

## Growth analysis of sweet chestnut burr in two seasons with differing weather conditions

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**Abstract:** In Italy, most of the traditional sweet chestnut (*Castanea sativa* Mill.) orchards are still non-irrigated since they are located in mountain-hill areas with climate conditions that used to be optimal to sustain the vegetative and reproductive growth of this nut tree species. Nowadays, the increase of summer temperatures and the decrease of rainfall (due to climate change) are affecting negatively chestnut physiological performances. The aim of this experiment was to study sweet chestnut burr growth in two seasons, one warm/dry and one mild/rainy (2017 and 2018, respectively). The study was carried out in a traditional rainfed chestnut orchard. The seasonal burr growth was measured weekly from 30 days after full bloom (DAFB) to the beginning of burr valves opening. Air temperature and daily precipitation were measured at a nearby weather station. The results of this study highlighted that chestnut burr growth seems to be affected by seasonal weather conditions. Indeed, in 2017, the high summer temperatures and the moderate rainfall in summer (227 mm) and winter-spring (385 mm) appeared to affect negatively burr absolute growth rate (AGR;  $0.31 \text{ mm day}^{-1}$ ) and consequently final burr size (46.2 mm). The mild and rainy weather conditions that occurred in 2018 (663 and 340 mm of winter-spring and summer precipitation, respectively) positively influenced burr AGR ( $0.54 \text{ mm day}^{-1}$ ) and therefore its final size (60.8 mm). These preliminary results suggest that the introduction of irrigation as a common management practice for chestnut orchards may promote their resilience to climate change with a positive effect on their productivity and fruit quality.

**Keywords:** burr development; burr absolute growth rate; *Castanea sativa* Mill.; environmental physiology.

### 1. Introduction

In many Italian mountain-hill areas, sweet chestnut often represents a secondary crop that integrates the annual income of farmers and its cultivation follows mainly traditional practices. Nowadays, sweet chestnut production is regaining significant importance in the global market, with completely unexploited potentialities of development and better perspectives for farmers. Unfortunately, since the last decades, Italian chestnut production has suffered because of a multiplicity of factors, such as the general abandonment of mountain areas and the diffusion of ink disease (*Phytophthora cambivora* (Petri) Buis and *Phytophthora cinnamomi* Rand.), wood canker (*Cryphonectria parasitica* (Murr.) Barr.), and gall wasp (*Dryocosmus kuriphilus*).

In Italy, the most common orchard system used for sweet chestnuts includes vigorous trees planted at low densities (80-100 trees  $\text{ha}^{-1}$ ). Most of these orchards are located in the “*castanetum*” phytoclimatic area (Philippis, 1937), where air temperatures and

precipitations have always been optimal for moderate thermophilic species like chestnut. These conditions are characterized by annual rainfalls of 600-1500 mm, mean annual air temperatures of 9-13 °C, mean maximum air temperatures in summer time of 27 °C (Heiniger and Conedera, 1992; Gomes-Laranjo et al., 2008), and thermal unit accumulations between May and October of 1900-2200 GDD (growing degree-days) (Dinis et al., 2011). Climate change, with an overall increase of temperatures and a decrease in precipitation intensity and frequency in summer (Fernández-López et al., 2005), is having a negative impact on the yield performance of traditional rainfed chestnut cultivations. This leads to significant consequences on tree vegetative and reproductive activities affecting plant phenology and productivity (Gomes-Laranjo et al., 2008). As reported by Gomes-Laranjo et al. (2018), the productivity of chestnut trees is mainly correlated to total precipitations (Mota et al., 2018b), particularly in August (Vigiani, 1941), and to minimum, maximum and mean air temperatures in specific months (e.g. September; Pereira et al., 2011). Ferrini and Nicense (2000) reported that *Castanea sativa* Mill. trees require a minimum of around 30 mm of rainfall during the three summer months to avoid losses in nut yield. However, this threshold value may vary depending on other factors such as soil type, orchard exposure, winter rainfall, soil water holding capacity, planting density, leaf area index, etc. In any case, water stress was reported to decrease tree vegetative growth and consequently fruit productivity in chestnut trees (Breisch et al., 1995). The lack of rain at the end of summer or in autumn (IPMA, 2017) is known to be the main constrain for chestnut development and production, bringing income losses to the chestnut sector (Vida Rural, 2017). Even though soil water availability is one of the main factors driving chestnut productivity (Mota et al., 2018a), Martins et al. (2010) found that, independently of leaf water potential, leaf photosynthetic rate of chestnut trees is sharply decreased at air temperatures of 33 °C. Almeida et al. (2007) reported that mature trees presented maximum photosynthetic activity when air temperature ranged between 24 and 28 °C, whereas a significant thermo-inhibition effect occurred for air temperature higher than 32 °C (Gomes-Laranjo et al., 2007, 2008). The impact of the weather conditions could be reflected on burr seasonal growth. To date, there are no published studies about the seasonal development of chestnut burr. Previous research demonstrated that tree water stress affects negatively fruit growth and harvested yield in several fruit crops, such as apple, peach, kiwifruit and pear (Manfrini et al., 2018). These effects are generally attributed to a reduced stomatal conductance and the consequent decrease in canopy carbon assimilation (Tozzi et al., 2018). Monitoring burr growth, by means of the analysis of absolute growth rate (AGR), could represent a good indicator of chestnut tree performances along the whole season. The introduction of irrigation (when and where possible) as a common management practice for chestnut orchards may improve fruit yield and quality. The use of smart irrigation could also help to promote the resilience of chestnut orchards to climate change. Indeed, introducing the use of irrigation for the management of chestnut orchards can allow more regular and greater production over the years (Breisch et al., 1995; Gomes-Laranjo et al., 2018). Irrigation is considered to be a suitable strategy to increase the commercial value of the chestnut chain, not only because of the increased production, but also because of the improved nut size (Gomes-Laranjo et al., 2018). The aim of the research was to study the response of chestnut burr growth to different weather conditions.

## **2. Materials and Methods**

### *2.1. Chestnut orchard location and weather conditions*

The study was performed during 2017 and 2018 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), commercial, rainfed 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., 1979; Mellano et al., 2012). The area was very steep with typical soil slopes ranging between 20% and 80%. The soil is mostly classified as deep Haplic cambisols (Dystric), with a sandy loam texture. In both years, average ( $T_{med}$ ) and maximum ( $T_{max}$ ) air temperatures and daily precipitation ( $P$ ) were measured at a nearby (2 km) weather station belonging to the Regional Agency for Environmental Control (Arpae, 2019). The recorded autumn-spring (November-May) precipitations were 385 and 663 mm, while the summer precipitations (June-September) were 227 and 340 mm in 2017 and 2018, respectively. The area where the experiment was carried out is characterized by an annual rainfall and a mean air temperature of 960 mm and 12.7 °C, respectively (averages were calculated in the period 1991-2015).

In both years, full bloom occurred in the middle of June (12 June). The nut set was uniform in both years, suggesting a regular bearing tendency of the ecotype used in this study, differently from most wild nut trees (Rutter et al., 1991).

## 2.2. Burr growth

The maximum equatorial diameter of 25 burrs was measured weekly throughout the growing season. Measurements started 19 and 28 days after full bloom (DAFB) in 2017 and 2018, respectively. Last burr measurement was taken around one week before the beginning of burr fall, at burr valves still completely closed (111 and 103 DAFB in 2017 and 2018, respectively). Burr diameters were measured using a digital calliper (Calibit, HK-Horticultural Knowledge s.r.l., Bologna, Italy). The absolute growth rate (AGR, mm day<sup>-1</sup>) of the burrs was then calculated as follows:  $AGR = (D_{t1} - D_{t0}) / (t1 - t0)$ ; where  $D_{t1}$  and  $D_{t0}$  are the burr diameters measured on a given day of year ( $t1$ ) and on the previous sampling date ( $t0$ ), respectively (Bastias et al., 2012).

Burr development was hypothesized to follow the stages reported by Okello et al. (2015) and Chen et al. (2017). Indeed, in the present study each season was divided in three phenological stages: stage I (0-50 DAFB) mainly characterized by cell division, stage II (50-80 DAFB) mainly characterized by cell expansion and the beginning of starch accumulation, and stage III (80 DAFB – burr valves opening) mainly characterized by cell expansion and rapid starch accumulation in the fruit. The mean ( $T_{med}$ ) and maximum ( $T_{max}$ ) air temperatures, the cumulated precipitation (mm stage<sup>-1</sup>), and the percentage of rainy days (%) were calculated separately for the three stages.

## 2.3. Statistical analysis

For each phenological stage, the significance of the differences in burr diameter and AGR between the two years was assessed with one-way ANOVA. In addition, a Principal Components Analysis (PCA) was performed using the data of AGR,  $T_{med}$ ,  $P$ , and the number of days after the last precipitation event (DAP; number of days without rain following each rain event) from the end of stage I to the beginning of burr valves opening (103-111 DAFB). Maximum air temperature ( $T_{max}$ ) was not included in the PCA analysis because positively correlated to  $T_{med}$ .

# 3. Results and discussion

## 3.1. Weather conditions

Winter-spring precipitations (November of the previous year - May of the current year) were almost double in 2018 (663 mm) compared to 2017 (385 mm), whereas 2017 was drier than 2018 throughout the three burr development stages (Table 1). This was particularly the case for stages I (rainfall of 0 mm) and II (one rainfall event of 47.8 mm

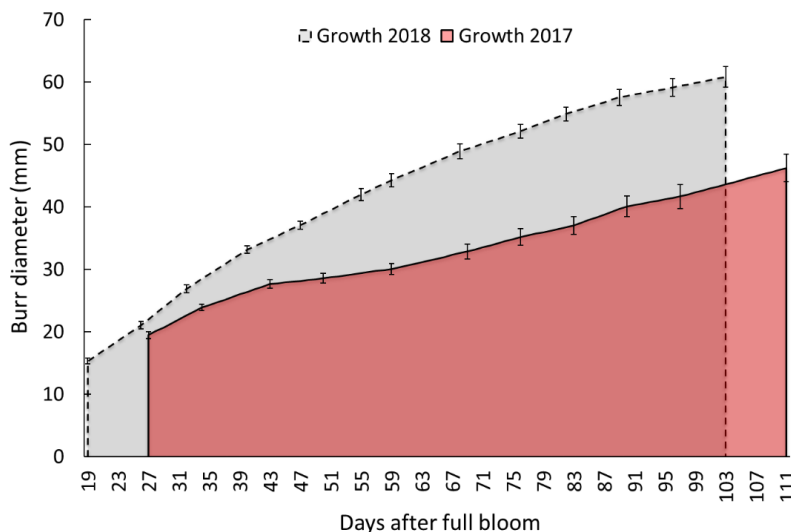
registered on 11 August, 60 DAFB). Indeed, in these two stages, the percentage of rainy days was lower in 2017 (0% and 6%, respectively) than in 2018 (40% and 17%, respectively). In addition, in the same two stages,  $T_{mean}$  was higher and more fluctuating in 2017 compared to 2018 (Table 1), whereas  $T_{max}$  and  $T_{mean}$  were higher in 2017 than in 2018, with a higher variability ( $\pm 4$  °C) and almost 2 °C of difference in stage II (28.6 °C and 26.8 °C, respectively). In 2018,  $T_{max}$  never exceeded 32 °C, that was reported as the temperature threshold above which thermo-inhibition occurs in chestnut trees (Gomes-Laranjo et al., 2008). On the contrary, in 2017 air temperatures were above 32 °C for nine continuous days (1-9 August; 50-58 DAFB) with a maximum of 36 °C (2 August; 51 DAFB).

**Table 1.** Mean and maximum air temperature  $\pm$  standard deviation, total precipitation and percentage of rainy days (precipitation ratio) measured in the three burr development stages in 2017 and 2018.

Stage	Mean air temperature (°C)		Maximum air temperature (°C)		Precipitation (mm)		Precipitation ratio (%)	
	2017	2018	2017	2018	2017	2018	2017	2018
I	22.4 $\pm$ 2.3	22.0 $\pm$ 1.8	26.7 $\pm$ 2.8	25.9 $\pm$ 1.5	0	29.4	0	40
II	24.0 $\pm$ 3.7	22.9 $\pm$ 2.9	28.6 $\pm$ 4.0	26.8 $\pm$ 2.7	48.4	70.2	6	17
III	15.9 $\pm$ 3.8	19.7 $\pm$ 2.1	19.4 $\pm$ 4.4	23.1 $\pm$ 2.2	124	171	49	30

### 3.2. Burr size development

In 2018, burrs grew steadily throughout the growing season, whereas, in 2017, burr growth slowed down in the central part of the growing season (44-60 DAFB) (Figure 1).



**Figure 1.** Seasonal pattern of burr diameter (mm)  $\pm$  standard error of the mean, measured in 2017 and 2018.

Burr diameter at 27 DAFB did not differ between 2017 and 2018, with mean values of 19.5 and 21.0 mm, respectively (Figure 1). In 2018, at the end of stage I, burr diameter was significantly higher compared to 2017 (37.1 mm and 28.6 mm, respectively; Table 2). In addition, in the stage II, burr size differences between the two seasons increased (52.1 and 35.2 mm in 2018 in 2017, respectively). At the end of stage III, the final burr diameter was

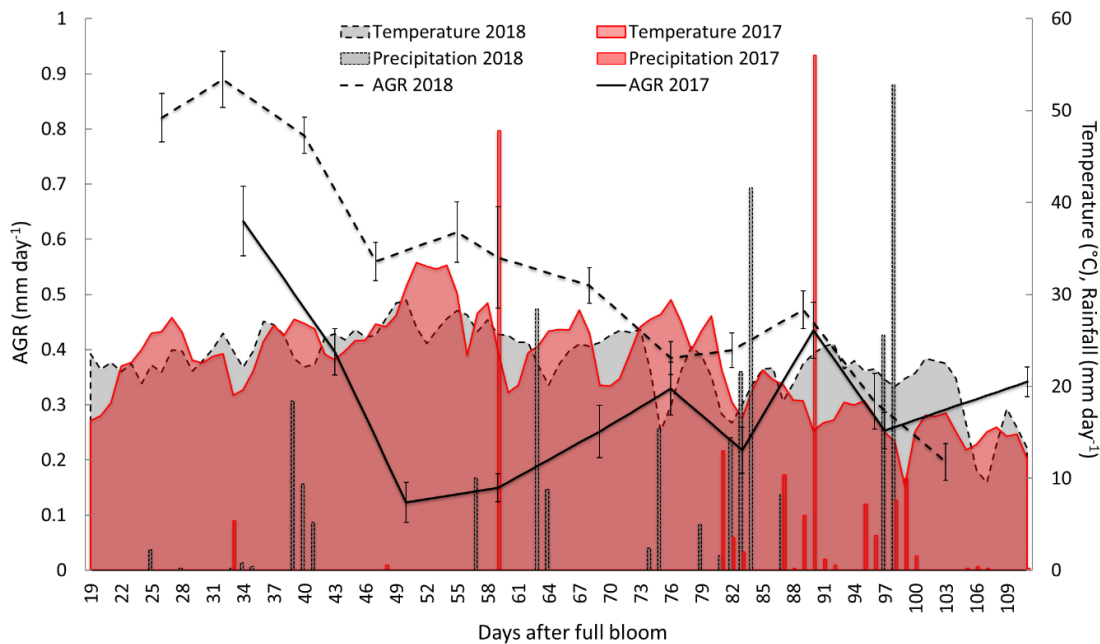
60.8 and 46.7 mm in 2018 and 2017, respectively. This difference in diameter was still significant but slightly restrained in comparison to stage II (Table 2).

**Table 2.** Burr absolute growth rate (AGR) and final burr diameter in the three development stages in 2017 and 2018. Within each line, means followed by different letters indicate significant differences between years (ANOVA,  $p \leq 0.01$ ).

Stage	Burr AGR (mm day <sup>-1</sup> )		Burr Diameter (mm)	
	2017	2018	2017	2018
I	0.38 b	0.76 a	28.6 b	37.1 a
II	0.24 b	0.52 a	35.2 b	52.1 a
III	0.31	0.35	46.7 b	60.8 a

3.3. Seasonal AGR trend

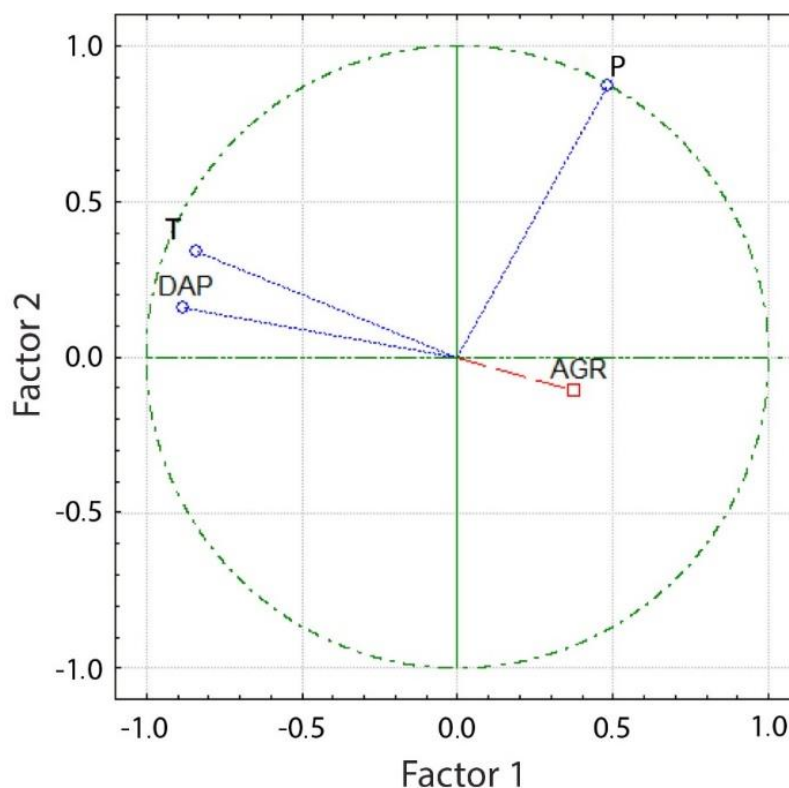
In 2017 (characterized by drier and warmer conditions compared to 2018), burr growth rate slowed down especially between 44 and 60 DAFB (Figure 2). An opposite trend was observed in 2018 when the overall burr AGR was probably enhanced by (a) the abundance of spring-winter precipitations, (b) the occurrence of regular summer precipitations and (c) the mild summer temperatures.



**Figure 2.** Seasonal patterns of burr absolute growth rate (AGR; mm day<sup>-1</sup>) ± standard error, mean air temperature (°C) and precipitation (mm day<sup>-1</sup>) measured in 2017 and 2018.

In 2017, AGR in stages I and II (0.38 and 0.23 mm day<sup>-1</sup>, respectively) was significantly lower than in 2018 (0.76 and 0.52 mm day<sup>-1</sup>, respectively). The lack of replenishment of soil water reservoir during winter/spring time, the higher summer temperatures (>32 °C) and the significant water deficit in July and August (Table 1) probably caused low values of AGR (0.12 mm day<sup>-1</sup>; 1 August; 50 DAFB). High temperatures and water deficit are among the main environmental factors affecting negatively photosynthetic activity and causing a decrease in assimilate availability in

chestnut trees (Bukhov and Mohanty, 1999; Gomes-Laranjo et al., 2006). This hypothesis is supported by the results of the PCA (Figure 3), that showed that AGR is inversely correlated to the mean air temperature (T) and to the number of days without rainfall after the last precipitation event (DAP) and it is positively correlated to rainfall (P).



**Figure 3.** Principal component analysis carried out using the data of mean air temperature (T), precipitation (P), number of days without rainfall following each precipitation event (DAP) and burr absolute growth rate (AGR) measured in 2017. Factors 1 and 2 explained 57% and 30% of the total variance, respectively.

Most of the orchard management practices (*e.g.* no-tillage with grass covering, late season grass mowing, conservation of organic residual for increasing soil organic matter) currently adopted in traditional chestnut orchards (Ferrini and Pisani, 1993) are nowadays insufficient to mitigate the impact of climate change and to allow a satisfactory level of productivity. The results of our preliminary study suggest that the use of irrigation is becoming essential also in mountain areas (especially in Mediterranean conditions) where summer water deficit is becoming one of the main constraints to chestnut tree growth and productivity.

Only the rainfalls recorded at 60 DAFB (11 August), and especially during stage III, induced an increase in AGR until burr valves opening. At this latter phenological stage, no significant difference in AGR was detected between the two seasons (Table 2). Indeed, in stage III, the difference in burr diameter between 2017 and 2018 (Figure 1 and Table 2) was slightly smaller than at the end of stage II. This suggests that tree physiological conditions improved during the last part of fruit development. Furthermore, this study suggests that burr seasonal growth could be likely used as a mirror for monitoring tree physiological status and for managing smart irrigation practices. However, additional and more specifically designed studies will be necessary to confirm this hypothesis. It is well known that, in chestnut, the availability of rain at the end of summer, or in autumn, seems to affect significantly chestnut development and productivity (Vigiani, 1941; Breisch et al., 1995;



Bounous, 2002; Gomes-Laranjo et al., 2007; Vida Rural, 2017; Mota et al., 2018b). Almost half of the fresh weight of chestnut kernel is made of carbohydrates (mainly starch), being the major component of its dry matter (Dinis et al., 2012). Chen et al. (2017) reported that, in *C. mollissima* Blume, the increase in endosperm dry mass starts at 60 DAFB and reaches a peak at 80 DAFB, when nuts are mature. It is possible to hypothesize that starch endosperm content is correlated to soil water availability between late summer and early autumn. Thus, the absence of limiting weather conditions during this period (e.g. no thermo-inhibition and enough water availability) can directly enhance tree physiological performances and fruit growth. In any case, it cannot be excluded a possible negative effect of tree water stress on cytokinesis and thus on burr final size.

The phenological stage of full burr fall occurred 4-5 days earlier in 2017, the warmer year (5-6 October; 115-116 DAFB), compared to 2018. According to Sparks et al. (2001), an increase of 2.5 °C in air temperature may anticipate the occurrence of plant phenological stages. Similarly, Dinis et al. (2011) reported that fruit harvest was carried out earlier in locations where air temperatures, between May and October, were higher. In 2018, burr valves opening started eight days earlier than in 2017 (23 September; 103 DAFB).

#### 4. Conclusions

The results of this study suggest that sweet chestnut burr growth is significantly influenced by seasonal weather conditions. In 2017, the high summer temperatures coupled with the low summer and winter-spring rainfall, affected negatively the overall burr AGR with negative consequences on final burr size. Conversely, in 2018 the mild weather conditions enhanced AGR and thus final burr size. Further studies should be carried out at a burr scale level to better investigate its growth mechanism and consequently define tailored irrigation practices for enhancing chestnut productivity.

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