on burr daily growth rate responses and on differential nut phenological sensitivity) during the most sensitive stages of nut growth, for achieving better yield and nut quality (Perulli et al., 2022).

5. Conclusions

This study indicates that burr and nut developments take place in two different phases: burr mainly in the initial period of the season, while the nut in the last part of the season. Furthermore, burr seems to be an important resource of assimilates for feeding nut in the second period of the season. Indeed, in the burrs where the number of nuts is maximum (three), nuts experienced a limited growth compared to burrs with two or one nuts, but only at the beginning of nut filling phase. Lateral nuts evidenced, at the beginning of the season, a higher growth rate in weight with occasionally reduced dry matter compared to central nuts. Although the different seasonal growth pattern between burrs and nuts, the burr diameter could be used to know and predict the internal nut size and the total nut weight. These preliminary results for this species need to be fourthly assessed and corroborated also in other *C. sativa* ecotypes and for different growing seasons.

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Conflicts of Interest: The authors declare no conflict of interest.

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