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Genotype × environment interactions influence early fruit traits in young Mediterranean sweet chestnut orchards

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Abstract

Climate variability requires site-specific evaluation of sweet chestnut (*Castanea sativa* Mill.) cultivars under Mediterranean conditions. This study assessed phenology, early production, commercial and sanitary quality, nut size, and kernel composition in young orchards across four contrasting Portuguese environments during 2024. Three traditional cultivars (Judia, Longal and Martaínha) and ColUTAD were compared.

Strong cultivar × site interactions affected most traits. Some combinations initiated reproductive development but failed to produce harvestable nuts. Commercial performance, sanitary quality, nut size, soluble carbohydrates, and starch varied markedly among sites. ColUTAD showed consistent early productivity but poorer sanitary quality under the warmest conditions, whereas Martaínha and Judia showed more favourable sanitary profiles at productive sites.

These findings demonstrate that genotype × environment interactions jointly shape early production, fruit quality, and kernel composition. Integrating these traits can improve site-specific cultivar selection and support more resilient Mediterranean chestnut production under increasing climatic variability.

1. Introduction

Mediterranean chestnut production is increasingly challenged by higher temperatures, irregular rainfall patterns and increasing biotic pressure, making cultivar adaptation a major concern for sustainable orchard management. Under these changing climatic conditions, identifying cultivars capable of maintaining stable productivity and fruit quality across contrasting environments has become a priority for the long-term resilience of chestnut production systems. Sweet chestnut (*Castanea sativa* Mill.) is one of the most important nut-producing tree species in southern Europe, supporting traditional agroforestry systems, rural economies and culturally valued landscapes. Beyond nut production, it also provides valuable timber, contributes to biodiversity conservation and delivers important ecosystem services (Conedera et al., 2016). However, increasing environmental variability across Mediterranean and sub-Mediterranean regions is exposing orchards to conditions that can impair tree growth, reproductive development, and fruit production, highlighting the need to identify cultivars capable of maintaining stable performance across contrasting environments. Such adaptation largely depends on genotype × environment interactions, which determine how individual cultivars respond to local growing conditions.

The adaptation of chestnut cultivars to contrasting Mediterranean environments is largely determined by genotype × environment interactions, which regulate physiological performance and ultimately influence fruit production and quality. Previous studies have demonstrated substantial differences among sweet chestnut cultivars in physiological performance, water relations, photosynthetic capacity and carbohydrate metabolism under contrasting environmental conditions (Borges et al., 2008; Marques et al., 2025a, b). While these physiological responses provide valuable insight into plant adaptation, their agronomic relevance ultimately depends on whether they are reflected in fruit-level traits.

For growers and the chestnut industry, successful adaptation is determined not only by tree performance but also by early bearing capacity, commercial yield, fruit quality, sanitary status, and kernel nutritional composition. These traits are particularly informative during orchard establishment, when differences in reproductive development among cultivar × site combinations may already reveal contrasting levels of adaptation.

Fruit quality is one of the main determinants of chestnut market value. Commercial performance depends on the proportion of marketable nuts, nut calibre, mean nut weight, and the incidence of defects such as hollow, malformed, rotten, or insect-damaged fruits. Among the principal causes of economic losses in European chestnut production, carpophagous moths of the genus *Cydia* are responsible for substantial insect damage (Ferracini et al., 2021), while fungal diseases remain major constraints to post-harvest quality. In recent years, chestnut brown rot has been increasingly associated with *Gnomoniopsis smithogilvyi* (syn. *G. castaneae*), now recognised as one of the principal causal agents of nut rot in several European producing regions, including Portugal (Lema et al., 2023; Possamai et al., 2023). Disease incidence may vary according to cultivar susceptibility, orchard management, local environmental, and fruit maturity at harvest, suggesting that sanitary performance also reflects genotype × environment interactions.

Besides commercial quality, chestnut kernels possess a distinctive nutritional profile. Unlike most tree nuts, chestnuts are characterised by high carbohydrate concentrations, particularly starch, together with relatively low lipid content and moderate protein levels (Borges et al., 2008; Santos et al., 2022). Carbohydrate composition strongly influences nutritional value, technological behaviour, and sensory characteristics. Previous studies have reported substantial variation in kernel composition among cultivars and growing regions, indicating that carbohydrate accumulation is regulated by both genetic background and environmental conditions (Borges et al., 2008; Mert and Ertürk, 2017; Santos et al., 2022). Such variation indicates that environmental conditions may influence not only productivity and fruit appearance but also the biochemical quality of harvested nuts, providing additional insight into genotype × environment responses beyond conventional yield traits.

Although considerable progress has been made in understanding chestnut physiology and responses to abiotic stress, relatively little information is available linking genotype × environment interactions with phenology, early fruit production, commercial quality, sanitary performance and kernel composition under contrasting Mediterranean field conditions. Most previous studies have focused on individual aspects of cultivar performance, whereas integrated assessments combining agronomic, sanitary, and nutritional traits remain scarce, particularly during the early productive stage of orchard establishment. Yet, this period is especially relevant because differences in reproductive development and fruit production may provide an early indication of long-term cultivar adaptation to local environmental conditions.

To address this knowledge gap, the present study evaluated the influence of four contrasting Mediterranean environments on phenology, early fruit production, commercial quality, sanitary status,

and kernel nutritional composition in three traditional Portuguese chestnut cultivars (Judia, Longal, and Martaínha), together with the ColUTAD genotype/rootstock cultivated as a nut-producing material. This work represents the first fruit traits assessment within the ongoing ClimCast experimental network, integrating reproductive phenology, commercial performance, sanitary quality, and kernel composition during the 2024 harvest season across four Portuguese experimental orchards (Carrazedo de Montenegro, Parada, Penela da Beira, and Marvão). We hypothesised that genotype × environment interactions would already be expressed during the early productive stage through differences in fruit production, commercial quality, sanitary performance, and kernel composition. Specifically, we tested whether (i) early fruit production, marketable yield, and nut size differed among site × cultivar combinations; (ii) sanitary traits, including rot and insect damage, were jointly influenced by cultivar and growing environment; and (iii) kernel composition, particularly starch and soluble carbohydrate contents, varied among environments in a cultivar-dependent manner. By integrating these complementary traits, this study provides a comprehensive early assessment of cultivar adaptation and contributes to the identification of chestnut cultivars better suited to specific Mediterranean growing conditions under increasing environmental variability.

2. Materials and Methods

This study evaluated the early fruit-level responses of four chestnut cultivars grown under contrasting Mediterranean environments. Cultivar performance was assessed through an integrated experimental workflow comprising sequential evaluations of phenology, fruit production, commercial quality, sanitary status and kernel composition. The overall experimental design and workflow are summarised in Fig. 1.

2.1. Sites description

The study was conducted within the ongoing ClimCast field trial during the 2024 growing season, corresponding to the first fruit-level assessment of young chestnut orchards established in climatically contrasting regions of mainland Portugal (Fig. 1). The selected sites represent a gradient of altitude and temperature and include both traditional and emerging chestnut-growing areas. All orchards belonged to the ClimCast network. Carrazedo de Montenegro, Parada, Penela da Beira and Marvão were managed under rainfed conditions.

Table 1
Geographic location and main climatic characteristics of the four chestnut orchards evaluated in this study

(adapted from Instituto Português do Mar e da Atmosfera n.d.).

Orchards	Coordinates	Mean Temperature (°C)	Mean Precipitation (mm)	Altitude (m a.s.l.)
Carrazedo de Montenegro	41°33’41.76”N; 7°25’51.41”W	13.7	50.5	765
Parada	41º38’12.53”N; 6º42’42.94”W	12.8	65.1	740
Penela da Beira	41°01’38”N; 7°26´38”W	12.5	52.9	885
Marvão	39°21'19.5"N 7°21'40.1"W	15.5	49.1	583

Mean temperature and mean precipitation correspond to the period 2020–2024. Altitude is expressed as metres above sea level (m a.s.l.).

2.2. Plant material and experimental setup

The plant material consisted of three traditional Portuguese chestnut cultivars, namely Judia, Longal and Martaínha, grafted onto the ColUTAD rootstock, together with ColUTAD grown as a non-grafted genotype. All orchards included the same set of cultivars, ensuring comparable genetic material across the different study sites. The orchards were established in 2019, and trees were approximately 4 to 5 years old at the time of the study. All sites were managed under similar agronomic practices, including comparable planting density, pruning, fertilization and pest management, thereby minimizing management-related variability. For each cultivar at each site, three representative trees were selected as biological replicates. In total, 12 trees were evaluated per site, corresponding to 48 trees overall.

2.3. Phenological observations and harvest timing

Phenological monitoring was conducted across all sites following the BBCH scale for chestnut (Medič and Kunc, 2025). Observations were carried out weekly from June to July 2024, covering the main reproductive stages, including male catkin emergence, catkin elongation, female flowering, fruit initiation and early fruit development. Additional observations were conducted in October 2024 to assess fruit maturation, ripening onset and harvest timing. Photographic records were taken throughout the monitoring period to document phenological differences among cultivar × site combinations. These records were used as supporting evidence for the interpretation of early reproductive performance, particularly in cases where some cultivars showed visible bur development whereas others had limited or absent fruit production. Representative images of the main phenological stages and contrasting cultivar responses are provided as Supplementary Material.

2.4. Fruit harvest and post-harvest handling

Chestnut burs were harvested at physiological maturity within 10 days between late October and early November 2024. For each sampled tree, approximately 15 burs were randomly collected to minimize sampling bias. Samples were pooled by cultivar and site and transported to the laboratory. Nuts were manually extracted from the burs, counted and stored at 4°C for one week before fruit quality evaluation and biochemical analyses.

2.5. Fruit quality assessment and defect classification

Chestnuts were visually inspected and classified according to external and internal quality traits into the following categories: well-formed, wrinkled, hollow, rotten and insect-damaged. Well-formed chestnuts corresponded to fruits with normal size and morphology, whereas wrinkled chestnuts showed surface shrinkage or deformation affecting commercial value. Hollow chestnuts were underdeveloped or empty fruits. Rotten chestnuts showed visible fungal decay, and insect-damaged fruits were identified by the presence of larval galleries or exit holes.

Based on symptomatology and regional pest prevalence, insect damage was mainly attributed to *Cydia* spp., which account for the vast majority of cases in the study area.

Commercial marketable chestnuts were calculated as the sum of well-formed and wrinkled fruits. The sanitary commercial fraction was obtained by excluding rotten and insect-damaged chestnuts. Fruit calibre was expressed as the number of well-formed chestnuts per kilogram.

2.6. Sample preparation for biochemical and nutritional analyses

For each cultivar × site combination, chestnut samples were processed separately. Fruits were manually peeled, sliced, frozen in liquid nitrogen and freeze-dried using a VirTis Benchtop Pro freeze-drier (SP Industries, Warminster, PA, USA) at − 80°C. Dried samples were ground into a fine powder and stored in airtight containers at − 80°C until analysis, for no longer than two weeks. All biochemical determinations were expressed on a dry matter basis.

2.7. Chemicals and reagents

All chemicals and reagents used were of analytical grade. Sodium hydroxide, boric acid, sulfuric acid, ethanol, petroleum ether, glucose, α-amylase, amyloglucosidase, sodium acetate and phosphoric acid were obtained from Sigma-Aldrich (Steinheim, Germany). Kjeldahl digestions were performed using a selenium catalyst. Milli-Q purified water (Millipore, Bedford, MA, USA) was used throughout all extraction and analytical procedures.

2.8. Determination of soluble carbohydrates and starch

Soluble carbohydrates were determined in triplicate from lyophilized chestnut samples. Soluble sugars were extracted with 80% ethanol (*v*/*v*) in conical centrifuge tubes placed in a water bath at 80°C for 40 min. After cooling and centrifugation, the supernatant was collected for analysis. Quantification was based on the anthrone colorimetric method using glucose as standard. The anthrone reagent was prepared by dissolving 0.2 g of anthrone in 100 mL of 80% ethanol. Appropriate aliquots of the extracts were diluted as necessary, mixed with 3 mL of anthrone reagent, heated in a water bath at 100°C for 10 min, cooled to room temperature, and the absorbance was measured at 625 nm (Andrade et al., 2021).

Starch content was determined in triplicate from the extraction residue remaining after soluble sugar extraction. The pellet was subjected to enzymatic hydrolysis with *α*-amylase and amyloglucosidase, and the released glucose equivalents were quantified colorimetrically. Results for both soluble carbohydrates and starch were expressed as mg g^{-1} dry matter (Mccleary et al., 1997).

2.9. Nutritional composition of chestnut fruits

Lyophilized chestnut samples were analysed in triplicate according to AOAC procedures (AOAC International, 1990). Dry matter and ash were determined according to AOAC method 942.05. For dry matter determination, samples were oven-dried at 105°C to constant weight. Ash content was determined by incineration of the dried material in a muffle furnace at 550°C for 3 h.

Crude protein was determined according to AOAC method 954.01 using the Kjeldahl procedure. Ground samples were digested in sulfuric acid in the presence of a selenium catalyst at 420°C. The released ammonia was distilled and collected in boric acid, and nitrogen content was determined by titration. Crude protein content was calculated by applying a nitrogen-to-protein conversion factor of 6.25.

Total fat was determined according to AOAC method 920.39 by Soxtec extraction with petroleum ether. Samples were extracted under boiling and rinsing steps with the organic solvent, followed by solvent evaporation, oven-drying of the residue at 105°C, cooling in a desiccator and gravimetric determination of total fat content.

Total dietary fibre was determined using an enzymatic-gravimetric assay based on AOAC method 985.29. Samples were subjected to sequential enzymatic digestion with heat-stable α-amylase, protease and amyloglucosidase under controlled pH and temperature conditions. After ethanol precipitation, the residues were filtered, washed, dried and weighed. Dietary fibre content was obtained after correction for residual protein and ash.

2.1. Statistical analysis

The experimental unit corresponded to each cultivar × site combination, with biological replicates depending on fruit availability within each site. Results are presented as mean (M) ± standard deviation (SD), as indicated in tables and figure legends. Differences among cultivars within each site were initially evaluated using one-way analysis of variance (ANOVA) followed, whenever possible, by Tukey's honestly

significant difference (HSD) post hoc test. Prior to analysis, assumptions of normality and homogeneity of variances were assessed using the Shapiro-Wilk and Levene's tests, respectively.

When the assumption of homogeneity of variances was violated, Welch's ANOVA followed by Games-Howell post hoc comparisons was performed. In cases where the assumptions of parametric analysis were not met, non-parametric analyses (Kruskal-Wallis test followed, whenever possible, by Dunn's pairwise comparisons with Bonferroni correction) were additionally performed to support the interpretation of treatment effects.

All statistical analyses were performed using jamovi v. 2.7.26, and statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Phenological development across sites and cultivars

Phenological observations revealed marked differences in reproductive timing and bur development among cultivar × site combinations. Although Judia at Parada and Marvão and Martaínha at Penela da Beira did not produce harvestable nuts, these combinations exhibited reproductive initiation and the formation of developing burs during the observation period. However, bur development was generally later or less pronounced than in productive combinations. Therefore, the absence of harvestable yield could not be attributed solely to a lack of bur formation and may have resulted from limited or interrupted subsequent fruit development. Representative phenological sequences recorded during four observation weeks between mid-June and late July 2024 are presented in Supplementary Figures S1–S4.

Clear differences in reproductive development were also observed among cultivars and study sites. ColUTAD consistently exhibited earlier and more frequent bur formation across the experimental network, whereas trees grown in Marvão generally showed more advanced bur development during the observation period.

3.2. Nut yield, size traits and commercial quality parameters

The percentage of marketable nuts differed significantly among cultivars within each study site (Fig. 2a). Based on the separate within-site comparisons, ColUTAD was included in the highest statistical group at all four sites, with observed values ranging from 60.7% in Penela da Beira to 77.9% in Marvão. In Carrazedo de Montenegro, ColUTAD produced a significantly higher percentage of marketable nuts (68.1%) than Martaínha (34.8%), whereas Judia (63.2%) and Longal (39.0%) presented intermediate values and did not differ significantly from either cultivar. Martaínha was included in the highest statistical group in Parada (66.6%) and Marvão (73.3%), where it did not differ significantly from ColUTAD but showed significantly higher percentages than Longal (35.8% and 32.6%, respectively). In

Penela da Beira, ColUTAD (60.7%) and Longal (50.1%) produced significantly higher percentages of marketable nuts than Judia (30.5%), with no significant difference between ColUTAD and Longal.

The proportion of healthy nuts within the marketable fraction also differed among cultivar × site combinations (Fig. 2b), revealing differences in sanitary quality. Based on the separate within-site comparisons, Longal was included in the highest statistical group at all sites, reaching 100.0% in Carrazedo de Montenegro, 87.0% in Penela da Beira and 86.9% in Marvão. Martaínha was also included in the highest statistical group wherever it was evaluated, reaching 100.0% in Carrazedo de Montenegro and 98.2% in Marvão. Judia showed a high proportion of healthy marketable nuts in Penela da Beira (96.1%), where it did not differ significantly from Longal, while in Carrazedo de Montenegro it presented an intermediate value that did not differ significantly from the other cultivars. In contrast, ColUTAD was included in the lowest statistical group in Carrazedo de Montenegro, Penela da Beira and Marvão, with values of 68.5%, 63.4% and 41.0%, respectively. No significant differences among cultivars were detected in Parada, where the proportion of healthy marketable nuts ranged from 50.7% in Longal to 72.6% in ColUTAD.

Significant differences in morphological calibre (number of nuts kg^{-1}) were observed among cultivars at most study sites (Fig. 3a), although the magnitude of these differences varied with the growing environment. ColUTAD consistently exhibited among the highest calibre values, indicating the production of smaller nuts. This pattern was particularly evident in Carrazedo de Montenegro, where ColUTAD (81.0 nuts kg^{-1}) and Judia (85.9 nuts kg^{-1}) produced significantly smaller nuts than Longal (61.8 nuts kg^{-1}) and Martaínha (56.8 nuts kg^{-1}). Likewise, in Marvão, ColUTAD (105.9 nuts kg^{-1}) exhibited significantly higher calibre values than Longal (77.0 nuts kg^{-1}) and Martaínha (68.9 nuts kg^{-1}). In Penela da Beira, Judia showed the highest calibre value (162.1 nuts kg^{-1}), significantly exceeding ColUTAD (110.1 nuts kg^{-1}), whereas Longal (118.7 nuts kg^{-1}) presented intermediate values. By contrast, no significant differences among cultivars were detected in Parada, where calibre values ranged from 86.8 to 101.7 nuts kg^{-1}.

Mean marketable nut weight showed the expected inverse trend (Fig. 3b). Martaínha consistently produced some of the heaviest nuts, reaching 18.1 g in Carrazedo de Montenegro, where it significantly exceeded ColUTAD (12.9 g) and Judia (12.2 g), while Longal (15.4 g) showed intermediate values. In Marvão, both Martaínha (14.9 g) and Longal (13.4 g) produced significantly heavier nuts than ColUTAD (10.2 g). In contrast, Penela da Beira was characterised by generally smaller nuts, although ColUTAD (7.4 g) still produced significantly heavier nuts than Judia (4.5 g), with Longal (5.0 g) showing intermediate values. No significant differences among cultivars were detected in Parada, where mean marketable nut weight ranged from 9.0 to 10.9 g. Across all environments, variations in mean nut weight closely reflected the opposite pattern observed for morphological calibre.

Whereas fruit size varied consistently among cultivar × site combinations, sanitary quality exhibited greater variability, with statistically significant differences detected only at specific locations (Fig. 4). Rot incidence showed substantial variation across environments (Fig. 4a), ranging from the complete absence of rotten nuts in several cultivar × site combinations to a maximum of 38.5% in ColUTAD at

Marvão. Relatively high rot incidence was also observed in Martaínha at Parada (22.5%). Significant cultivar differences were identified only in Parada, where Martaínha exhibited higher rot incidence than ColUTAD and Longal, and in Marvão, where ColUTAD showed significantly higher rot incidence than both Longal and Martaínha.

A similarly variable pattern was observed for insect-damaged nuts (Fig. 4b). The highest incidence occurred in Longal at Parada (49.3%), followed by ColUTAD in Penela da Beira (26.4%) and Marvão (20.5%). Significant cultivar differences were detected only in Penela da Beira, where ColUTAD exhibited a higher proportion of insect-damaged nuts than Judia, while Longal presented intermediate values. No significant differences among cultivars were observed in Carrazedo de Montenegro, Parada, or Marvão.

3.3. Kernel chemical composition and nutritional profile

Kernel proximate composition and carbohydrate profile differed significantly among the available site × cultivar combinations (Table 2). Significant differences were detected for all analysed variables ($p < 0.001$), including dry matter, organic matter, protein, fat, soluble carbohydrates and starch.

Dry matter showed relatively limited variation among combinations, ranging from 93.9% in Longal from Penela da Beira to 96.4% in ColUTAD from Marvão. A similar pattern was observed for organic matter, with the lowest values recorded in Judia and Longal from Penela da Beira and the highest values in ColUTAD from Carrazedo de Montenegro and Marvão.

Protein content varied considerably among cultivar × site combinations. The highest protein concentration was recorded in ColUTAD from Penela da Beira (9.38% DM), while similarly high values were observed in Judia and Longal from the same site and in Judia, Longal and Martaínha from Carrazedo de Montenegro. Lower protein contents were recorded in ColUTAD and Martaínha from Parada and in ColUTAD from Marvão. Fat content showed an opposite tendency, with ColUTAD from Carrazedo de Montenegro (3.47% DM) and Marvão (3.16% DM) presenting the highest concentrations, whereas most of the remaining cultivar × site combinations contained approximately 1–2% fat on a dry matter basis.

The greatest variability was observed for carbohydrate-related traits. Soluble carbohydrate concentration ranged from 31.6 mg g^{-1} DM in Judia from Carrazedo de Montenegro to 94.8 mg g^{-1} DM in Longal from Marvão. High soluble carbohydrate concentrations were also recorded in Longal and Martaínha from Carrazedo de Montenegro and Parada, whereas comparatively low values were observed in ColUTAD from Parada and Judia and Longal from Penela da Beira. Starch content varied from 47.0 mg g^{-1} DM in ColUTAD from Parada to 71.0 mg g^{-1} DM in Judia from Penela da Beira. Relatively high starch concentrations were also recorded in Longal and Martaínha from Parada and in Longal and Martaínha from Marvão, whereas lower values were generally associated with ColUTAD from Carrazedo de Montenegro and Parada.

Table 2
Proximate composition and carbohydrate profile of chestnut kernels (*Castanea sativa* Mill.) across different site × cultivar combinations.

Site	Cultivar	Dry matter (%) M ± SD	Organic matter (%) M ± SD	Protein (% DM) M ± SD	Fat (% DM) M ± SD	Soluble carbohydrates (mg g^{-1} DM) M ± SD	Starch (mg g^{-1} DM) M ± SD
Carrazedo de Montenegro	ColUTAD	95.0 ± 0.01 d	98.0 ± 0.06 a	6.37 ± 0.73 b	3.47 ± 0.23 a	76.1 ± 0.82 b	55.6 ± 2.74 ab
	Judia	95.3 ± 0.01 c	97.2 ± 0.05 c	8.51 ± 0.13 ab	1.19 ± 0.12 b	31.6 ± 1.36 c	65.9 ± 0.45 a
	Longal	95.7 ± 0.04 b	97.3 ± 0.04 c	8.09 ± 0.01 ab	1.26 ± 0.02 b	88.0 ± 0.62 a	60.4 ± 0.73 b
	Martaínha	96.3 ± 0.02 a	97.7 ± 0.01 b	8.45 ± 0.06 a	1.07 ± 0.06 b	88.2 ± 0.60 a	62.6 ± 0.47 b
Parada	ColUTAD	95.4 ± 0.06 b	97.4 ± 0.03 a	5.16 ± 0.01 c	2.08 ± 0.08 b	50.3 ± 1.14 c	47.0 ± 2.43 b
	Longal	95.7 ± 0.01 a	97.2 ± 0.05 a	7.81 ± 0.14 a	1.26 ± 0.03 c	81.0 ± 1.05 b	68.3 ± 0.64 a
	Martaínha	94.9 ± 0.05 c	97.5 ± 0.07 a	5.52 ± 0.03 b	2.60 ± 0.01 a	86.9 ± 1.29 a	66.9 ± 0.56 a
Penela da Beira	ColUTAD	95.0 ± 0.08 a	97.6 ± 0.03 a	9.38 ± 0.23 a	1.03 ± 0.08 c	63.2 ± 1.79 a	67.2 ± 0.66 a
	Judia	94.8 ± 0.01 a	95.0 ± 0.09 b	8.79 ± 0.69 a	1.58 ± 0.01 b	54.2 ± 0.98 b	71.0 ± 1.29 a
	Longal	93.9 ± 0.04 b	94.6 ± 0.07 c	8.95 ± 0.25 a	2.13 ± 0.11 a	42.9 ± 1.42 c	56.8 ± 1.09 b
Marvão	ColUTAD	96.4 ± 0.01 a	98.0 ± 0.03 a	5.86 ± 0.11 c	3.16 ± 0.06 a	84.2 ± 3.29 b	63.0 ± 0.37 b

Values are means ± standard deviation

Site	Cultivar	Dry matter (%) M ± SD	Organic matter (%) M ± SD	Protein (% DM) M ± SD	Fat (% DM) M ± SD	Soluble carbohydrates (mg g^{-1} DM) M ± SD	Starch (mg g^{-1} DM) M ± SD
	Longal	95.5 ± 0.06 b	97.1 ± 0.01 c	8.26 ± 0.09 a	1.31 ± 0.06 b	94.8 ± 1.71 a	66.2 ± 0.08 a
	Martaínha	95.1 ± 0.03 c	97.6 ± 0.01 b	7.51 ± 0.14 b	1.32 ± 0.14 b	72.5 ± 1.06 c	66.1 ± 0.42 a
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Values are means ± standard deviation							

Different letters within each column indicate significant differences among cultivars within each site ($p < 0.05$).

4. Discussion

Understanding how chestnut cultivars respond to contrasting Mediterranean environments during orchard establishment is becoming increasingly important under current climate change scenarios. While previous studies have mainly focused on mature orchards, the present work demonstrates that genotype × environment interactions already influence fruit production, commercial quality, sanitary performance and kernel composition during the first productive years. These findings extend previous evidence that environmental variability affects chestnut adaptation not only through phenological and physiological responses but also through fruit traits directly associated with commercial performance (Dinis et al., 2011; Fernandes et al., 2022; Marques et al., 2025a, 2025b; Pereira et al., 2011; Portela et al., 2015). Collectively, they demonstrate that cultivar adaptation to contrasting Mediterranean environments can already be assessed during orchard establishment, when differences in reproductive development and fruit quality first become apparent.

4.1. Phenological responses in contrasting environments

The absence of bur formation in several cultivar × site combinations provides strong evidence that differences in early fruit production were primarily associated with reproductive development rather than fruit abortion or harvest losses. This finding is particularly relevant during orchard establishment, when reproductive maturity varies considerably among cultivars and early productivity may already provide valuable information on environmental adaptation.

Previous studies have shown that chestnut phenology is strongly regulated by temperature accumulation and local environment, with important consequences for flowering, fruit set and yield formation (Dinis et al., 2011; Larue et al., 2021; Medič and Kunc, 2025). The phenological differences observed in our results therefore support the view that climatic conditions can influence reproductive development from the earliest productive stages of orchard establishment, ultimately determining whether trees successfully initiate fruit production.

ColUTAD consistently exhibited earlier reproductive development than the grafted cultivars across the study sites. However, this apparent precocity should not be attributed exclusively to genetic characteristics. Unlike the remaining materials, ColUTAD was established as a non-grafted tree and did not require replacement after planting, whereas several grafted cultivars experienced delayed establishment following graft failure or mechanical damage. Consequently, differences in orchard establishment likely contributed to the observed variation in reproductive precocity, together with genotype-specific responses. Similar relationships between establishment success, vegetative vigour and early reproductive performance have been reported for other perennial fruit crops (Kendall et al., 2022).

Marvão, the warmest and lowest-altitude site, consistently exhibited the earliest reproductive development and fruit maturation. This response agrees with the well-established influence of temperature accumulation on chestnut phenology Dinis et al., 2011; Fonti et al., 2007; Larue et al., 2021). Nevertheless, earlier phenological development was not necessarily associated with superior fruit quality, as several cultivar × site combinations at Marvão also exhibited increased sanitary losses. These phenological differences therefore provide the first indication that cultivar adaptation to contrasting Mediterranean environments is already expressed before differences in fruit quality become evident.

4.2. Effect of genotype × environment interactions on chestnut fruit quality and size

Commercial fruit quality emerged as an early expression of genotype × environment interactions, indicating that cultivar adaptation is already reflected in fruit traits during orchard establishment. Although cultivar identity clearly contributed to marketable yield, its expression depended on local growing conditions, highlighting that commercial performance cannot be considered an intrinsic cultivar trait alone. This finding agrees with previous studies showing that chestnut productivity and fruit quality result from the combined effects of genetic background, environmental conditions and annual climatic variability (Borges et al., 2008; Pimentel-Pereira et al., 2007; Poljak et al., 2021; Portela et al., 2015). This work further demonstrates that these interactions are already evident during orchard establishment, before trees reach full productive maturity.

The contrasting responses observed among cultivars illustrate the strong influence of the growing environment on commercial performance. ColUTAD consistently produced a high proportion of marketable nuts across the productive sites, suggesting good early productive potential. However, this apparent advantage should be interpreted together with its more advanced establishment stage and the

higher incidence of sanitary defects observed under some environmental contexts. Martaínha showed a stronger environmental response, with relatively low marketability in Carrazedo de Montenegro but substantially better performance in Parada and Marvão. In contrast, Longal generally exhibited lower marketable percentages under warmer environments, whereas Judia displayed a more site-dependent response, performing well in Carrazedo de Montenegro but less favourably in Penela da Beira. Collectively, these observations reinforce the importance of evaluating cultivar performance across multiple environments before establishing recommendations for commercial planting.

Fruit size also varied markedly among environments, indicating that local environment influenced fruit growth in addition to reproductive success. The smaller fruits recorded in Penela da Beira, reflected by higher morphological calibre values and lower mean nut weight, contrast with the larger fruits produced in Carrazedo de Montenegro and Marvão, particularly for Martaínha and Longal. Fruit size is one of the main determinants of chestnut commercial value and depends not only on genetic characteristics but also on the capacity of developing fruits to accumulate assimilates during kernel growth (Borges et al., 2008; Bounous and Marinoni, 2004; Pereira-Lorenzo et al., 2006; Poljak et al., 2021).

Several physiological mechanisms may contribute to these differences. Final fruit size in chestnut is determined by the balance between assimilate supply and sink demand, which is influenced by carbon availability, water status, mineral nutrition and crop load (Génard et al., 2007; Gomes-Laranjo et al., 2008; Mota et al., 2018). Environmental conditions that restrict photosynthesis or increase competition among developing fruits may reduce assimilate allocation to the kernels, limiting kernel filling and ultimately producing smaller nuts. The consistent inverse relationship observed between morphological calibre and mean nut weight supports this interpretation.

The observed differences suggest that commercial quality and fruit size cannot be attributed to cultivar identity alone but result from the interaction between genotype, growing environment and orchard establishment. Evaluating cultivar performance under Mediterranean conditions requires the integration of productivity, fruit size and commercial quality, as these traits provide early evidence of environmental adaptation.

4.3. Sanitary quality, insect damage and rot incidence

The present results demonstrate that commercial quality and sanitary quality should not be considered synonymous. A high proportion of marketable nuts did not necessarily correspond to high sanitary quality, indicating that commercial classification alone may overestimate the effective value of the harvested crop. This distinction was particularly evident in Marvão, where ColUTAD combined high marketable yield with the highest rot incidence, whereas Martaínha achieved similarly high marketability while maintaining excellent sanitary quality. These contrasting responses highlight that cultivar adaptation should be assessed using both productive and sanitary criteria.

Sanitary defects were strongly influenced by the interaction between cultivar and environment. Insect damage varied markedly among sites, consistent with previous reports identifying *Cydia* spp. as the

principal carpophagous pests of European chestnut orchards and showing that infestation depends largely on local climatic conditions and phenological synchrony between the host and the insect (Cuestas et al., 2020; Ferracini et al., 2021; Marrão et al., 2021; Pombo et al., 2018). Rot incidence followed a similarly heterogeneous pattern. Although *Gnomoniopsis smithogilvyi* is currently recognised as the main causal agent of chestnut brown rot in several European producing regions, including Portugal (Lema et al., 2023; Possamai et al., 2023), chestnut decay may also involve other fungal species, and the associated microbiota can be influenced by cultivar, orchard management and post-harvest conditions (Rodrigues et al., 2022). The elevated rot incidence observed in ColUTAD at Marvão is consistent with the influence of the local environment on disease development, as warmer temperatures may favour pathogen activity or accelerate fruit maturation, while physiological stress can increase host susceptibility to infection (Desprez-Loustau et al., 2006; Lema et al., 2023; Lione et al., 2018). Because pathogen isolation and insect identification were beyond the scope of the present study, sanitary assessments were based exclusively on visible symptoms. The observed patterns should therefore be interpreted as indicators of sanitary performance rather than definitive evidence of the causal agents involved.

Sanitary quality emerged as an essential component of cultivar evaluation. Early productive potential alone does not ensure commercial suitability if high sanitary losses accompany it. Incorporating the proportion of healthy marketable nuts into cultivar assessment provides a more realistic measure of early cultivar adaptation under contrasting Mediterranean environments.

4.4. Kernel composition and nutritional quality

Kernel composition responded markedly to genotype × environment interactions, confirming that the nutritional quality of chestnuts is shaped not only by genetic background but also by local growing conditions. Although chestnut kernels are characteristically rich in carbohydrates and contain relatively low lipid concentrations compared with other edible nuts (Borges et al., 2008; Künsch et al., 2001; Santos et al., 2022), our results show that the relative proportions of protein, fat and storage carbohydrates varied among cultivar × site combinations. These results demonstrate that environmental effects extend beyond fruit production, influencing the biochemical composition and nutritional quality of chestnut kernels.

Protein accumulation exhibited a clear environmental component, supporting previous observations that kernel nitrogen content depends on cultivar, nitrogen availability, fruit development and the balance between carbon and nitrogen allocation during kernel filling (Borges et al., 2008; Mert and Ertürk, 2017; Pereira-Lorenzo et al., 2006). By contrast, differences in lipid concentration were comparatively small, reflecting the naturally low fat content of chestnuts. Nevertheless, the consistently higher lipid concentration observed in ColUTAD at two locations suggests that genotype-specific patterns of carbon allocation may also contribute to kernel composition. As discussed previously, this response should be interpreted together with the more advanced establishment of ColUTAD, which may have influenced assimilate availability during fruit development.

The greatest compositional variation was observed for soluble carbohydrates and starch, indicating that carbon partitioning was particularly sensitive to environmental conditions. Although starch represents the principal storage compound in chestnut kernels, soluble sugars also play important roles in reserve mobilisation, osmotic regulation and the acquisition of sensory characteristics. Previous studies have likewise reported substantial variation in carbohydrate composition among cultivars and growing regions, reflecting the combined influence of genotype and environment (Borges et al., 2008; Künsch et al., 2001; Mert and Ertürk, 2017; Santos et al., 2022). The partial dissociation observed between starch and soluble carbohydrates suggests that site conditions influenced carbon partitioning during kernel filling rather than simply altering total carbohydrate accumulation. Similar responses have been described in other fruit tree species, where assimilate partitioning depends on source–sink relationships, environmental constraints and developmental stage (Génard et al., 2007).

These observations reinforce that assessing chestnut adaptation under Mediterranean conditions requires the integration of productive, commercial, sanitary and nutritional traits, as each contributes complementary insight into cultivar performance during orchard establishment.

5. Conclusions

This study demonstrates that genotype × environment interactions influence chestnut performance from the earliest productive stages of orchard establishment. Differences among cultivar × site combinations were already reflected not only in fruit production but also in commercial quality, sanitary status, fruit size and kernel composition, indicating that cultivar adaptation becomes evident well before orchards reach full productive maturity.

Rather than identifying a universally superior cultivar, the results revealed distinct adaptive profiles across the evaluated materials. Productive performance was not consistently associated with commercial quality, sanitary stability or kernel composition, emphasising that cultivar suitability depends on the interaction between genotype and local environmental conditions. These findings highlight the importance of evaluating fruit quality, sanitary performance and nutritional attributes together with yield when selecting cultivars for Mediterranean environments.

Although continued monitoring within the ClimCast experimental network will be essential to determine whether these responses remain stable as orchards mature, the present findings indicate that fruit traits are sensitive early indicators of cultivar adaptation to contrasting Mediterranean environments. Integrating productive, commercial, sanitary and nutritional traits provides a robust framework for site-specific cultivar selection and supports the development of more resilient chestnut production systems under increasingly variable Mediterranean climates.

Declarations

Author Contributions

T.M. conceived and conducted the study. T.M., H.F. and A.F.-P. performed analyses. T.P., E.C., L.F. and J.G.-L. supervised the project. All authors reviewed and approved the final manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Data will also be deposited in a public repository following article acceptance, in accordance with the *Applied Fruit Science* data sharing policy.

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Conflicts of Interest

The authors declare no conflict of interest.

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Figures

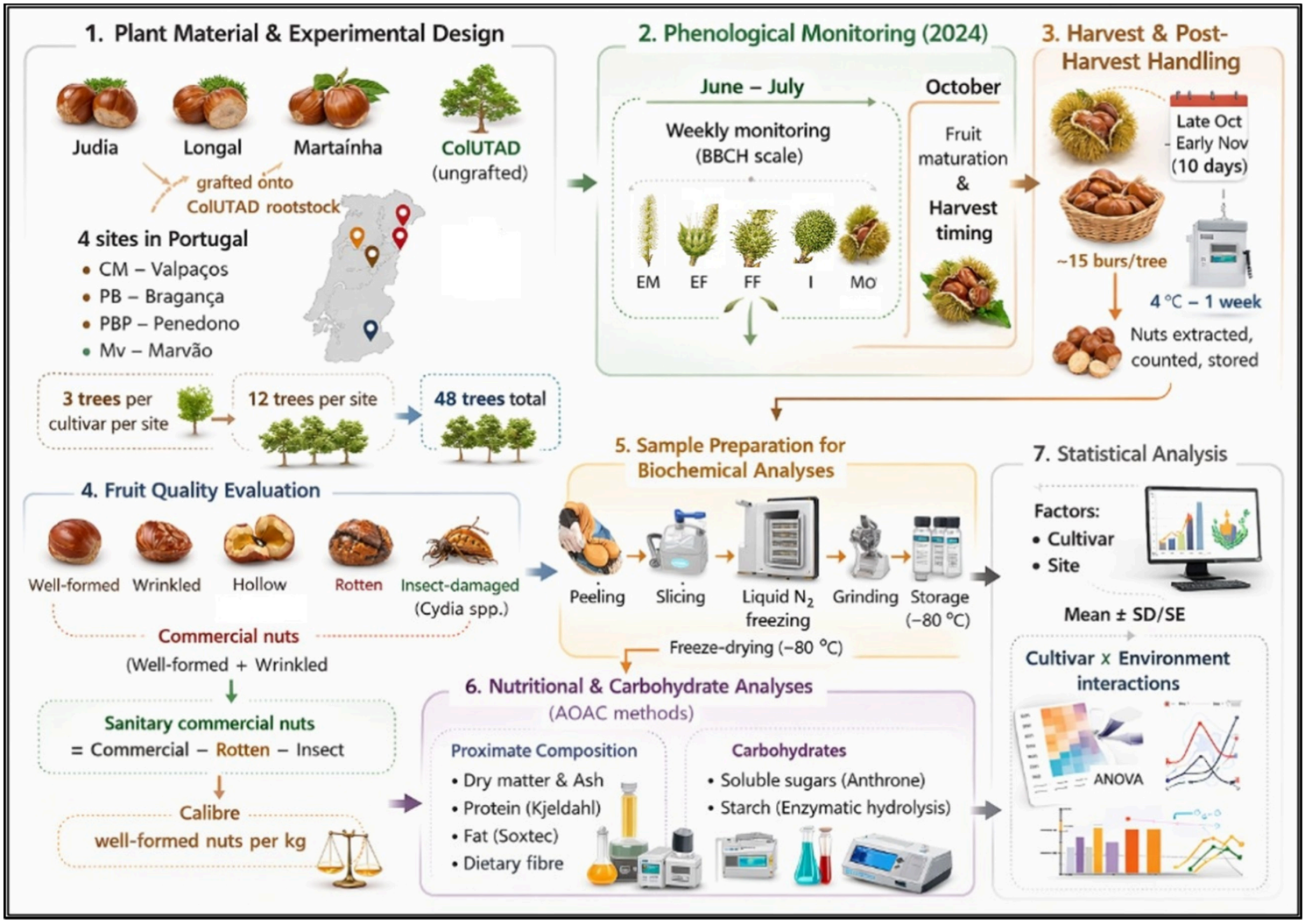


Figure 1

Integrated experimental workflow linking field phenology, fruit production and kernel composition in young chestnut orchards under contrasting environmental conditions.

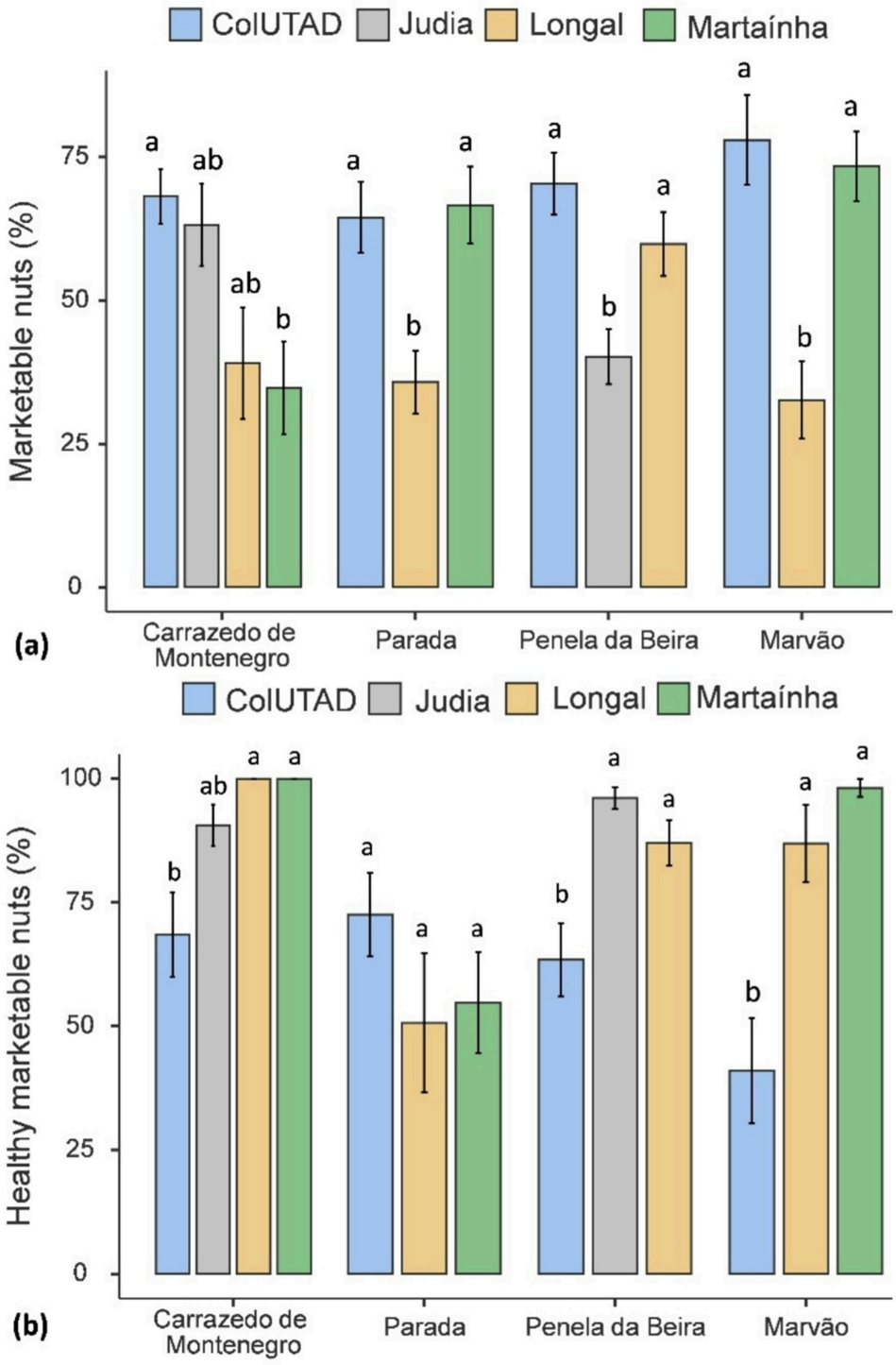


Figure 2

Early fruit production and commercial quality of chestnut cultivars across the study sites. **(a)** Percentage of marketable nuts. **(b)** Percentage of healthy marketable nuts within the marketable fraction. Error bars indicate standard deviation (SD). Letters indicate significant differences among cultivars within each site according to Games–Howell or Dunn's tests ($p < 0.05$).

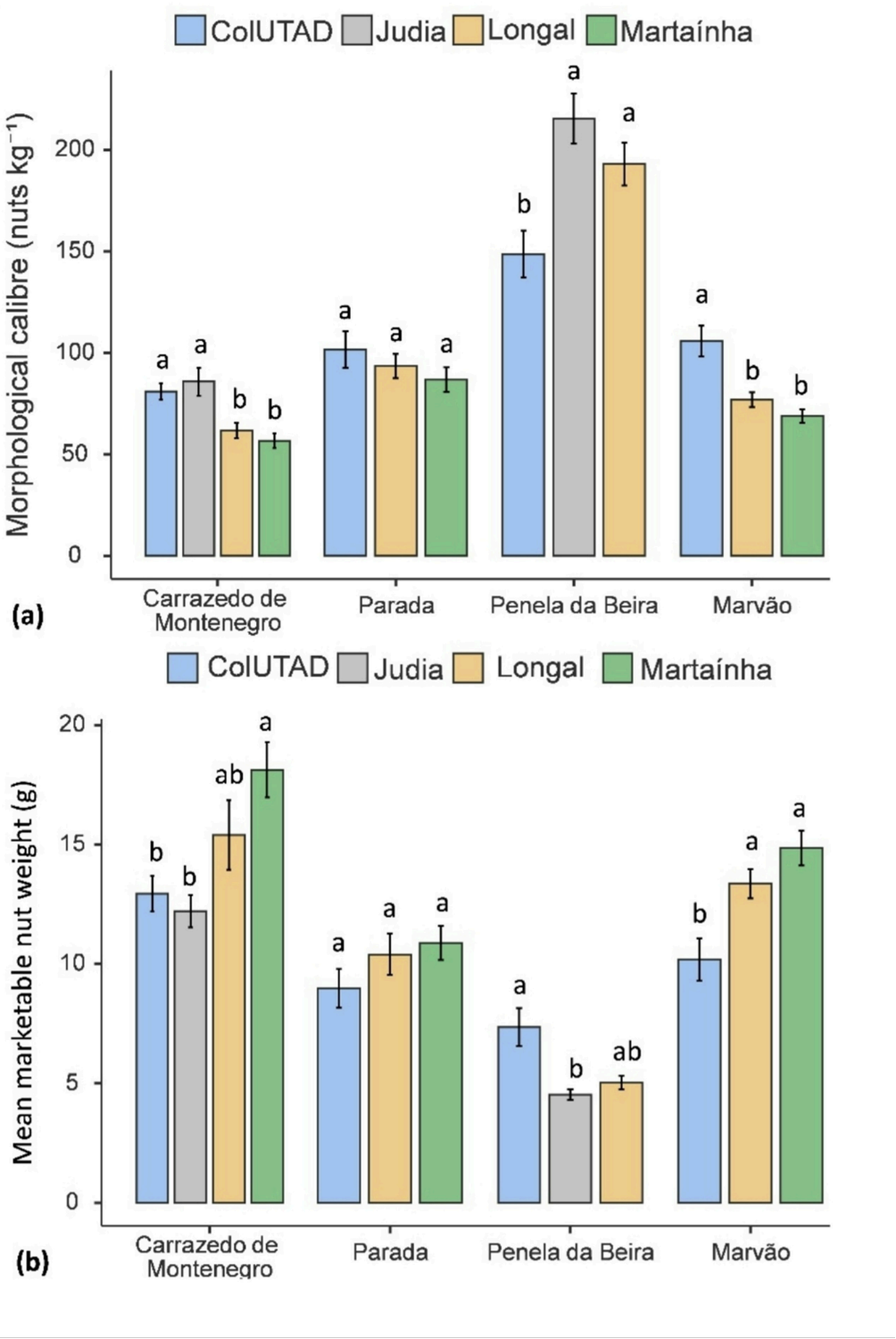


Figure 3

Calibre **(a)**, expressed as the number of nuts per kilogram, and mean marketable nut weight **(b)** of chestnut cultivars across the study sites. Letters indicate significant differences among cultivars within each site according to Tukey's HSD, Games-Howell or Dunn's tests, as appropriate ($p < 0.05$). Higher calibre values correspond to a greater number of nuts per kilogram and therefore to smaller nut size.

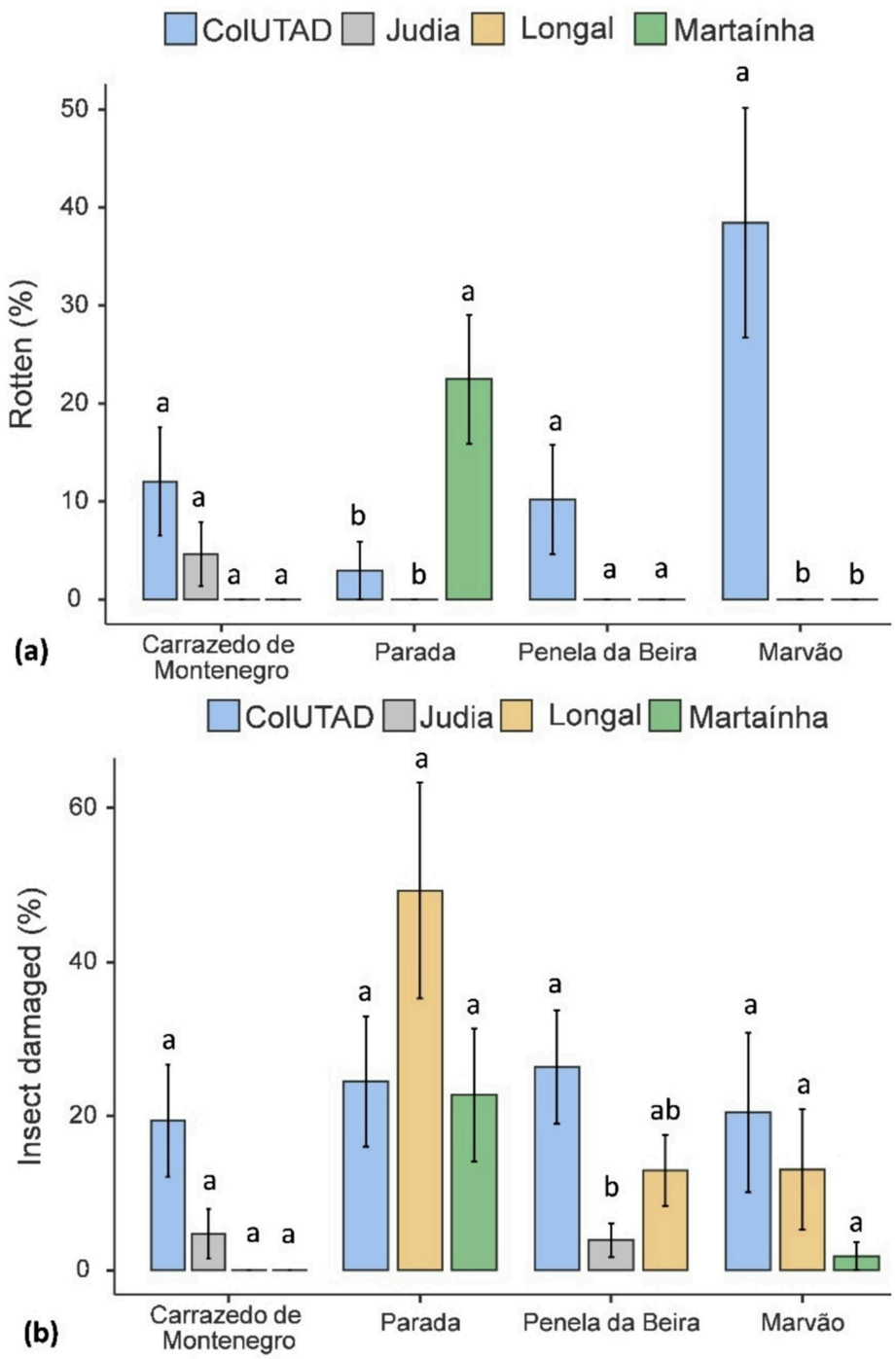


Figure 4

Sanitary quality of marketable chestnuts across cultivar × site combinations. **(a)**Rotten nuts (%) and **(b)**insect-damaged nuts (%), expressed as a percentage of the marketable nut fraction. Letters indicate significant differences among cultivars within each site according to Tukey's HSD, Games–Howell or Dunn's post hoc tests, as appropriate ($p < 0.05$).

Supplementary Files

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