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SUSTAINABLE CULTIVATION OF HOPS (*Humulus lupulus* L.) FOR THE EXPANSION OF CROP BIODIVERSITY IN MEDITERRANEAN AGRO-ECOSYSTEMS

AGR/02 – Agronomia e Coltivazioni erbacee

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DOTTORATO

6. OTHER ACTIVITIES DEVELOPED DURING THE PhD COURSE

5.1. Forage Mixture Productivity and Silage Quality from a Grass/Legume Intercrop in a Semiarid Mediterranean Environment

5.2. *Asparagus officinalis* (L.): Yield and Field Performance of 10 Genotypes Cultivated in a Semi-arid Environment

7. ACKNOWLEDGMENTS

INTRODUCTION

Agricultural biodiversity, also known as agrobiodiversity, can generally be defined as biodiversity within agricultural contexts, encompassing the variety of living organisms and their variability within agricultural and livestock production systems (Heywood, 1999). Agrobiodiversity is closely associated with crop cultivation and animal husbandry, as well as the complex ecology found within these production systems. Agroecosystems should therefore be understood as the product not only of the physical elements of the environment and biological resources but also of the cropping systems and associated management techniques. The concept of agrobiodiversity is not static but dynamic, influenced by numerous factors including not only agronomic but also cultural and human ones. As early as the late 1990s, within the framework of the "Global Plan of Action" proposed by the FAO and endorsed in the "Convention on Biological Diversity" (CBD), the introduction of new crops was identified as a tool to promote the sustainability of modern agricultural activities by increasing the complexity of agroecosystems. In this regard, a recent line of research aims to study the implications of climate change on the economic sustainability of traditional agricultural systems. The need to assess climatic effects on quality is particularly relevant for certain crops (such as grapes, tea, and coffee), where technological quality primarily derives from aromatic, sensory, and taste components (Ahmed et al., 2014). The study by Ahmed & Stepp (2016) further emphasized how crop diversification, involving the introduction of new crops, is emerging as a promising strategy for the climate resilience of agricultural areas, helping to mitigate the impact of climate change effects on the qualitative parameters of traditional crops in a given region.

This Ph.D. research project aimed to investigate the productive potential of hops (*Humulus lupulus* L.), considering the introduction of the crop into the Mediterranean environment as an important tool for expanding the agrobiodiversity of local agricultural systems. Improved agronomic performances even in new growing areas, have allowed the spread of the crop into warm-arid climate zones (Carr, 2017), and within reduced-input systems. Furthermore, several studies have shown the benefits of specific farming practices (i.e., low-height trellis systems). In fact, compact hop cultivation in low-trellis systems offers environmental benefits by enabling more efficient and less dispersed phytosanitary treatments due to the dense plant canopy and limited vertical growth (Darby, 2005; Beatson et al., 2009). Additionally, it facilitates more effective biological control of key pest insects and mites (Gunn & Darby, 1987; Lilley et al., 1999; Darby, 2004). Also, the use of resilient hop varieties in low-trellis systems has proven to be highly advantageous, ultimately enabling the adoption of organic farming practices. This represented a tangible economic opportunity for organic hop growers in the United Kingdom (Darby & Seigner, 2007). In Italy, in recent years, systematic studies have been conducted on the suitability of modern commercial hop varieties to growth conditions in the Mediterranean environment (Mongelli et al., 2015; Rossini et al., 2016; Ruggeri et al., 2018; Marceddu et al., 2020; Rossini et al., 2021), confirming the excellent productive and qualitative response of some selected hop varieties.

Although domestic hop cultivation still occupies a relatively small area (Carbone & Cherubini, 2016), Italy has already produced examples of excellence in hop production. However, there remains a gap in scientific understanding regarding the yield potential of hops in Mediterranean environments, particularly under low-input growing conditions. Despite these challenges, the hop-growing sector is expanding, driven by the rising demand for craft beer. Since 2020, the cultivated area has steadily increased (Licciardo et al., 2021), reaching 97.5 hectares across the country (Licciardo & Carbone, 2023).

This growth represents an opportunity to further integrate local raw ingredients, especially hops, into beer production, offering numerous benefits that include enhancing sustainability, improving product quality, and fostering economic resilience. By focusing on regionally adapted hop varieties and cultivation techniques, Italy has the potential to strengthen both its hop and brewing industries while capitalizing on the global demand for unique, locally sourced products. From an environmental perspective, sourcing hops locally helps to reduce the carbon footprint by minimizing transportation needs, which is especially significant as agriculture faces increasing pressure to adopt sustainable practices (Liu et al., 2016). Certain hop varieties are often better adapted to regional climates and soils, reducing the need for chemical inputs such as pesticides and fertilizers, further enhancing sustainability (Rossini et al., 2021; Turner et al., 2011).

Economically, the use of local ingredients might support regional agriculture and strengthens local economies by creating demand for hops and fostering relationships between brewers and farmers. It could also enhance resilience against global supply chain disruptions, such as those caused by climate change or geopolitical instability, ensuring that breweries can continue production without being reliant on distant sources (Tukamuhabwa et al., 2017). Additionally, close proximity between growers and brewers also allows for greater customization of hop varieties to suit specific brewing needs, driving innovation and tailored production (Dobis et al., 2019).

Also, the terroir—the unique combination of soil, climate, and environmental conditions— might play a crucial role in shaping the flavor and aroma profiles of hops, much like it does in the production of wine (Van Leeuwen et al., 2022). Local hops reflect the specific characteristics of their growing region, creating distinct sensory qualities in the beer that are directly tied to the place of cultivation. By incorporating local hops, breweries can develop beers with unique flavor profiles that are difficult to replicate elsewhere, fostering a stronger connection between the product and its region of origin. Indeed, this differentiation, driven by the terroir effect, could provide a strong marketing edge for breweries, as consumers are increasingly drawn to authentic, locally crafted products. By adopting these practices, breweries can eventually produce unique beers that capture the essence of their region while also promoting ecological and economic sustainability, securing the long-term success of both the brewing and hop-growing sectors.

Hence the need for further research aimed at highlighting the potential of organic cultivation of the crop, in terms of productivity and economic sustainability, potentially conferred using new varieties within reduced-input management systems.

With this purpose, experimental setups were established in three different sites across Sicily, operating under organic farming conditions, and conducting a comparison between hop varieties and agronomic techniques. In the first year, a botanical survey was conducted to identify plant material that was potentially more adaptable to the Mediterranean region. Two of the most suitable varieties for semi-arid Mediterranean environments were selected, namely Cascade and Chinook (Forteschi et al., 2019; Gresta et al., 2023; Carrubba et al., 2022), which were subsequently planted in three distinct experimental sites. Suitable experimental designs with replications were adopted, with the main investigated agronomic treatment assigned as the primary experimental factor (i.e., irrigation system, mulching, trellis height, and trellis design). In each location, trellises were constructed for hop plant cultivation, reinforced at the top with a galvanized iron wire, serving the dual purpose of stabilizing the structure and providing support for vertical plant growth. Each orchard was also equipped for drip irrigation, with estimated seasonal water requirements of approximately 200 mm ha^{-1} per growing season. Climate conditions were monitored at each site using specific meteorological stations to optimize irrigation interventions and evaluate the effect of key climatic variables on the biometric and qualitative characteristics of the crops. Specifically, daily minimum and maximum temperatures (used for calculating Growing Degree Days - GDD), daily rainfall, wind speed, relative humidity, evapotranspiration, and solar radiation were monitored. Field observations recorded the start and end dates of major phenological phases (leaf apparatus development, lateral shoot emergence, inflorescence formation, flowering, cone swelling, and ripening) according to the BBCH scheme developed by a group of German researchers from the Federal Biological Research Centre for Agriculture (Meier et al., 2009). The analysis of α- and β-acids was conducted using Fast High-Performance Liquid Chromatography (HPLC), following the methodology proposed and codified by the Analytica European Brewery Convention (Analytica EBC, 1975), carried out at the "CEntro di Ricerca sulla Birra" (CERB), belonging to the Department of Agricultural, Food and Environmental Science of the University of Perugia. The oil content was determined through distillation followed by gas chromatographic analysis, conducted at the laboratories of UPV, according to the protocols reported by Marceddu et al. (in press). The collected data, including yields, biometric and qualitative parameters of the cones, and growing degree days (GDD) required for overcoming major phenological stages, underwent statistical analysis using leading statistical software (Minitab, Systat, Sigmaplot, etc.).

The data collected throughout the PhD course were reported in peer-reviewed papers and presented to congresses and meetings at national and international level.

To implement the doctoral course, I participated in the application process for a Fulbright scholarship (2022) applying for a "Visiting Student Researcher" grant. The project presented has taken its cue from my current PhD project with a greater focus on issues related to the adaptation of a new crop such as hops in sub-tropical climate environments. Following an extensive selection procedure, I was honored to receive the grant, officially becoming a Fulbright fellow. From October 2022 until March 2023, I carried out my research at the Gulf Coast Research and Education Center (Wimauma, FL), a research facility belonging to the University of Florida. This Fulbright scholarship indeed represented an important opportunity for my personal and professional growth as a PhD student, further expanding my vision skills and building a training path for the transfer of acquired knowledge. Starting from December 2023 until March 2024, I also conducted research activities at the Universitat Politècnica de València (UPV), carrying out gas chromatographic analysis on hop oils and hydrolates.

The structure of this PhD thesis follows a systematic approach, first aimed at evaluating the most relevant issues in the cultivation of hops in the Mediterranean environments, and subsequently reporting the outcomes of the trials performed throughout the PhD period. Whenever these outcomes have been published, the corresponding papers have been inserted into this PhD thesis in their final editorial form.

CHAPTER I: presents a published paper and a review of the current state of hop farming in the Mediterranean region, focusing on its response to primary abiotic stressors, particularly emphasizing cultivation management in semi-arid environments and within low-height systems. This can be considered an initial exploratory step for approaching the investigation subsequently conducted during my doctoral studies.

CHAPTER II: explores the physiological, biometric, and qualitative responses of hops cultivated in a controlled environment, as detailed in the two referenced papers. The simplification of cultivation techniques was aimed at rigorously assessing their impact on both productivity and quality, considering the introduced sources of variation.

CHAPTER III: reports the responses of hops subjected to different agronomic treatments in open field conditions, to delve into the effect of agronomy on the potential performances of American varieties within the reference Mediterranean agroecosystems. Therefore, the first research work focuses on the productive performances of the species, assessing the efficacy of the applied agronomic treatments. Also, a preliminary assessment of the impact of extreme heat events, expressed as Extreme Degree Days (EDD), on hop farming in a semi-arid environment such as Sicily is presented. This involved evaluating their potential effect on the qualitative performance of the species, considering variations in genotype and trellis design.

CHAPTER IV: focuses on the adaptation of hop species in subtropical climate environments. The first work of research investigated whether off-season cropping of hops, achieved through "multiple harvests," could facilitate the sustainable and economically viable expansion of this crop into new growing areas like southwest Florida. In the second paper, an integrated approach was taken to explore the relationship between vertical growth rates and vegetative development in hops, with a specific focus on the Cascade cultivar in two distinct growing environments: semi-arid Sicily, Italy, and a subtropical environment in Florida, USA. Understanding the growth characteristics associated with developmental stages is crucial for comprehending interactions between plant development and external factors such as pests and diseases. This integrated perspective offers a valuable tool for gaining insights into hops' cultivation dynamics and suggests targeted interventions to improve crop health and yield.

Finally, conclusions and perspectives derived from the thesis are provided. This comprehensive structure reflects the ambitious scope of the thesis, aiming to give a significant contribution to the field of sustainable agriculture. It addresses critical aspects of implementing Mediterranean agro-biodiversity through the introduction of cultivation of a key crop for the growing Italian craft beer industry.

In the final part of the thesis, three articles and one review paper are appended, published as outcome of additional research carried out with the Department of Agricultural, Food and Forest Sciences of Palermo University during the doctoral years.

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1. CHAPTER I

This chapter presents a published paper and a review of the current state of hop farming in the Mediterranean region, concentrating on how it responds to primary abiotic stressors, with a particular focus on cultivation management in semi-arid environments and low-height systems. While the modern brewing industry relies heavily on hops for product characterization, there is limited information on their suitability for semi-arid Mediterranean areas, where they could provide valuable resources for farmers. The perennial nature of hops presents challenges due to the limited understanding of the hypogeal system's development in relation to cone yield.

The first publication (DOI 10.17660/ActaHortic.2023.1366.17) discussed above details experimentation conducted in Sicily during 2018 and 2019. This study aimed to evaluate the growth and yield performance of three hop cultivars: ‘Cascade’, ‘Chinook’, and ‘Nugget’. It assessed biometrical characteristics, phenological stages, and development rates in relation to heat accumulation (GDD; °C). Different mulching techniques were tested, including organic (fragmented pine bark) and synthetic (PE plastic film) options. Sensory analysis of the cones was performed by brewing small batches of beer, which revealed excellent organoleptic characteristics and promising crop potential for semi-arid Mediterranean environments. However, the results underscored the importance of selecting the appropriate variety and cultivation technique, as these factors significantly influenced the accumulation trends of hypogeal biomass and overall yield.

Additionally, a review paper (doi: 10.3389/fpls.2022.1064922) is introduced, focusing on recent studies conducted in the southern regions of Europe, particularly Spain and Italy, to examine how hops respond to abiotic stresses. These investigations are pivotal for comprehending hops' adaptability to new cultivation environments and their long-term economic implications, given the perennial nature of the species. The phytochemical makeup of hops, influenced by both genetic and environmental factors, underscores the necessity for a thorough understanding of the effects of abiotic stresses. This review offered valuable insights into the challenges and strategies for coping with hop cultivation in semi-arid Mediterranean environments, laying the groundwork for further exploration in my doctoral studies.

1.1.Cultivation Trials of Hop (*Humulus lupulus* L.) in a Mediterranean Environment

Cultivation trials of hop (*Humulus lupulus* L.) in a Mediterranean environment

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Abstract

In the modern brewing industry hops are an essential element for the characterization of the brewing product. Although several works have been addressed to evaluate the yield performance of hop in Northern Italian environments, scarce information is available about the suitability of hop to the semi-arid Mediterranean areas, where this crop could represent a valuable resource for farmers. Hop being a perennial crop, a major issue is the lack of knowledge about the mechanisms underlying the development of the hypogeal system related to cones yield. An experimentation was conducted in Sicily in 2018 and 2019, to test the growth and yield performance of three hop cultivars ('Cascade', 'Chinook', and 'Nugget'). Biometrical characteristics, phenological stages, and plants' development rates were evaluated, and put in relation to heat accumulation (GDD; °C). Furthermore, organic (fragmented pine bark) and synthetic (PE plastic film) mulches were applied. Finally, sensory analysis of the cones collected was carried out, by using them to brew small batches of beer. The results showed a high variability of yields and biometrical characteristics of the tested hop varieties according to the cropping management. Indeed, the cultivation technique was able to influence the accumulation trends of the hypogeal biomass, but this effect proved genotype-dependent. The development rates had a direct influence on the hypogeal biomass accumulation, even though they could not be considered a reliable indicator of productivity: the faster the growth in the first two phases of plants' elongation, the lower the yield in both cones and total epigeal biomass. The sensory analyses assessed the excellent organoleptic characteristics of Sicilian hops, as well as of beers produced. Hop demonstrates to be a promising crop for semi-arid Mediterranean environments although a proper choice of the variety demonstrated crucial.

Keywords: craft beer, crop production, crop yields, development stages, growth rate, hop productivity, agro-ecosystems, mulching treatment, thermal sums

INTRODUCTION

Hop plants (*Humulus lupulus* L.) are mainly cultivated for the beer industry as a source of secondary metabolites (i.e., alpha-acids, beta-acids, and the so-called "oils"), providing the characteristic bitterness and aroma, as well as antimicrobial properties, to the finished product (Neve, 1991; Mishra et al., 2015). These substances are contained in the lupulin glands (glandular trichomes) that develop mainly -but not only- in the female inflorescences of hops (cones). Recent studies have shown the existence of glandular trichomes, although present with a lower density, on the lower foliar epidermis, which differ morphologically from those present in the cones, and also for the lower content of secondary metabolites detected (Sugiyama et al., 2006; Mishra et al., 2019). In addition, hops have been known for a long time in traditional medicine and recently several compounds contained in the lupulin metabolome have received particular attention given their medicinal properties (Zanoli and Zavatti, 2008; Magalhães et al., 2008). Several studies have been carried out on the factors influencing the biosynthesis of these compounds, concluding that, as with other secondary metabolites of

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plant origin, both the genotype and the environment play a key role in determining their final concentration (Almaguer et al., 2014; Moore et al., 2014; Morcol et al., 2020).

The biosynthesis of alpha-acids, beta-acids, and prenylated flavonoids involves complex biosynthetic pathways (Paniego et al., 1999; Okada and Ito, 2001), as well as equally articulated is the influence of external factors (cultivation environment, phytosanitary status, and age of crops) (Jelínek et al., 2012; Matoušek et al., 2016; Morcol et al., 2020; Eriksen et al., 2021). Morcol et al. (2020) found that the environmental factor had a great influence on the concentration levels of aromatic glucosides, leading for the first time to hypothesize a "terroir" effect for hop production. In Italy, in recent years, systematic studies have been carried out on the adaptability of modern commercial varieties of hops to growing conditions in the Mediterranean (Mongelli et al., 2015; Rossini et al., 2016; Ruggeri et al., 2018; Marceddu et al., 2020), confirming the excellent productive and qualitative response of hop productions obtained from the numerous varieties tested. This work aims to summarize the cultivation experiences carried out in Sicily, in two growing seasons. A first trial was conducted in 2018 and 2019 in two locations, to test the growth and yield performance of three hop varieties ('Cascade', 'Chinook', and 'Nugget'), with already proven adaptability to the cultivation environments (Rossini et al., 2016; Forteschi et al., 2019). Details about the experimental sites and the followed experimental protocols can be retrieved in a previously published paper (Marceddu et al., 2020). Briefly, biometrical characteristics, phenological stages, and plants' development rates were evaluated, and put in relation to heat accumulation, expressed in growing degree days (GDD; °C). Also, being hop a perennial crop, the study tried to deepen the mechanisms underlying the development of the hypogeal system, potentially related to the productive performance of the crop within the economically viable lifespan of the hop orchard. Two experimental sites were adopted, allowing to get some preliminary information on crop management in two environments of the Sicilian territory. Low-input management was so used, adopting low-height systems ("low-trellis"). As stated by several Authors (Darby, 2004, 2005a, b; Beatson et al., 2009; Gunn and Darby, 1987; Lilley et al., 1999), the adoption of low-trellis farming systems showed to be more suitable for lowering productive inputs, without hampering the achievement of adequate cones yield and hop quality. Mulching treatments were also tested, namely organic (fragmented pine bark) and synthetic (PE plastic film). Finally, sensory analysis of the cones collected was carried out, by using them to brew small batches of beer, then subjected to a panel test. The adaptive responses of the different genotypes to the applied experimental factors were therefore investigated, by monitoring the productive performances both in terms of quantity (cone production) and quality (aromatic contribution given to the beers brewed).

MATERIALS AND METHODS

Experimental sites

A first trial was carried out in 2018 and 2019, at the "Orleans" farm (Palermo, Italy, 38°06'27"N; 13°21'01"E; 0 m a.s.l.) of the Department of Agricultural, Food and Forest Sciences (D/SAAF) of the University of Palermo. A separate cultivation trial was also conducted at the "Sparacia" experimental farm (Cammarata, AG, Italy, 37°38'07" N; 13°45'47" E; 450 m a.s.l.), also belonging to the D/SAAF, to compare 'Cascade's adaptive response to two different cropping systems and growing environments (pots in Orleans, Palermo, PA, and open field in Sparacia, Cammarata, AG). Both study locations are characterized by a warm temperate climate, according to the Köppen-Geiger classification (Kottek et al., 2006).

Experimental plantings

In the first two-year trial carried out at the "Orleans" farm, three of the most popular commercial cultivars have been selected, namely 'Cascade', 'Chinook', and 'Nugget'. The orchard was set up on 9th April 2018. Each plant was placed in a 40-L pot, provided on the bottom with a draining layer of expanded clay and gravel (1:1), then filled with a brown peat and perlite (3:2) mixed substrate. The growing support was arranged through the installation of a low-trellis system made of iron three-meter poles (0.5 m underground and 2.5 m emerging from the ground).

To evaluate the response of the three genotypes to the use of different mulching materials, 15 pots for each variety were covered with organic material (organic mulch in pine bark), and black PE film (1 mm), and as many have been kept uncover and used as a control treatment.

A second experiment was also set up, with the purpose to compare the growth and yield performance of 'Cascade' farmed with two contrasting cropping systems (pots and open field) and in different environments (Orleans and Sparacia). The pots were arranged as previously described for the preceding trial, whereas plants grown directly in the field were managed using supporting structures made up of iron poles 2.6 m high, planted into the ground to set up two different rows. Distances of 3 m between rows and 1.5 m between plants in the row were adopted to allow mechanical weeding. The experimental design involved 30 hop plants ('Cascade'); two third of these (20 plants) were propagated by a specialized nursery in northern Italy ("*MrHops*"), and the others were micro-propagated in D/SAAF laboratories. The cultivation started on 21st June 2019. Pre-transplant fertilization was carried out, burying about 5 kg of mature bovine manure at a depth of 30-40 cm for each plant pocket.

For both trials, a localized irrigation system was set up with a single drip wing along each row. The system was equipped with a shut-off valve and a pressure gauge to control the operating pressure and water flow, allowing the irrigation volumes to be provided according to the needs of the crop. Frequent shifts and reduced dispensing intensity were adopted to ensure the amount of available water close to the water field capacity.

Field measurements

During both tests, hop plants were subjected to periodic surveys to check their phytosanitary and developmental conditions, identifying the dates of achievement of the main phenological phases.

The assessment of several phenological phases was carried out using the BBCH scale (Rossbauer et al., 1995), by carrying out the surveys with a 3-day shift. The harvesting period was identified by evaluating the hop cones' visual-sensory parameters and their moisture content (Calderwood and Post, 2015). Eventually, in all plants included in each experiment, the total biomass obtained for each plant was harvested, weighed, and sorted by hop cones, annual stems, and leaves. Samples of each plant fraction were taken, weighted to obtain the mass of fresh matter (g f.m.) and subsequently dried in stove at 104°C for 24 h, to determine the dry matter (g d.m.) content. All botanical fractions, except roots, were reported into dry matter values.

Sensory evaluation

Fresh cones were used for the production of beer, in two test batches for 'Cascade' and 'Chinook', respectively. Only one basic malt (Pils malt) was used, with mono-step mashing at a temperature of 70°C, a boil of 60 min, and a range of fresh cones (100 g L^{-1}) in the next whirlpool phase. The fermentation was carried out by pitching a neutral strain of *S. cerevisiae* at 17°C for 7 days, whereas the maturation phase lasted two weeks at 20°C. Both test batches were refermented in the bottle with the addition of fresh yeast and sucrose (4.2 g L^{-1}) until reaching 1.90 volumes of CO_2. Once the target carbonation volume was reached, two test panels were set up, adopting a discontinuous scale, evaluating the intensity of each attribute with categorical scores ranging from 1 to 9 (Sortino et al., 2017).

Statistical analysis

All collected data have been subjected to variance analysis (ANOVA), using the statistical software Minitab (version 19.2.0.0). For both experimentations, the GLM (General Linear Model) procedure was used. At the Orleans experimental farm, we set as dependent variables the measured durations (in days) of plants' elongation stages on the trellis, the corresponding GDD values (in °C), and all the measured plant traits in g of d.m. (cones yield, and total epigeal biomass, hypogeal biomass). The factors "year" (2018 and 2019), "cultivar" ('Cascade', 'Chinook', and 'Nugget'), and "mulching treatment" (organic, synthetic, and no mulch) were set as independent variables. Whereas, for the trials carried out in Sparacia, the factors "locality" (Sparacia and Orleans) and "origin of plant material" (purchased – "Extra" – and managed by the UNIPA laboratories – "SAAF") were set as independent variables. When the ANOVA offered statistically significant results, the differences between mean values were appreciated through Tukey's test (P≤0.05) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Biometric data and hop growth rates

According to the year and cropping management, the results showed a high variability of yields and biometrical characteristics of the tested hop varieties (Table 1).

Table 1. Results of ANOVA (mean values) for yield of epigeal biomass (g d.m.), cones yield (g d.m.), root system biomass (g f.m.) and GDDs (°C dds) of three hop varieties in a two-year trial at "Orleans" farm (Palermo, Italy).

	Epigeal biomass (g d.m.)	Cones yield (g d.m.)	Root system biomass (g f.m.)	GDD (°C dds)
Mean effect of year				
2018	181.45 a	33.18 a	890.00 b	1797.05 a
2019	110.69 b	25.90 b	1023.50 a	1263.90 b
Significance of p-value	***	*	*	***
Mean effect of variety				
Chinook	183.18 a	39.64 a	1221.92 a	1269.19 b
Cascade	134.14 b	30.07 a	899.42 b	1630.06 ab
Nugget	120.92 b	18.91 b	748.92 c	1692.18 a
Significance of p-value	***	***	***	*
Mean effect of the mulching treatment				
No mulch	150.23	26.55	1060.67 a	1612.97
Plastic mulch	148.20	34.04	901.21 b	1352.53
Organic mulch	139.79	28.04	908.38 b	1625.93
Significance of p-value	n.s.	n.s.	*	n.s.

*, ***: significant differences at P<0.05, P<0.001, respectively; n.s.: not significant. Means that do not share a letter are significantly different according the Tukey method and 95% confidence.

The "year" factor exerted a strong and significant influence on all the parameters observed, with decreases in cones yield of about 21% in 2019 compared to 2018; similarly, the epigeal biomass appeared significantly influenced by the "year" factor (with decreases of about 39% in 2019 compared to 2018).

This aspect would seem to suggest that climatic patterns played a key role in influencing crop responses in the first years of planting. The "cultivar" factor allowed to appreciate the better adaptability of 'Chinook', which was found to have highest yield in epigeal and root system biomasses, showing also an earlier development, considering the lower requirement in GDD (°C) for the attainment of the maximum growth along the trellis (p≤0.05). The "mulching treatment" did not determine a significant influence in cones and epigeal biomass, whereas it significantly influenced the accumulation trends of the root system biomass over the two year-trial (p≤0.05). It was also observed in both trial years that the greater tendency to the accumulation of root biomass had a direct influence on the productive performances. This assertion is confirmed by the regression analysis carried out, revealing the positive correlation between cones yield and hypogeal reserve organs of hop plants (Figure 1).

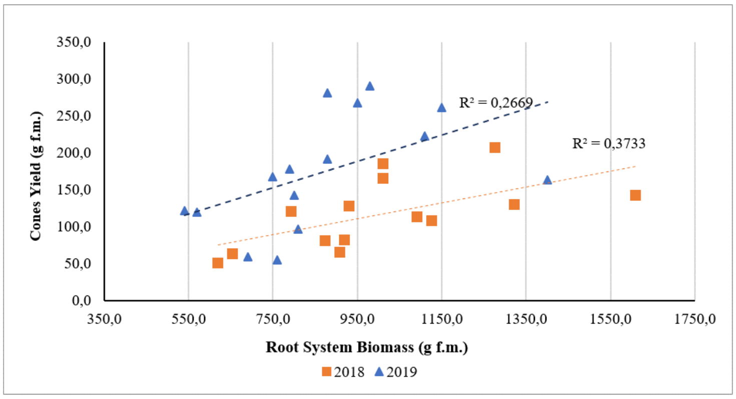

Figure 1. Regression lines estimated between cones yield (g f.m.) and root system biomass (g f.m.).

It emerges therefore the absolute relevance of all those cultivation techniques that can promote the development of the root system, considering that hop is a perennial crop and also given the close linkage between reserve organs and the productive performances during the economic viability of the crop.

The trial carried out in 2019 in two contrasting cropping conditions has also allowed to highlight the better performance of hop in open field, and the good vocational nature of the Sicilian countryside (i.e., the Sparacia farm, located in the central Sicily countryside) (Table 2).

Table 2. Results of ANOVA (mean values) for yield of epigeal biomass (g d.m.) and cones yield (g d.m.) obtained in a cultivation trial of 'Cascade' hop in 2019, grown in two different sicilian locality (i.e. Orleans: plants cultivated in pots at "Orleans" farm (Palermo, Italy); Sparacia: plants cultivated in open field at "Sparacia" farm (Cammarata, Italy). Plant material was also marked according to its origin (i.e. "Extra": purchased; "SAAF": managed by the UNIPA laboratories).

	Epigeal biomass (g d.m.)	Cones yield (g d.m.)
Mean effect of locality		
Sparacia	210.27 a	8.94
Orleans	67.88 b	8.19
Significance of p-value	***	n.s.
Mean effect of plant's origin		
Extra	194.08 a	13.45 a
SAAF	84.06 b	3.68 b
Significance of p-value	***	***

***: significant differences at P<0.001; n.s.: not significant. Means that do not share a letter are significantly different according the Tukey method and 95% confidence.

Although no significant differences in cones yield were found, the average epigeal biomass produced was significantly higher at the "Sparacia" farm. The choice of the propagation material has also proved crucial, effectively influencing the performance of the different treatments, and potentially making the hops plantations in a new cultivation area such as Sicily sustainable or not. However, the availability of good quality commercial material on a local scale is improving, and this could be a good opportunity for farmers to cope with this challenging new crop.

Sensory evaluation

Analogies for the main "Tropical fruit", "Grassy" and "Floral" flavour descriptors were detected in the two single-hop Pale Ale with Sicilian 'Cascade' and 'Chinook' so compared (Figure 2a). However, a greater impact on the aromatic bouquet of single-hop Chinook ale than 'Cascade' one was reported by panelists. The analysis of beer taste also confirmed the similarities for the previously emerged flavor descriptors (Figure 2b), highlighting a lesser marked resinous note in the single-hop batch with 'Chinook' hops, characteristic perfectly in line with the classical descriptors for 'Chinook', paneled in a previous study (Schott, 2018).

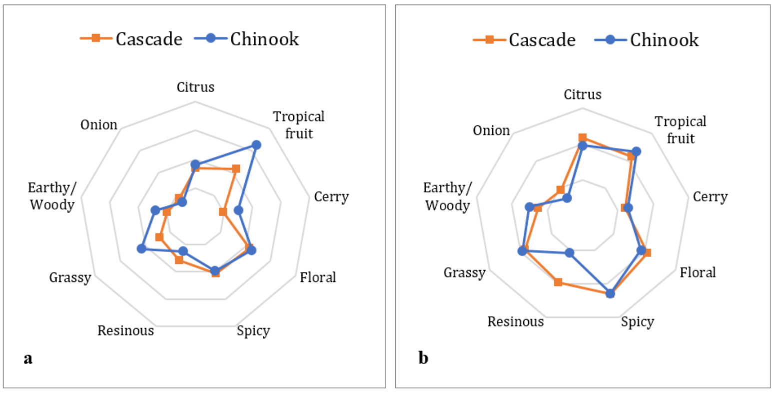

Figure 2. Spider plot of aroma evaluation (a) and taste evaluation (b) of two Pale Ale single hop batch beers, crafted using the locally-grown hop 'Cascade' and 'Chinook'.

Thus, the sensory analyses assessed the excellent organoleptic characteristics of Sicilian hops, as well as of the beers produced. Hop demonstrates to be a promising crop for semi-arid Mediterranean environments although a proper choice of the variety demonstrated crucial.

CONCLUSIONS

According to our findings, it emerged the absolute relevance of all those cultivation techniques that can promote the development of the root system, considering that hop is a perennial crop and also given the close linkage between reserve organs and the productive performances during the economic viability of the crop. The propagation material has also proved crucial, effectively influencing the performance of the different treatments. The sensory analyses assessed the excellent organoleptic characteristics of Sicilian hops. Hop demonstrates to be a promising crop for semi-arid Mediterranean environments although a proper choice of the variety demonstrated crucial.

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1.2.Resilience of hop (*Humulus lupulus* L.) to salinity, heat and drought stresses: A mini-review

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Resilience of hop (*Humulus lupulus* L.) to salinity, heat and drought stresses: A mini-review

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Over recent years, the cultivation of hops (*Humulus lupulus* L.) has spread widely in the Mediterranean, also affecting the southern regions of Spain and Italy with a typical semi-arid climate. Several and recent studies have investigated the responses of this species to the main abiotic stresses, which is an aspect of absolute relevance to the knowledge of the adaptive capacity of hops to the growing conditions of a new cultivation environment. Moreover, given the fact that hops' phytochemical composition is determined primarily by genetic and environmental factors, and that the species is perennial, the lack of knowledge on the effects of abiotic stress could be reflected in subsequent years, which means multi-year economic risks. This review work therefore aims to showcase, based on an in-depth investigation of the available literature, the response of hop to the main abiotic stresses, and the effect of these on productive and qualitative crop performances. The data presented will be useful to the understanding of constraints and to the identification of useful coping strategies to the cultivation of hops in semi-arid Mediterranean environments.

KEYWORDS
abiotic stress, multiple stresses, crop performances, plant physiology, proteomics

Introduction

According to the prediction on climate models for the southern Mediterranean regions, a decrease in winter precipitation and an increase in the number of heat waves compared to previous decades will take place in next years (Beniston et al., 2007). Indeed, Earth surface temperature is expected to rise gradually on a global scale, and major changes are likely to occur in the hydrological and energy cycles (IPCC, 2001). Therefore, in the next decades, mankind will probably experience dramatic and threatening changes in regional extreme weather and climate events. Being "fixed" to their growth substrate, plants are supposed to be particularly exposed to changes in the frequency and intensity of extreme events (i.e., heat waves, heavy precipitation, droughts, *etc.*) (Christensen and Christensen, 2003; Goyette et al., 2003; Beniston, 2004; Schär et al., 2004; Ulbrich et al., 2001). An additional constraint is linked to the frequent simultaneous occurrence of two, or three, abiotic (and biotic) stressors, whose combination is mostly lethal to crops (Mittler, 2006), as much as that this "combined stress" is considered as a new and special complex stressor (Bai et al., 2018).

However, almost all vascular plants have developed in time several morphological and physiological adaptations to enable their survival under harsh conditions. Open field crops are almost always subjected to stressors coming from the surrounding environment, and that is the reason why it's very hard that field crops on farms achieve their full yield potential, and huge yield gaps are highlighted, e.g., when field crops are compared to crops grown for experimental purposes (Lobell et al., 2009; Van Ittersum et al., 2013).

On the other side, the need to assess the climatic effects on quality is particularly relevant for some crops in which technological quality derives mainly from the aromatic-sensory and gustatory components (Ahmed et al., 2014).

In hop (*Humulus lupulus* L.) several breeding programs have focused on improving agronomic performances, introducing characteristics such as resistance to biotic and abiotic stressors, reduced growth capacity ("dwarf" varieties), together with the improvement of yields and organoleptic characteristics of production (Hampton et al., 2001). To date, although numerous studies have already confirmed the excellent productive and qualitative response of hops grown in the Mediterranean environments (Mongelli et al., 2015; Rossini et al., 2016; Ruggeri et al., 2018; Marceddu et al., 2020; Rossini et al., 2021; Figures 1, 2), there's still the lack of knowledge on the effects of abiotic stresses on hop yield and quality. Since hop is a perennial crop, abiotic stresses might reflect in cone yields in subsequent growing seasons, representing a multi-year economic risk for farmers who decide to invest in this crop in a new environment such as the southern regions of the Mediterranean.

Hops are mainly cultivated for the beer industry as a source of secondary metabolites (i.e., α-acids, β-acids and aromatic oils), which give the characteristic bitterness and aroma, as well as antimicrobial properties to the brewing products (Neve, 1991). These substances are contained in the lupulin glands that develop mainly in the female inflorescences, and their

FIGURE 1
Pictures taken during the first year of hop cultivation in a Sicilian semi-arid environment at the "Sparacia" farm (37°38'07" N; 13°45'47" E; 450 m a.s.l.), Department of Agricultural, Food and Forest Sciences (D/SAAF), University of Palermo.

FIGURE 2
Hop bines before 2022 harvest at the "Sparacia" farm (37°38'07" N; 13°45'47" E; 450 m a.s.l.), Department of Agricultural, Food and Forest Sciences (D/SAAF), University of Palermo.

amount increases as reproductive plant stages go on (i.e., from the appearance of cones to their full growth) (de Keukeleire et al., 2007).

Several studies have been carried out on the factors influencing the biosynthesis of these compounds. The biosynthesis of α-acids, β-acids and prenylated flavonoids involves extremely complex biosynthetic pathways (Goese et al., 1999; Paniego et al., 1999; Okada and Ito, 2001), that can be affected in many steps by several external factors, such as area of cultivation, phytosanitary status and age of crops (Jelínek et al., 2012; Matoušek et al., 2016; Morcol et al., 2020; Eriksen et al., 2021). It appears that, as with other plant secondary metabolites, both the genotype and the environment play a key role in determining their final concentration (Almaguer et al., 2014; Moore et al., 2014; Morcol et al., 2020).

This review work, therefore, aims to present the response of hop to three common abiotic stresses (salinity, heat, and drought), and the effects of these on crop productive and qualitative performances.

Salinity stress

Among all the abiotic stressors, salinity is one of the major threats to crops' productivity in semi-arid environments, due to its increased occurrence in farms with irrigated crops (Flowers, 2004). Salt stress persistence throughout plants' growth cycles might lead to co-occurrence with other stressors, either abiotic or biotic, way more than drought or heat stress, which often occurs at various intermittent periods either preceding or following pathogen infection (Kissoudis et al., 2014). In general, all plants respond to saline stress with morphological and physiological modifications, responsible for the adaptation of the osmotic potential, thanks to the involvement of different genes and pathways (Julkowska and Testerink, 2015). Also, plant hormones appeared to have a key role in the response to salinity stress as reported by several researchers in different plant species (Golldack et al., 2014; Ryu and Cho, 2015; Tao et al., 2015).

However, limited research has been devoted to studying the basis of salinity tolerance in hops. Unquestionably, this species might adapt to multiple hostile conditions, determined by the presence of abiotic stressors such as salinity, activating all those mechanisms already well defined for most vascular plants, i.e., the alteration of cytoplasmic free Ca^{2+}, the activation of Ca^{2+}/calmodulin-dependent kinase, the production of secondary signaling molecules such as reactive oxygen species (ROS) and abscisic acid (ABA), and the activation of the salt overly sensitive (SOS) pathway for regulation and maintenance of ion homeostasis (Julkowska and Testerink, 2015).

In a field study with hops cultivars over a range of different cultivation sites, Dabbous-Wach et al. (2021) assessed the good acclimatization of German hop cultivars to the Corsican environment one year after planting, even though high levels of soil salinity were detected in most of the growing sites. A noticeable change in plant morphology was detected after the first year of cultivation: some plants showed a shortening of side shoots, and, considering the lower number of cones in the side shoots per plant, also reduced cone yields. From the phytochemical point of view, the essential oil of these genotypes showed a higher amount of myrcene (characterized by spicy and balsamic herbal notes) and α–humulene (woody notes), and lower α–selinene (herbal notes). Hence, a definite compositional and aroma difference was assessed between hops grown in the coastal areas and others from inner Corsica; although salinity could not be claimed as the only cause of these variations, it was certainly involved in the combined co-presence of the high salinity of soils, water scarcity, and wind, acting as a multiple stressor on cultivated plants. Interestingly, it appeared that not all the varieties reacted in the same way, as they have not been directly improved for salinity resistance, showing also very important information for growers to make a proper choice of hop cultivars that suit better to the local growing conditions, and that would be consistent with the outcomes of a recent research carried out in a semi-arid Mediterranean environment (Carrubba et al., 2022).

Drought and heat stress

Numerous molecular and metabolic-related studies found that plants' responses to the combination of heat and drought stress are unique and should be evaluated jointly rather than individually (Pnueli et al., 2002; Rizhsky et al., 2002; Rizhsky et al., 2004; Suzuki et al., 2005; Potopová et al., 2021). That is the reason for these stressors to be presented jointly in this paragraph.

In a Mediterranean environment, characterized by high temperatures throughout all hop growth season, an inverse correlation was shown between plant growth and productivity and heat accumulation, especially in the vegetative development stages (Marceddu et al., 2020). High temperatures (HT) and low-water (LW) stress during the growing season have consistently been shown to decrease hop cone yield and bitter acid content of cones (Srečec et al., 2004; Mozny et al., 2009; Nakawuka et al., 2017; Donner et al., 2020).

A recent study carried out by Donner et al. (2020) found a significant negative correlation between summer air temperature and α–acids content in several Czech cultivars (cv. "Saaz", "Sladek" and "Premiant"). Mozny et al. (2009) also highlighted a decrease in cone yield in growing seasons with low precipitation and a decreased α-acids content in cv. "Saaz" hops during high-temperature years. Similar reductions in cones and α-acids yields under combined LW and HT conditions were also found in the cv. "Aurora" in Croatia (Srečec et al., 2004) and Slovenia (MacKinnon et al., 2020). In a study carried out by Nakawuka et al. (2017) in the Washington State (U.S.), a significant decrease in cone yields under reduced irrigation was assessed, even though no significant effect was assessed on bitter acid content in several American varieties (e.g., "Mt Hood", "Columbus", "Chinook", and "Willamette").

Nevertheless, limited research has been carried out on hop mechanisms and structural traits resulting from drought stress (Gloser et al., 2013; Korovetska et al., 2014; Korovetska et al., 2016).

On a general basis, it is well known that the main effects of drought stress on plant growth and development are determined by water relation disorders as well as modification of water use efficiency, with a major impact on the relative water content in green tissues, leaf water potential, osmotic potential, pressure potential and transpiration rate (Farooq et al., 2009; Farooq et al., 2012). Changes in the pH, ABA, and sulphate concentration in xylem sap were suggested as long-distance drought signals also for hop plants (Gloser et al., 2013; Korovetska et al., 2014; Korovetska et al., 2016), even though there is still no certainty about the role of these metabolites in the drought response of the species. In a trial carried out by Kolenc et al. (2016), the drought stress response of two Slovenian hop cultivars grown in pots was assessed by combining physiological studies and proteomic analysis. According to these findings, hop plants showed decreased transpiration rate and water potential during reduced water availability, experiencing a decrease in photosynthesis due to stomatal and non-stomatal limitation and a strong decrease in photosynthetic proteins and proteins of the energetic metabolism, affecting plant fitness in general. Also, a very interesting study was carried out by Eriksen et al. (2021) who looked at physiological traits and differential gene expression in leaf, stem, and root tissue in plants of the cv "Cascade" exposed to HT stress, LW stress, and a combination of both. In the above experiment, the cultivation trial took place in growth chambers where the imposed stress conditions were able to impress substantial changes to the transcriptome. Significant reductions in the expression of numerous genes were detected, which resulted in a decrease in agronomically important secondary metabolite biosynthesis, e.g., bitter acids. However, as reported by the same Authors, other studies found no reductions in α–acids content under LW stress (Nakawuka et al., 2017) or cultivar-specific reactions to LW and HT stresses (Donner et al., 2020), suggesting possible cultivar-based differences in the temperature tolerance range, that could be exploited to develop breeding lines with increased resilience to abiotic stress.

Heat stress-wise, it is known that plants exposed to high temperatures might manifest various symptoms, deriving from the drastic limitation of their photosynthetic activity (Berry and Bjorkman, 1980). Allakhverdiev and coworkers (2008) identified three components of the photosynthetic system that are sensitive to heat damage, namely the photosystems themselves, the ATP-generating electron transport chain, and the carbon assimilation process. According to other research also, heat resulted to affect photosystem II by causing the dissociation of Manganese (Mn) from the oxygen-evolving complex, but also by disrupting the distribution of absorbed light energy from the light-harvesting complex (Enami et al., 1994; Enami et al., 1998; Nash et al., 1985; Pastenes and Horton, 1996).

The disruption of membrane fluidity caused by heat, also, determines the breakdown of the thylakoid membrane integrity, which leads to disruptions in the electron transport chain and ATP synthesis (Gounaris et al., 1983; Inaba and Crandall, 1988). Moreover, HT stress was found responsible for the destruction of the Rubisco activase protein, leading to the inactivation of the carboxylating enzyme and, therefore, to the disruption of carbon assimilation (Salvucci et al., 2001; Salvucci and Crafts-Brandner, 2004; Sharkey, 2005). In six hop cultivars exposed to HT (within a range of temperatures from 15 to 45°C), Eriksen et al. (2020) highlighted that all the tested plants achieved maximal carbon assimilation at temperatures ranging from 21 to 39°C without the availability of water being a limiting factor. When the temperatures reached and overpassed 41°C, all plants experienced severe stress, showing decline in carbon assimilation, due to multiple effects on the cell, including damage to photosystem II (PSII), damage to membrane integrity as reflected in electrolyte leakage at high temperatures, and declines in Rubisco activity probably due to deactivation of Rubisco-activase enzyme. According to these findings, "Cascade" and "Southern Brewer" appeared to be better candidates for use as breeding lines to improve abiotic stress tolerance than "Chinook", which appeared to be particularly susceptible to extreme heat stress.

Conclusions

Biosynthesis pathways of secondary metabolites in hops, and therefore concentration levels in cones, proved very sensitive to stress conditions such as salinity, LW and HT. Such sensitivity is variable according to many factors, including genotype, intensity and duration of stress, the simultaneous occurrence of two or more stressors, but also phenology of the plant. For example, the stage of cone development is one of the most crucial for flavor compounds biosynthesis in hops, therefore representing a key moment for the success of hop cultivation. Even though HT stress is difficult to avoid in the field, LW stress should be minimized in irrigated systems during this period. In this sense, agrotechnical care is an essential tool to obtain satisfactory production levels, above all when those to be managed are new crops in new areas of cultivation. The findings from the research above might represent valuable information for growers developing hopyards in the increasingly warm regions of the Mediterranean. However, it also emerged that some hop genotypes are better adapted than others to environmental constraints, and are, therefore, more suitable to highly demanding cultivation areas. The proper choice of the right variety given the environmental context is a key point to successfully manage this challenging crop.

Author contributions

AC conceived the project. RM and AC wrote the manuscript. RM, AC, and MS revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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1.3.Conclusions

Our findings highlight the absolute importance of cultivation techniques that promote root system development in hop plants. Since hops are perennial and a close relationship exists between reserve organs and productive performance throughout the crop's economic lifespan, root health is critical. Additionally, the choice of propagation material plays a significant role in influencing the outcomes of various treatments.

Sicilian hops exhibited excellent organoleptic characteristics, proving that this crop holds promise for semi-arid Mediterranean environments. However, the proper selection of hop varieties is essential, as different genotypes respond variably to environmental conditions. This sensitivity extends to the biosynthesis of secondary metabolites, crucial for cone quality, which is highly influenced by stress conditions such as salinity, limited water (LW), and high temperatures (HT). The degree of sensitivity varies depending on factors such as genotype, stress intensity, stress duration, and plant phenology. Specifically, the cone development stage is critical for the biosynthesis of flavor compounds, making it a pivotal period for ensuring the success of hop cultivation.

While heat stress may be unavoidable in the field, minimizing drought stress in irrigated systems during cone development is essential. In this regard, proper agrotechnical management becomes indispensable, particularly when introducing hops as a new crop in previously unexplored regions. This research provided valuable insights for growers aiming to establish hopyards in increasingly warm Mediterranean areas. It also revealed that some hop genotypes are better adapted to environmental stressors, making them more suitable for cultivation in challenging conditions. Therefore, selecting the appropriate variety for the specific environmental context is crucial for the successful management of hops in demanding regions.

In conclusion, optimal root system development, careful variety selection, and tailored stress management strategies are key factors that will determine hop productivity and quality in non-traditional growing areas like the Mediterranean.

2. CHAPTER II

This chapter investigates the physiological, biometric, and qualitative responses of hops to various agronomic treatments in controlled environments, as detailed in two studies. The objective is to refine cultivation practices and assess their effects on hop productivity and quality, considering different sources of variation.

The first study, published in HortScience, 57(11), 1409-1415; https://doi.org/10.21273/HORTSCI16754-22) evaluates the suitability of low-trellis systems for hop cultivation in semi-arid Mediterranean regions, focusing on three traditional hop genotypes (i.e., Cascade, Chinook, and Nugget). Conducted in 2018 and 2019, the research assessed the impact of low-trellis heights and mulching treatments (organic and synthetic) on plant development, cone yield, root biomass, and total epigeal biomass. Results indicated that rapid early growth correlated with lower cone and above-ground biomass yields but increased root biomass. Synthetic mulching notably enhanced root development, particularly for the Cascade variety, revealing variability in the suitability of low-trellis systems among different hop varieties in Mediterranean climates.

The second study has been scheduled for submission at the XIII International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems, that will be held in Napier (New Zealand), on January 19-24, 2025. The study explores the effects of localized irrigation systems—surface drip irrigation (SDI) and sub-surface drip irrigation (SSDI)—on potted Cascade hops. The experiment, conducted in 2023, assessed evapotranspiration, photosynthetic efficiency, and gas exchange rates under different irrigation regimes. The findings show that SSDI significantly improved cone quality, biomass yields, and physiological responses compared to SDI, providing valuable insights into optimizing irrigation practices and enhancing hop production and quality standards in semi-arid environments.

Together, these studies offer a comprehensive understanding of how agronomic practices, environmental conditions, and genetic differences influence hop cultivation. They underscore the importance of tailored cultivation strategies to optimize hop production and quality, particularly in non-traditional and challenging climates.

2.1. Hop (*Humulus lupulus* L.): Suitability of Traditional Cultivars to a Low-Trellis Farming System in a Semiarid Environment

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Hop (*Humulus lupulus* L.): Suitability of Traditional Cultivars to a Low-Trellis Farming System in a Semiarid Environment

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Additional index words. crop production, hop growth rate, hop productivity, low-trellis system, mulching treatment, thermal sums

***Abstract.* *Humulus lupulus* is a dioecious twining herb, with an outstanding vertical development capacity. Hop plants are usually grown on trellises up to 4.5 to 6.0 m high, whose management requires intense use of water, fertilizers, pesticides, and labor. In semiarid Mediterranean areas, where native resources are often scarce, the adoption of low-trellis farming systems could be a sustainable option for hop cultivation. With the aim of evaluating hop suitability to low-trellis cultivation in a Mediterranean environment, in 2018 and 2019 three traditional hop genotypes ('Cascade', 'Chinook', and 'Nugget') were grown, and their development rate was evaluated and put in relation with the plants' cone, root, and biomass yield. Moreover, organic (fragmented pine bark) and synthetic [black polyethylene (PE) plastic film] mulches were applied on the same cultivars, and both epigeal and hypogeal development were evaluated. The results showed that the faster the growth in the first two phases of plant elongation (up to 50% of the height of the upper wire), the lower the yield in both cones and total epigeal biomass. A fast growth rate was instead associated with a higher hypogeal biomass production. Mulching was able to significantly affect the hypogeal biomass, specifically for cv. Cascade, where the use of synthetic mulching allowed significant root biomass increases. The analysis of the results obtained showed that hop's suitability to a low-trellis farming system is highly variable among varieties in the semiarid Mediterranean environment.**

In northern Italy, hops grow as spontaneous species (Mongelli et al., 2015), and those environments are thought to be the most suitable for hop cultivation. However, several preliminary studies have successfully explored the possibility of introducing this crop to semiarid Mediterranean environments (Forteschi et al., 2019; Marceddu et al., 2020; Rossini et al., 2016, 2021). Several genotypes have been selected that are best adapted to the local growth conditions (Rossini et al., 2016). Recent studies have also been carried out to assess the response of hops in terms of crop productivity and cone quality. A survey conducted by Rossini et al. (2016) has shown that some varieties obtained higher yields, justifying the spread of varietal groups that, although heterogeneous, guarantee sustainability of hop cultivation.

From a qualitative point of view, both panel tests (Marceddu et al., 2020; Rossini et al., 2016) and laboratory analyses (Forteschi et al., 2019) demonstrated the excellent potential of hop production obtained in the Mediterranean environment.

However, high investment costs, and time-consuming and resource-consuming crop management, are major constraints to a wider expansion of hops in Mediterranean areas. Traditional hop cultivations usually adopt climbing supports up to 4.5 to 6.0 m high, where plants are allowed to grow up until harvest, forming a dense vegetation wall. In these conditions, demand for water and fertilizer, pest and disease control, and harvest management are outstanding. Hence, many experiments have been conducted to explore the feasibility of growing hops on reduced 2- to 3-m climbing structures ("low trellis"), with many advantages related to easier crop management, lower initial expenses, and enhanced sustainability. Several studies have shown that low-trellis hop farming systems offer benefits related to environmental protection, because phytosanitary treatments can be carried out more efficiently and with less dispersion because of reduced vertical development (Beatson et al., 2009; Darby, 2005). The cultivation of hops on lower trellises also allows a more effective biological control of major pests and diseases (Darby, 2004; Gunn and Darby, 1987; Lilley et al., 1999). A few dwarf varieties have been selected to fit these reduced production systems, but significant research is also addressed to evaluate the performances of traditional hop varieties under those constrained conditions.

With the aim to evaluate the yield and growth rate of hops in a low-trellis system in a Mediterranean environment, three American hop varieties (Chinook, Nugget, and Cascade) were compared in 2018 and 2019, with or without the application of two mulches (organic and synthetic). The progression of plants' climbing on the vertical supports was monitored by reference to the phenological development scale pointed out for hop (Rossbauer et al., 1995). Furthermore, hypogeal and epigeal biomass were measured and compared in all treatments.

Materials and Methods

Experimental site. The experiment was carried out in 2018 and 2019 at the University of Palermo, Department of Agricultural, Food and Forest Sciences (D/SAAF) "Orleans" farm (Palermo, Italy, 38°060 2700 N; 13°210 0100 E; 22 m a.s.l.). In both years, climatic patterns (Fig. 1) during the cultivation periods were characterized by scarce rainfall and high summer temperatures (maximum values >30 °C).

Plant management. For research purposes, a single hop row was built, with 2.5-m-high iron poles supporting structures; horizontal iron wires run at the top of the poles, and coconut fiber ropes provided vertical supports, onto which the plants were allowed to climb. All plants were purchased from a nursery in northern Italy. Five plants for each variety (Cascade, Chinook, and Nugget) at the development stage of four to six true leaf development stage (≈10 cm high), were used for the trial. In both years, each plant was placed in a 40-L pot, filled with a brown peat and perlite (3:2) mixed substrate, with a deep draining layer composed of expanded clay and gravel (1:1). In each variety, a mulch-free control, a synthetic mulch treatment (black PE film), and an organic mulch treatment (pine bark) were compared. Synthetic mulching used a 1-mm-thick black PE plastic film; organic mulching was covered with a 5-cm layer of fragmented pine bark (diameter 10–20 mm).

The experiments started on 9 Apr 2018, and 6 May 2019, in the first and in the second year, respectively. In both years, a supporting mineral fertilization was applied to plants (50 g per plant) using a ternary fertilizer (15N–7.3P–21.3K), also containing microelements (Mg, Fe, and B, at amounts of 2%, 0.1%, and 0.01%, respectively). A localized irrigation system was set up with a single dripline along the row. The irrigation system was equipped with a closing valve and a pressure gauge to control the water pressure and flow rate needed for the crop requirements. Frequent irrigation cycles at low volumes were adopted to ensure available water amounts close to field capacity.

The harvest period was assessed through the evaluation of the cones' dry matter content. Cones were harvested once they reached a 20% dry matter value (Calderwood and Post, 2015). At harvest time, the total biomass obtained for each plant was weighed, and sorted by marketable (hop cones) and unmarketable biomass (annual stems and leaves). In addition,

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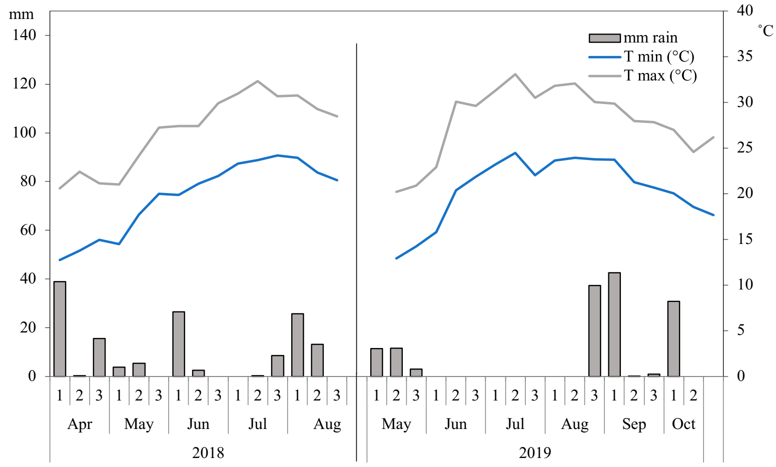

Fig. 1. Ten-day values of rainfall and temperatures recorded at the "Orleans" farm during the 2018 and 2019 growing seasons (Palermo, PA, Italy).

in both years of experimentation (2018 and 2019), during the dormancy of rhizomes, the total root biomass was weighed after both growing seasons.

Calculation of hop growth rate and growing degree days. In each year, hop plants were periodically checked to monitor their phytosanitary and developmental conditions. The main phenological phases were assessed using the BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) scale (Rossbauer et al., 1995). Vertical development of the crop was monitored at stage 3 ("Elongation of bines") of the BBCH scale twice per week and expressed as a percentage of top wire height (2.50 m).

Growing degree days (GDDs; °C) were calculated starting on the day of transplant, corresponding to the BBCH phase 1.2 (second pair of leaves unfolded). The GDDs accumulated when plants had reached the threshold height values of 62.5 cm, 125.0 cm, 187.5 cm, and 250.0 cm (corresponding to 25%, 50%, 75%, and 100% of wire length) were recorded. All calculations were performed according to the general formula:

$$\mathrm{GDD} = \sum_{i=1}^{k} (\mathrm{T_{avg}} - \mathrm{T_{base}})_i,$$

where *i* and *k* indicate the starting and ending date of each growth stage (i.e., first and last days of measurement, respectively), T_{avg} is daily average temperature, and T_{base} is base temperature (i.e., temperature value below which plant growth is assumed to be zero).

T_{avg} was calculated based on daily temperature measurements, as follows:

$$\mathrm{T_{avg}} = \frac{(\mathrm{T_{max}} + \mathrm{T_{min}})}{2},$$

where T_{min} is the minimum day temperature; when $T_{min} < T_{base}$, then T_{base} should be used for calculation. This, however, never happened in either trial season, occurring in late spring and summer. T_{max} is the maximum day temperature.

In the choice of the base temperature (T_{base}), reference was made to the evidence shown in a recent work. To accurately calculate the GDDs for hops in a Mediterranean environment, Marceddu et al. (2020) discussed the idea of differentiating the T_{base} based on the phenological crop phases; a $T_{base} = 0\,°C$ was found more suitable in the vegetative phases, whereas, after the phase BBCH 2.1, the adoption of base temperatures of 5 °C (as proposed by Srečec et al., 2008) or 10 °C were substantially the same.

In the two trial years, all hop plants reached the maximum height during the vegetative phases (between BBCH 1.2 and 2.1). Thus, following the results obtained in a previous work (Marceddu et al., 2020), it was opted for a calculation of GDDs through the adoption of 0 °C as base temperature (T_{base}).

Hop Growth Rates (HGRs) were calculated according to the following formula:

$$\mathrm{HGR} = \mathrm{H_t} / \sum_{i=1}^{k} \mathrm{GDD},$$

where H_t is the height value reached on the wire by the plants in cultivation, and the $\sum_{i=1}^{k} GDD$ represents the corresponding thermal sum necessary to reach the same height, with i and k representing the starting and ending date of each vertical growth interval. Similar to GDDs, HGRs were calculated for the whole duration of the plants' elongation on ropes (HGR_{tot}), as well as for the four stages (0% to 25%, 25% to 50%, 50% to 75%, and 75% to 100%) to obtain the partial values termed $HGR_{0–25}$, $HGR_{25–50}$, $HGR_{50–75}$, and $HGR_{75–100}$, respectively.

Statistical analysis. All data collected were submitted to analysis of variance (ANOVA) using the statistical software Minitab (version 19.2.0.0). The general linear model procedure was used, setting as the dependent variable the measured durations (in days) of plants' elongation stages on the trellis, the corresponding GDD values (in °C), the calculated values of HGRs (cm/GDD), and all the measured plant traits in grams of DM (g DM) (hypogeal biomass, cone yield, and total epigeal biomass). The factors "year" (Y) (2018 and 2019), "variety" (V) (Cascade, Chinook, and Nugget), and "mulching treatment" (M) (organic, synthetic, and no mulch) were set as independent variables. When the ANOVA offered statistically significant results, the differences between mean values were appreciated through the Tukey's test ($P \leq 0.05$); no post hoc test was performed on the main effects, when the corresponding interactions proved to be significant ($P \leq 0.05$) at the ANOVA (Gomez and Gomez, 1984). To assess any significant association within the measured data, Pearson's correlation coefficients were calculated on pooled data between cone yields, plant biomass (hypogeal and epigeal), and HGR parameters.

Results

The duration of plants' elongation [days (dd) from transplant to achievement of maximum height, i.e., 2.50 m] was different between the two years, as plants reached, on average, the maximum height in ≈83 d in 2018 and 57 in 2019 (Table 1). Throughout the same time span, also GDDs accumulation (°C) was higher in 2018 (1753.4 GDD) than in 2019 (1263.3 GDD). Consequently, HGR was higher in 2019 than in 2018, because in the first trial year, plants gained ≈0.14 cm per each cumulated temperature degree, whereas plant growth was much faster (≈0.2 cm/GDD) in 2019. In the first year, more than 70% of the time requested for full elongation was occupied by the first half of climbing (up to 50% of top wire height), whereas in 2019 the same substage lasted

Table 1. Mean values of durations (days), thermal sums [growing degree days (GDDs)], and hop growth rates (HGRs) (cm/GDD) of plant elongation on a 2.50-m wire, in four intervals of hop climbing (up to 25%, 25% to 50%, 50% to 75%, 75% to 100% of top wire height, and total −0% to 100%) of three hop varieties (Cascade, Chinook, and Nugget) cultivated in 2 years (2018–19) under two mulching treatments (synthetic and organic) and without mulching (no mulch). Mean values of main effects (year, variety, and mulching treatment) and results of analysis of variance (*P* values) for main effects and first-order interactions. For each group of means, values followed by the same letter are not significantly different at $P \leq 0.05$ (Tukey's test). For interactions, significant *P* values ($P \leq 0.05$) are marked in bold.

	Percentage intervals of top wire ht														
	Total (0 to 100)			0–25			25–50			50–75			75–100		
Source	dd	GDD (°C)	HGR (cm/ GDD)	dd	GDD (°C)	HGR (cm/ GDD)	dd	GDD (°C)	HGR (cm/ GDD)	dd	GDD (°C)	HGR (cm/ GDD)	dd	GDD (°C)	HGR (cm/ GDD)
Year (Y)															
2018	83.3 a	1753.4 a	0.14 b	38.5	715.7	0.09 b	23.7 a	516.9 a	0.14 b	11.0	265.6	0.26	10.1	255.1	0.27 a
2019	57.3 b	1263.3 b	0.20 a	21.5	413.4	0.14 a	11.3 b	219.7 b	0.31 a	10.3	264.8	0.34	14.2	365.4	0.20 b
Significance of F test (P value)	*0.000*	*0.002*	*0.001*	*0.000*	*0.001*	*0.002*	*0.000*	*0.000*	*0.000*	*0.902*	*0.834*	*0.122*	*0.073*	*0.117*	*0.020*
Variety (V)															
Cascade	75.7 ab	1624.5	0.16	31.6	584.5	0.12	17.9	387.0	0.22	14.3	360.0	0.25 b	11.9	293.0	0.23
Chinook	61.1 b	1284.7	0.19	24.9	476.1	0.13	19.3	382.9	0.21	7.6	183.7	0.37 a	9.3	241.9	0.27
Nugget	76.0 a	1651.9	0.16	34.6	650.2	0.10	15.9	351.7	0.23	10.4	261.4	0.27 b	15.1	388.6	0.20
Significance of F test (P value)	*0.042*	*0.056*	*0.124*	*0.051*	*0.123*	*0.203*	*0.297*	*0.420*	*0.480*	*0.182*	*0.230*	*0.009*	*0.055*	*0.07*	*0.057*
Mulching treatment (M)															
No mulch	76.6	1649.0	0.16	29.8 ab	553.0 ab	0.11 ab	23.0	493.7	0.21	10.8	271.7	0.28	13.0	330.6	0.22
Synthetic	64.5	1352.5	0.19	25.3 b	469.3 b	0.14 a	16.2	322.2	0.23	10.2	241.6	0.28	12.8	319.4	0.23
Organic	74.6	1625.9	0.16	35.5 a	677.3 a	0.09 b	17.0	374.5	0.21	11.1	286.1	0.32	11.0	288.0	0.25
Significance of F test (P value)	*0.188*	*0.179*	*0.136*	*0.012*	*0.018*	*0.013*	*0.869*	*0.607*	*0.505*	*0.805*	*0.769*	*0.455*	*0.559*	*0.791*	*0.520*
Significance of *F* test (*P* value) for interactions															
Y × V	*0.397*	*0.354*	*0.585*	***0.001***	***0.002***	***0.001***	*0.515*	*0.347*	*0.196*	*0.221*	*0.293*	*0.167*	*0.317*	*0.548*	*0.911*
Y × M	*0.464*	*0.389*	*0.581*	*0.253*	*0.347*	*0.553*	***0.030***	***0.024***	*0.057*	*0.582*	*0.623*	*0.932*	*0.794*	*0.696*	*0.223*
V × M	*0.396*	*0.347*	*0.417*	*0.597*	*0.725*	*0.250*	*0.106*	*0.096*	*0.746*	*0.542*	*0.564*	*0.303*	*0.625*	*0.505*	*0.219*

≈50% of total duration. Significant differences show up, in fact, between the values of $dd_{0–25}$ and $GDD_{0–25}$, by one side, and $dd_{25–50}$ and $GDD_{25–50}$, by the other, measured in the two years (Table 1).

On average, irrespective of year and genotype, plant elongation on ropes was initially slow, and faster in the second half of climbing. The cv. Chinook had the fastest growth rate (Table 1), reaching the maximum height on the trellis in ≈61 d (i.e., 2 weeks before the other two varieties). The cv. Chinook had, in fact, the highest HGR values in all growth substages. This difference among varieties resulted in statistically significant $HGR_{50–75}$ (Table 1). In the earlier observations, however, a significant Y × V interaction showed up, demonstrating the different behavior of genotypes across years (Fig. 2A and B). Hence, in both years the cv. Chinook took approximately the same number of days to complete the first growth substage (0% to 25%), showing a high HGR value, whereas the other two varieties exhibited contrasting responses between years.

The mulching treatments (Table 1) did not show any significant effect on plant growth parameters calculated on the whole growth period, otherwise showing an influence on plants' growth rates in the first time span of plants' elongation (0% to 25%), with the synthetic mulch able to trigger a significantly faster growth of plants (25.3 dd), whereas the organic mulching seemed to slow down plant growth (35.5 dd). In the following substages, although the effect of mulching was always detectable, the differences between treatments were as small, as they could not be appreciated by the statistical analysis.

The yield of cones (Table 2) was affected by the year, which acted in association with both the genotypes and the mulching treatment (interactions Y × V and Y × M, significant at $P \leq 0.05$). In both years, the cv. Chinook allowed the highest yields, i.e., 49.3 g DM per plant in 2018, and 40.5 in 2019, and the cv. Nugget the lowest (less than 20 g DM per plant in both years), whereas the cv. Cascade exhibited a strong differentiation between years, expressing yields ranking among the highest in 2018 and among the lowest in 2019 (Fig. 3A). The observation of treatments showed, instead, the high reactivity of hop plants to the synthetic mulch, which allowed higher yields in 2018 and lower in 2019, whereas the other two treatments gained statistically no different yield values, independently of year.

Plant aerial biomass (Fig. 4) was the highest in the 'Chinook', followed by 'Cascade' and 'Nugget', respectively. Within each variety, no significant effect of the mulching treatment could be enlightened by the statistical analysis, and the significant V × M interaction (Table 2) can be evidenced between the highest ('Chinook' with organic mulch, 197.5 g DM per plant) and the lowest biomass values ('Nugget' with organic mulch, 92.1 g DM per plant). On average, no difference was detectable on the effect of mulching treatments.

The hypogeal biomass turned out significantly different ($P \leq 0.05$) between 2018 and 2019. The ANOVA also showed a significant

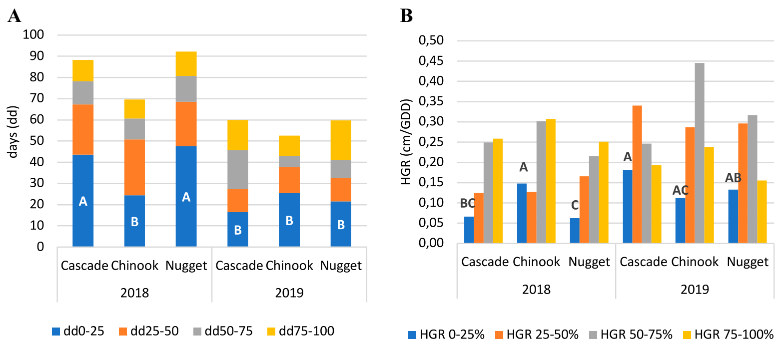

Fig. 2. Cumulated durations (**A**; days) and corresponding hop growth rate (HGR) values [**B**; cm/growing degree days (GDD)] calculated for subsequent intervals of plants' elongation (up to 25%, 25% to 50%, 50% to 75%, 75% to 100% of top wire height) in a 2-year cultivation trial of three hop varieties (Chinook, Cascade, and Nugget) at "Orleans" farm (Palermo, PA, Italy). Mean values of the interaction "year" × "variety." For each group of means, bars marked by the same letter are not significantly different at $P \leq 0.05$ (Tukey's test).

($P \leq 0.05$) V × M interaction (Table 2; Fig. 5), assessing a different response of genotypes to the mulching treatments. In fact, the adoption of synthetic mulching was a valuable instrument to positively enhance the root biomass yields for the cv. Cascade, different from what was found in the cv. Nugget, for which it showed to have a downward effect. Under both treatments (organic and synthetic mulch), as well as in the control, 'Chinook' emerged as having the best capability to accumulate hypogeal biomass, which averaged weight values significantly higher than the other two varieties (Fig. 5).

The analysis of correlations (Table 3) showed, at first instance, a strong and direct association (r = 0.695) between dry cone yield and dry epigeal biomass. These two characters did not evidence, on the other hand, any significant association with total growth duration and HGR. Otherwise, the biomass and cone yield of both plant demonstrated a close inverse association with HGR in the interval 25% to 50% of top wire height (r = −0.579, and r = −0.435, respectively). As expected, the same parameters demonstrated a direct association with the inverse measured values, that is, the duration (in days) of the corresponding plants' growth intervals. Cone yield showed, additionally, a significant inverse association with the duration of the final elongation stage of plants (75% to 100% of total wire height; r = −0.395).

Otherwise, a significant inverse relationship (r = −0.518) showed up between total cycle duration and hypogeal biomass; consequently, the hypogeal biomass resulted positively associated (r = 0.526) with HGR_{tot}. As shown, each partial HGR value demonstrated a positive association with hypogeal biomass values, whereas the corresponding partial durations ($dd_{0–25}$ to $dd_{75–100}$) always exhibited negative r values. Finally, all partial HGRs, as well as total HGR (from the beginning of trial to complete elongation on wire), demonstrated a close inverse correlation (r values always <0) with their corresponding stage duration (in days). Total duration of the plants' elongation was directly correlated with the first three stages, with decreasing r values, showing that the initial elongation stage is the most relevant part of total number of days.

Table 2. Mean values of yield of cones per plant (g DM), epigeal and hypogeal plant biomass (g DM), of three hop varieties (Cascade, Chinook, Nugget) cultivated in 2018 and 2019 at "Orleans" farm (Palermo, Italy), under two mulching treatments (synthetic and organic) and without mulching (no mulch). For each group of means, values followed by the same letter are not significantly different at $P \leq 0.05$ (Tukey's test). For interactions, significant P values ($P \leq 0.05$) are marked in bold.

	Cone yield (g DM)	Epigeal biomass (g DM)	Hypogeal biomass (g DM)
Year (Y)			
2018	38.4	177.4 a	240.30 b
2019	27.2	113.1 b	276.35 a
Significance of F test (P value)	*0.006*	*0.000*	*0.007*
Variety (V)			
Cascade	34.6	139.8	253.38
Chinook	44.9	184.5	321.62
Nugget	19.7	111.5	199.98
Significance of F test (P value)	*0.000*	*0.001*	*0.000*
Mulching treatment (M)			
No mulch	30.7	150.2	303.68
Synthetic	36.9	148.2	248.73
Organic	30.1	139.8	250.71
Significance of F test (P value)	*0.080*	*0.697*	*0.034*
Significance of *F* test (*P* value) for interactions			
Y × V	***0.012***	*0.981*	*0.794*
Y × M	***0.042***	*0.175*	*0.933*
V × M	*0.067*	***0.015***	***0.003***

Discussion and Conclusions

Significant variability was assessed in measured cone and biomass yields, according to the genotype and the treatments, but also dependent on year. This is not surprising in Mediterranean environments, where, even when irrigation is applied, climatic variability often results in wide yield oscillations (Arnon, 1992). Hence, regardless of the variety and the mulching treatment applied, in 2018, hop plants found more favorable climatic conditions, positively affecting both yields and plant growth rates.

The observed data also showed that the cultivar Chinook proved to be more stable, with significantly higher and constant production levels in both growing seasons. Oppositely, the cv. Cascade exhibited a higher reactivity to interannual variability, showing the best yield performance in the most favorable year, and dramatically lower productions in the second trial year. A better performance of the 'Chinook' variety compared with the other genotypes also emerged in a previous work (Marceddu et al., 2020), where this behavior was supposedly based on the higher precocity of this genotype. In this experiment also, the cv. Chinook was the earliest variety, with a remarkable homogeneity of growth rate between the 2 years.

The adoption of a low-trellis farming system could have played a significant role in this outcome, due to physiological imbalances eventually linked to the poor suitability of 'Cascade' and 'Nugget' to this constraining management. Although all three varieties are widely grown all around the world (Darby et al., 2017;

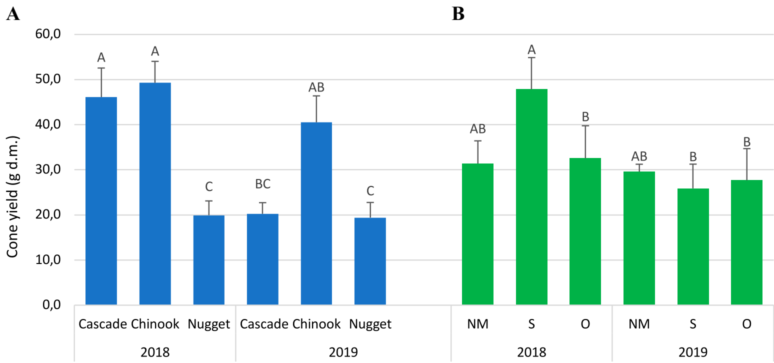

Fig. 3. (**A**) Cone yield (g of DM) obtained in a 2-year cultivation trial of three hop varieties at "Orleans" farm (Palermo, PA, Italy); (**B**) mean values + SEM of the interactions "year" × "variety" (**A**) and "year" × "mulching treatment" (**B**). For each group of means, bars marked by the same letter are not significantly different at $P \leq 0.05$ (Tukey's test). NM = no mulch; S = synthetic; O = organic.

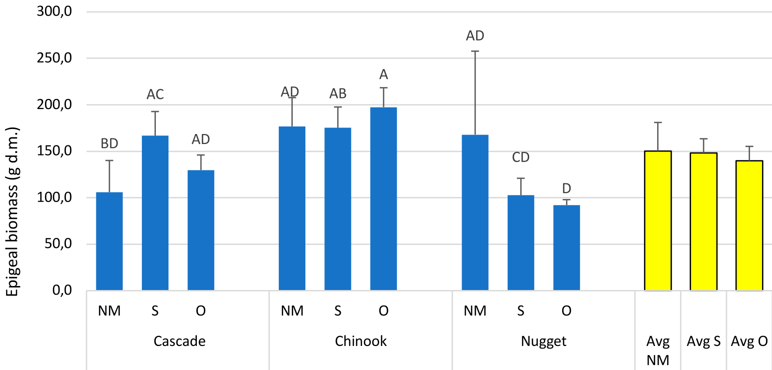

Fig. 4. Plant epigeal biomass (g of DM) obtained in a 2-year cultivation trial of three hop varieties at "Orleans" farm (Palermo, PA, Italy). Mean values + SEM of the interaction "variety" × "mulching treatment," and mean values of the mulching treatments. Bars marked by the same letter are not significantly different at $P \leq 0.05$ (Tukey's test). NM = no mulch; S = synthetic; O = organic.

Dodds, 2017), it appears that these two genotypes did not meet the best conditions to express their full yield potential.

The measured values of hypogeal biomass production also allowed some interesting considerations. In perennial crops such as hop, the storage capacity in root biomass, although not directly associated with yield, takes a special relevance. In this respect, our work demonstrated

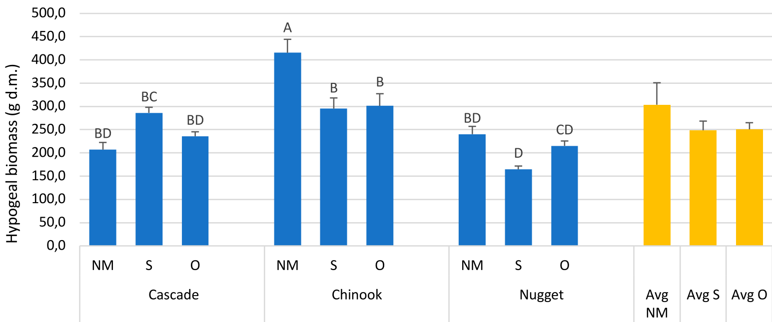

Fig. 5. Plant hypogeal biomass (g of DM) obtained in a 2-year cultivation trial of three hop varieties at "Orleans" farm (Palermo, PA, Italy). Mean values + SEM of the interaction "variety" × "mulching treatment," and mean values of the mulching treatments. Bars marked by the same letter are not significantly different at $P \leq 0.05$ (Tukey's test). NM = no mulch; S = synthetic; O = organic.

Table 3. Pearson's correlation coefficients and corresponding *P* values (in italic) between dry plant weight (g DM), root/rhizome system biomass (g DM), cone yield (g DM), and hop growth parameters [hop growth rate (HGR) and duration of elongation stages in four intervals of hop climbing on wire] in a 2-year cultivation trial of three hop varieties at "Orleans" farm (Palermo, PA, Italy). Significant *P* values (<0.05) are marked in bold.

	Epigeal biomass (g DM)	Hypogeal biomass (g DM)	HGR_{0-25}	HGR_{25-50}	HGR_{50-75}	HGR_{75-100}	HGR_{tot}	dd_{0-25}	dd_{25-50}	dd_{50-75}	dd_{75-100}	dd_{tot}
Cone yield	0.695	0.334	−0.017	−0.435	0.112	0.342	−0.018	0.007	0.370	−0.205	−0.395	−0.016
(g DM)	***0.000***	*0.077*	*0.928*	***0.018***	*0.562*	*0.069*	*0.926*	*0.973*	***0.049***	*0.286*	***0.034***	*0.934*
Epigeal biomass	–	0.336	−0.111	−0.579	−0.071	0.281	−0.247	0.198	0.614	−0.074	−0.309	0.311
(g DM)		*0.075*	*0.565*	***0.001***	*0.715*	*0.140*	*0.196*	*0.303*	***0.000***	*0.705*	*0.103*	*0.101*
Hypogeal biomass		–	0.360	0.242	0.532	0.205	0.526	−0.429	−0.074	−0.231	−0.303	−0.518
(g DM)			*0.055*	*0.206*	***0.003***	*0.286*	***0.003***	***0.020***	*0.705*	*0.228*	*0.110*	***0.004***
HGR_{0-25}			–	0.349	0.05	−0.052	0.692	−0.880	−0.175	0.108	0.073	−0.649
				0.064	*0.798*	*0.787*	***0.000***	***0.000***	*0.365*	*0.578*	*0.707*	***0.000***
HGR_{25-50}				–	0.312	−0.398	0.625	−0.440	−0.868	−0.057	0.324	−0.650
					0.099	***0.033***	***0.000***	***0.017***	***0.000***	*0.770*	*0.087*	***0.000***
HGR_{50-75}					–	0.115	0.640	−0.258	−0.274	−0.750	−0.274	−0.689
						0.553	***0.000***	*0.177*	*0.151*	***0.000***	*0.150*	***0.000***
HGR_{75-100}						–	0.066	0.169	0.358	−0.170	−0.859	−0.017
							0.733	*0.381*	*0.056*	*0.378*	***0.000***	*0.931*
HGR_{tot}							–	−0.734	−0.483	−0.440	−0.140	−0.965
								0.000	***0.008***	***0.017***	*0.469*	***0.000***
dd_{0-25}								–	0.231	−0.034	−0.185	0.760
									0.228	*0.860*	*0.337*	***0.000***
dd_{25-50}									–	0.044	−0.317	0.557
										0.820	*0.094*	***0.002***
dd_{50-75}										–	0.169	0.446
											0.380	***0.015***
dd_{75-100}											–	0.062
												0.748

that, opposite to what emerged for plant epigeal biomass and cone yield, a fast growth rate (as expressed by HGR values) was associated with a higher hypogeal biomass production. Mulching treatments had a crucial importance in this sense. The effect exerted by mulching treatments was strongly dependent on genotype: as shown, the use of synthetic mulching allowed significant hypogeal biomass increases in the cv. Cascade, but not in the other genotypes. A strong effect of mulches on soil temperature is widely acknowledged by the literature, and enhanced maximum and minimum temperatures are generally attributed to PE mulches (Bristow, 1988; Gu et al., 2019), whereas a lowering effect is recognized for organic mulches (Subrahmaniyan and Zhou, 2008; Yordanova, 2017). This remarkable thermal effect can justify the high HGR value recorded for synthetic mulching treatment in the first time span of plant elongation (0% to 25%), significantly higher than that relative to organic mulched and unmulched pots. Hence, synthetic mulch made plants' growth faster in the first stage of elongation (0% to 25%), whereas, oppositely, organic mulch made growth slower in the same stage. Eventually, this reduced duration of the 0% to 25% substage resulted in a higher root biomass.

In line with latest research findings (Marceddu et al., 2020; Rossini et al., 2016, 2021), it is therefore possible to state that, in the Mediterranean environment, the farming suitability of hops is highly variable among varieties. Further long-term research will focus on the relationship between root system development and plant productivity in hops, also pointing out the effects of cropping management on epigeal and hypogeal development, to ensure a higher sustainability in time of this crop.

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2.2. Impact of Different Localized Irrigation Systems on Physiological Responses and Quality Features of Cascade Hops

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Abstract

Global expansion of hop (*Humulus lupulus* L.) production has drawn the attention of breweries to explore the utilization of locally grown hops. Simultaneously, researchers have sought to investigate the physiological responses and adaptations of hops to novel cultivation regions, to meet production and quality standards through enhanced agronomic practices.

This study aimed to evaluate the yield, quality, and physiological responses of containerized Cascade hops grown under different localized irrigation systems, including surface drip irrigation (SDI) and sub-surface drip irrigation (SSDI). Evapotranspiration from potted plants was assessed through the gravimetric method, and irrigation was applied to maintain soil moisture between 90% and 100% of field capacity. The experiment was conducted in 2023 in Palermo (Sicily, Italy), a semi-arid Mediterranean environment. A randomized experimental design with three replications was employed, featuring one-year-old hop plants within a low-trellis orchard (2.60 m in height). Photosynthetic efficiency and gas exchange rate of hop plants were measured using SPAD-502 (Minolta Camera Co., Osaka, Japan) and CIRAS 3 (PP Systems®, Amesbury, MA, U.S.A.), respectively. The study explored correlations between the qualitative aspects of hop productivity and the species' physiological responses under the experimental conditions. The choice of irrigation system appeared to significantly influence cone quality, biomass yields, and the physiological responses of hop plants. The results offer insights into optimizing cultivation practices to achieve high production and quality standards, particularly in regions potentially involved in the expansion of hop production.

Introduction

Hop (*Humulus lupulus* L.) is native to northern temperate regions and belongs to the Cannabaceae family (Small, 1978). It is a perennial, climbing plant with rhizomes, whose shoots twist clockwise around available supports thanks to trichomes, reaching heights of up to 7 meters (Biancardi & Wagner, 1989).

The main historical hop-producing countries are Germany, the Czech Republic, England, and eastern Poland. The cultivation of a specific genotype in a given area contributes to the uniqueness of beer, highlighting the distinct characteristics of the particular genotype-environment combination. Supporting this hypothesis, a three-year comparative study was conducted between commercial hop varieties and ecotypes selected from northern Italy (Ghiselli et al., 2022). The study demonstrated how environmental influences generate variability in the phytochemical profile, with an increase in β-farnesene in some ecotypes. This compound is of particular interest in brewing processes, especially for beers with a more pronounced floral bouquet and in dry hopping (Mongelli et al., 2016).

However, the hop-growing area is currently expanding, driven by the increasing interest in craft beer production. In Italy, since 2020 (Licciardo et al., 2021), the cultivated area has steadily increased, reaching the current 97.5 hectares dedicated to hop cultivation nationwide (Carbone & Licciardo, 2023). In southern regions, various cultivation experiences have shown that modern hop varieties are suitable for open-field production (Marceddu et al., 2024; Ruggeri et al., 2023). Numerous other studies from central and southern Italy (including the main islands), revealed the high adaptability of American varieties such as Cascade, Chinook, Comet, and Nugget in terms of both yield and quality parameters (Carrubba et al., 2022; Gresta et al., 2023; Forteschi et al., 2019; Rossini et al., 2016).

The ongoing improvement of agronomic techniques, along with a deeper understanding of how hops respond physiologically to different soil and climate conditions, could greatly support the expansion of hop cultivation areas. Among these factors, the water response of hops is particularly important, yet it remains largely underexplored in current research. Appropriate irrigation management could significantly impact plant growth, yield, and the concentration of bitter acids in hops (Fandiño et al., 2015). There is currently limited information on the potential for hop production under various irrigation regimes in Mediterranean environments with semi-arid climates. This study aims to explore and optimize agronomic practices to successfully integrate hops into Mediterranean cropping systems. It specifically evaluates the impact of two irrigation methods—surface drip (SDI) and subsurface drip irrigation (SSDI)—on the growth, yield, and quality of potted hop plants (cv

Cascade). The assessment encompassed both production quantity and quality, focusing on critical parameters such as bitter acid concentration, which is essential for brewing applications, and the overall technological quality of hop production.

Materials and methods

The 2023 agronomic trial was conducted at the "Orleans" experimental field (38°06'27" N; 13°21'01" E; 0 m a.s.l.; Palermo, Italy), part of the Department of Agricultural, Food, and Forest Sciences (SAAF) at the University of Palermo. The field is located in a Mediterranean climate zone characterized by hot, dry summers and mild, rainy winters (Rivas & Mata, 2011).

The experiment employed a completely randomized design, with each block comprising six plants and replicated three times. Micropropagated Cascade hop plants, obtained from MrHops® (San Martino di Lupari, PD), were used for this trial. On April 27, 2023, a total of 36 plants were transplanted into 40-liter pots, each containing a drainage layer of expanded clay and filled with Vigorplant Terriccio Cinquestelle® (Vigorplant Italia srl), a substrate composed of superfine peat and calibrated silica sand. The pots were mulched with pine bark. A support system consisting of 2.5 m iron poles and galvanized wire was set up, with plant growth guided by coir fiber ropes attached to horizontal supports. The pots were arranged in alternating rows and equipped with localized irrigation systems (Figure 1).

Figure 1. Cascade hop orchard's installation at the "Orleans" experimental farm (Department of Agricultural, Food, and Forest Sciences, University of Palermo).

The setup featured two irrigation methods: surface drip irrigation (SDI) and sub-surface drip irrigation (SSDI). The irrigation system included a disk filter, solenoid valves, a pressure gauge, and a flow meter. The surface drip system used a PE main line (DN 20 mm) and a DN 16 mm PE pipeline with extruded drippers. Each pot received two self-compensating 4 L h^{-1} iDrop drippers (IRRITEC®). The subsurface system employed a similar main pipeline but included a double self-compensating drip line (Multibar Siplast IRRITEC®) with 2 L h^{-1} in-line drippers spaced 0.33 m apart. Four buried drippers per pot ensured uniform water distribution. Irrigation volumes were adjusted based on crop needs, determined gravimetrically, with a total seasonal volume of 22.46 m^3.

Fertilization was applied using Kelpstar® (Mugavero fertilizers, Palermo, Italy), a liquid extract of *Ecklonia maxima* with organic nitrogen (1%), organic carbon (10%), phytohormones (auxin 11 mg L^{-1}, cytokinin 0.03 mg L^{-1}), and organic substances < 50 kDa (30%), and Siapton® (Isagro spa, Milan, Italy), a liquid formulation containing organic matter

(567–720 g L^{-1}), organic nitrogen (8.7–9.1%), organic carbon (26%), and free amino acids (7.9–10%). Both biostimulants were applied on leaves at 150 ml ha^{-1} when plants had fully developed secondary shoots (stage 2.9 BBCH scale), using a 20 L shoulder sprayer pump (Record®, Volpi Originale, Casalromano, Italy).

Plants were monitored regularly for health and growth. Pest and pathogen levels remained below intervention thresholds, eliminating the need for pest control. Weed management was conducted manually within the pots. Phenological stages were tracked using the BBCH scale (Rossbauer et al., 1995), photosynthetic efficiency was measured biweekly with a SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan), and gas exchange was assessed during flowering (BBCH 5.1-5.9) using the CIRAS 3 system (PP Systems®, Amesbury, MA, USA).

Plants' height and SPAD values were measured be-weekly in all plants throughout the stages from 1.1 to 8.9 (23 and 24 measurements, respectively). Harvesting occurred on August 31, 2023, at full cone maturity (BBCH 8.9), indicated by compact cones and typical aroma (Calderwood & Post, 2015). Biomass measurements included above-ground biomass (cones, leaves, stems) and below-ground biomass (crown diameter, rhizome weight, root dimensions). Cone quality was analyzed for alpha and beta acids using A-EBC methods (Analytica-EBC, 2007).

Biometric data (i.e., "total biomass weight", "cone yield", "stems and leaves weight" and "roots and rhizome") were analyzed using ANOVA by means of the Minitab software (version 19.2.0.0). Before ANOVA, normality and variance homogeneity were checked using the Ryan-Joiner ($\alpha = 0.05$) and Levene's tests ($\alpha = 0.05$) (Gomez & Gomez, 1984). Non-normal parameters (i.e., "cone yield," "total biomass weight," "stems and leaves weight") were transformed to normality using the Johnson distribution system (Johnson et al., 1994). Measurements repeated over time (i.e., "plants height", "SPAD values" and all parameters related to photosynthetic efficiency) were analyzed using ANOVA for repeated measures. The tests were performed using SYSTAT software (version 13.2) (Graffiti LLC, Palo Alto, CA, USA).

Statistically significant results were further analyzed with Fisher's least significant difference (LSD) post hoc (Gomez & Gomez, 1984).

Results and discussion

During the trial, climatic conditions were marked by low rainfall and high summer temperatures, with peak values exceeding 30°C. Specifically, during the trial, days with maximum temperatures exceeding 45°C occurred right around the flowering period (Figure 2). This aspect significantly influenced the physiological response of the plants, as later shown by the gas exchange values recorded for the cultivated plants.

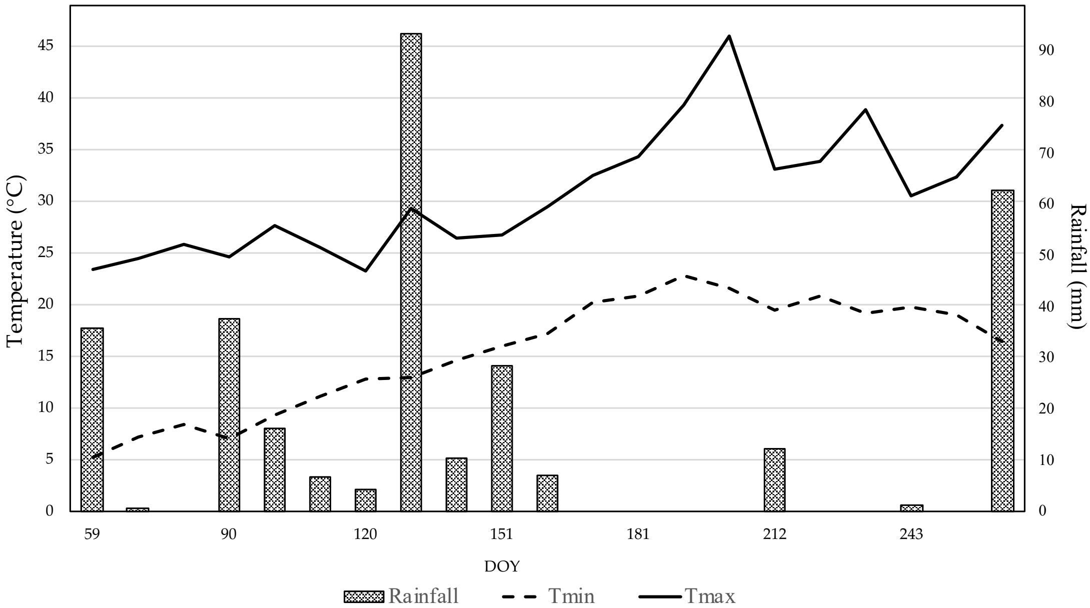

Figure 2. Ten-day values of rainfall and temperatures (minimum and maximum) recorded at the experimental site (Palermo, PA) in 2023.

Total biomass per plant was weighed, and further sorted in aerial part (weight of stems and leaves, and cone yield), and below-ground biomass. All collected data were expressed in grams of fresh matter at harvest (FM). As highlighted in table 1, the analysis of variance showed a significant effect of the irrigation system only for the cone yield variable, which averaged 55.01 g per plant in the treatment SSDI, showing an increase of 29% compared to plants with SDI. The average values of stem and leaf weight and total biomass per plant showed a similar trend to the previous one, although not significant and therefore not statistically distinguishable (Table 1). Similarly, for the last two variables, the data related to below-ground biomass weight also did not show a statistically significant differentiation, although, on average, higher absolute values were recorded for treatment SDI compared to SSDI, with an increase of about 6% from one treatment to the other (Table 1).

Table 1. Mean values + SEM of total biomass, cones yield, stems and leaves, roots and rhizomes (g FM $plant^{-1}$) of Cascade hops subjected to two irrigation systems (SDI and SSDI) at the end of the 2023 growing season in a semi-arid Mediterranean environment.

	Total biomass	**Cones yield**	**Stems and leaves**	**Roots and rhyzome**
Mean effect of Irrigation system				
SDI	138,89	42,50[b]	101,11	177,95
SSDI	134,71	55,01[a]	86,94	168,59
Significance of p-value	n.s.	*	n.s.	n.s.

SDI = surface drip irrigation; SSDI = sub-surface drip irrigation.

Note: Significance of the calculated p-value: * significant at $0.01 < p \leq 0.05$; n.s. not significant. Means with the same letter within a column are significantly not different according to Fisher's least significant difference (LSD) ($p < 0.05$).

Table 2 shows the results of the analysis of variance performed on the repeated measures of plant height (cm) and SPAD values between emergence and harvest. For both variables, the high significance of the measurement time ('time'; $p < 0.001$) is highlighted, while the interaction between time and irrigation treatment is significant only for the SPAD parameter ($p < 0.01$).

Table 2. ANOVA results and significance for repeated measures (Fisher's coefficient) for height and SPAD parameters, measured during the experiment.

Source of variation	**DF**	**Height**	**SPAD**
Between subjects			
IS	1	0.113 n.s.	0.339 n.s.
Error	33		
Within subjects			
Time	22	545.98***	25,167***
Time x IS	22	1,493 n.s.	2,383**
Error	726		

Note: Significance of the calculated F values: *** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; n.s. not significant.

The graphs in Figure 3 show the trend of plant heights during the observation period for each irrigation treatment. As it can be seen, the plants reached heights of 252 cm with sub-irrigation and 258 cm with traditional irrigation. Therefore, it is understandable that no statistical differences were observed between the two treatments regarding plant growth.

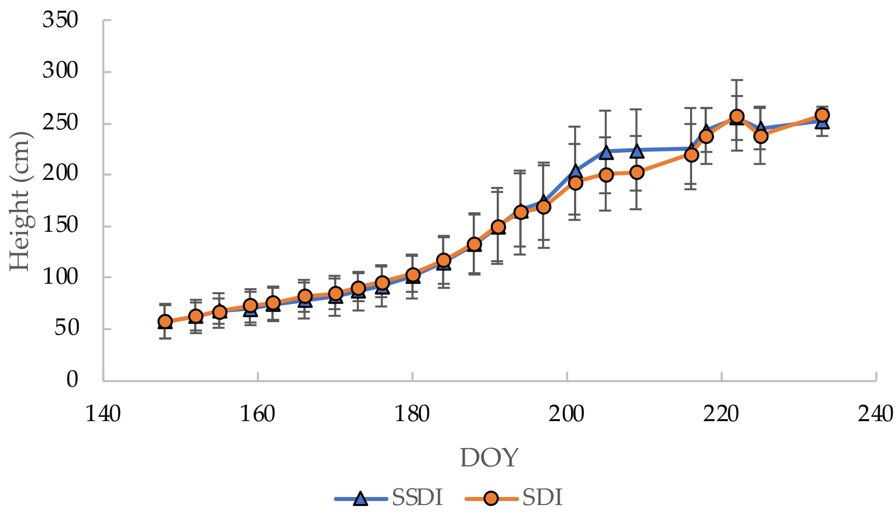

Figure 3. Mean values ± SE (n = 12) of plants' height with surface drip irrigation (SSI) and sub-irrigation (SSDI) during the 2023 growing cycle.

The SPAD values (Figure 4) varied from a minimum of 24.5 to a maximum of 38.1. In general, during the first part of plants' growth, SDI kept on slightly higher values than SSDI. It is worth noting, however, that between the 210th and 220th day (i.e., from July 20 to 29), a different response of the plants was observed depending on the irrigation treatment. During this period, as shown in Figure 2, exceptionally high maximum temperatures were recorded starting from the 200th DOY, peaking at 45.9°C, which induced severe stress conditions in the plants. In this case, the plants subjected to sub-irrigation were able to maintain a higher chlorophyll index compared to plants irrigated with the traditional treatment, with statistically significant differences at the 222nd ($p < 0.001$), 225th ($p < 0.001$) and 230th ($p < 0.001$) DOY (Figure 3). The difference between the two treatments diminished once the thermal stress was alleviated. This finding aligns with previous studies conducted on various crops (Zhou et al., 2015; Barutçular et al., 2016; Songsri et al., 2008), which observed that the SPAD index not only serves as a reliable indicator of nitrogen nutrition but is also positively correlated with non-limiting water availability for cultivated plants. The sub-surface drip irrigation system, therefore, appeared to provide a superior level of water availability within the pots compared to traditional surface drip irrigation. This aspect is further corroborated by the distinct behavior of Vapor Pressure Deficit (VPD) and Water Use Efficiency (WUE) observed between the treatments (Table 3). Interestingly, the response of hop plants subjected to intense thermal stress contrasted with the physiological mechanisms observed in other crops, such as tomato and potato, where increased thermal stress typically resulted in reduced leaf size and elevated SPAD index values (Bhattarai et al., 2021; Tang et al., 2018).

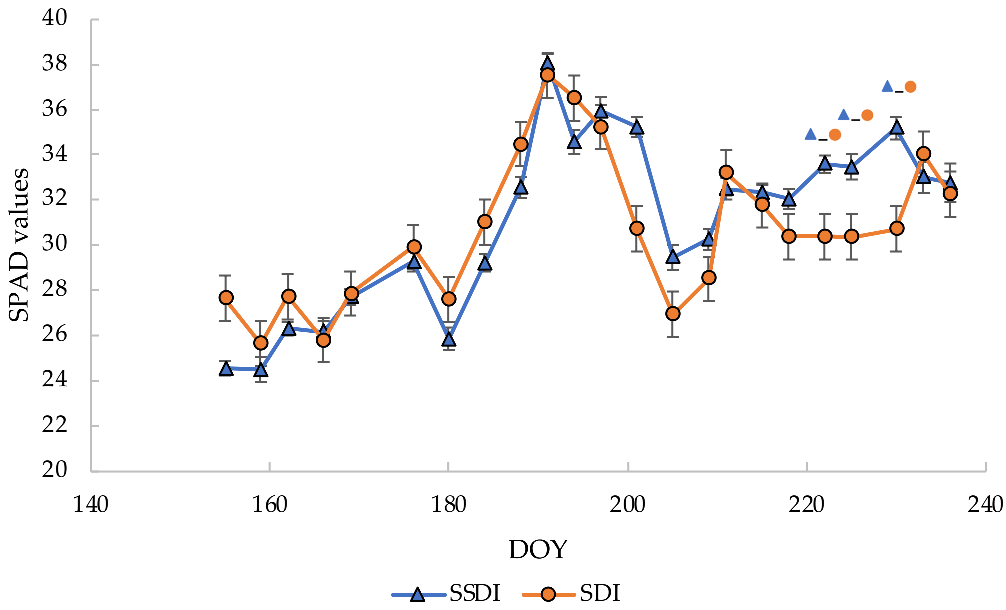

Figure 4. Mean values ± SE (n = 12) of SPAD values with surface drip irrigation (SSI) and sub-irrigation (SSDI) during the 2023 growing cycle. Treatment symbols joined by dashes indicate statistically different averages at $p < 0.05$ between these IS treatments within each time point, according to the Fisher's least significant difference (LSD) test.

The gas exchange measurements obtained using CIRAS 3 are presented in Table 3. No significant differences between treatments were detected for transpiration and stomatal conductance. However, changes in the physiological responses of hops became evident from the 200th day of the year (DOY), partially reflecting and potentially predicting the trends observed in SPAD values (Figure 5). Cellular respiration did not differ significantly between treatments, with notable changes occurring only over time. An increase in average respiration at the third measurement point indicated a severe stress response, likely altering normal photosynthetic processes and redirecting them towards enhanced respiratory activity (Plaxton et al., 2006; Tiwari et al., 2002). In contrast, significant effects were found for vapor pressure deficit (VPD) and water use efficiency (WUE) over time and in their interaction with other factors, though no other irrigation-related differences were detected. The observed variations in VPD (Figure 5e) and WUE (Figure 5f) correspond to the initial thermal stress period between the 200th and 210th days (July 20th to 29th). These results confirm that the SSDI system enabled plants to better withstand intense thermal stress, maintaining significantly distinct VPD and WUE values compared to those under traditional SDI treatment.

Table 3. ANOVA Results and Significance: Repeated measures analysis (Fisher's coefficient) for Respiration, Sub-stomatal CO_2 concentration, Transpiration, Stomatal Conductance, Vapor Pressure Deficit (VPD), and Water Use Efficiency (WUE), as measured during the experiment using the CIRAS 3 system (PP Systems®, Amesbury, MA, USA).

	DF	Respiration (µmol CO_2 m^{-2} s^{-1})	Sub-stomatal CO_2 concentration (µmol mol^{-1})	Transpiration (mmol H_20 m^{-2} s^{-1})	Stomatal conductance (mmol H_20 m^{-2} s^{-1})	VPD (kPa)	WUE (mmol CO_2 mol^{-1} H_2O)
Between subjects							
Irrigation system (IS)	1	0,217 n.s.	0,41 n.s.	1,717 n.s.	1,577 n.s.	0,280 n.s.	0,933 n.s.
Error	16						
Within subjects							
Time	3	6,761***	1,311 n.s.	94,734***	41,656***	115,064***	45,907***
Time x IS	3	0,493 n.s.	0,489 n.s.	2,144 n.s.	0,464 n.s.	5,006**	4,177**
Error	48						

Note: Significance of the calculated F values: *** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; n.s. not significant.

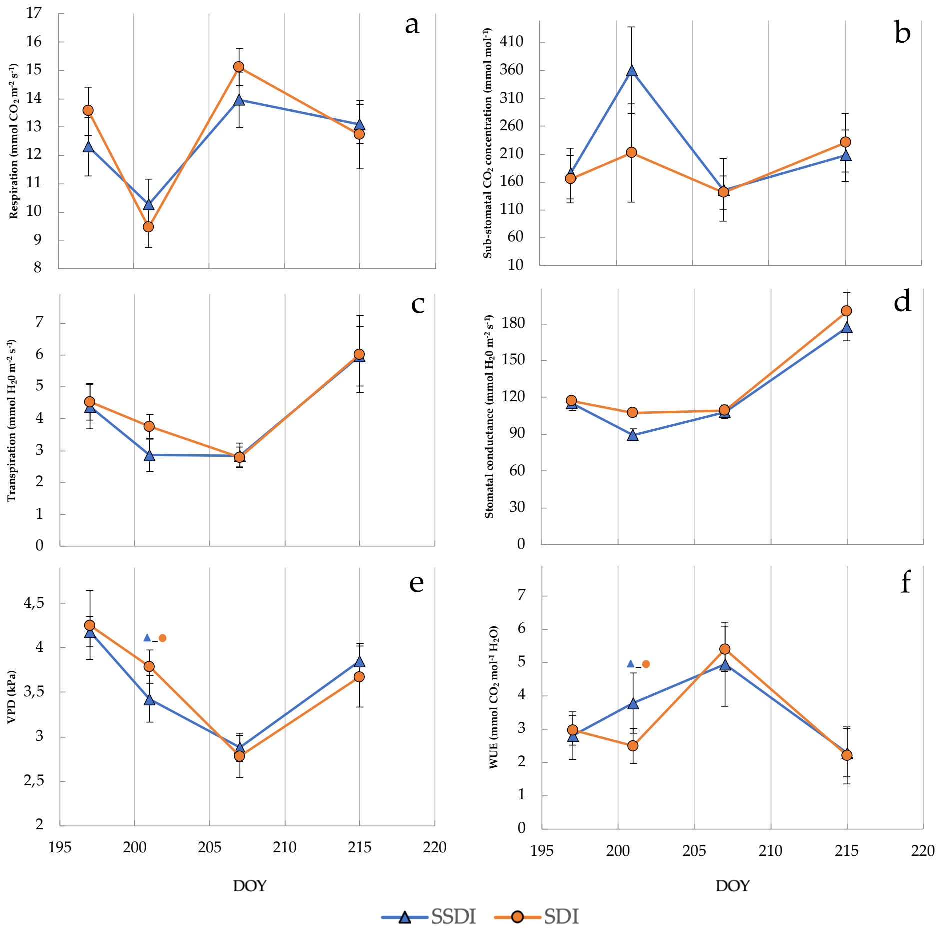

Figure 5. Mean values ± SE (n = 3) of (a) Respiration, (b) Sub-stomatal CO_2 concentration, (c) Transpiration, (d) Stomatal Conductance, (e) Vapor Pressure Deficit (VPD), and (f) Water Use Efficiency (WUE), as measured during the experiment using the CIRAS 3 system (PP Systems®, Amesbury, MA, USA) on plants with surface drip irrigation (SSI) and sub-irrigation (SSDI) during the 2023 growing season. Treatment symbols joined by dashes indicate statistically different averages at $p < 0.05$ between these IS treatments within each time point, according to Fisher's least significant difference (LSD) test.

Conclusions

The study shows that SSDI markedly improves the performance of hop plants under challenging climatic conditions, specifically high temperatures and low rainfall, characteristic of the semi-arid Mediterranean climate typical of southern Italy. SSDI increased cone yield by 29% compared to SDI, highlighting its superior water availability during periods of thermal stress. Although differences in total biomass, stem, leaf, and below-ground biomass between the treatments were not statistically significant, the overall trend favored SSDI.

During peak stress periods, SSDI-treated hop plants maintained higher SPAD values, indicating better chlorophyll functionality and improved physiological status. This response was further supported by significant differences in VPD and WUE, confirming that SSDI helps hops maintain critical physiological functions during extreme heat.

In conclusion, SSDI proves to be an effective irrigation strategy that enhances hop resilience and yield under high-temperature conditions, offering better water management than conventional SDI. These findings suggest that SSDI could play a crucial role in sustainable agriculture, particularly in drought-prone regions, by optimizing water use and supporting plant health in the face of global climate challenges.

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2.3. Conclusions

The studies demonstrated the importance of selecting the right hop genotypes, using appropriate cultivation techniques, and optimizing water management to improve hop yields in semi-arid Mediterranean climates. Significant variability in cone and biomass yields was observed among hop varieties, with 'Chinook' showing greater yield stability across different years, likely due to its early growth, while 'Cascade' exhibited more sensitivity to climatic fluctuations.

Mulching, especially synthetic mulches, played a crucial role in enhancing root biomass production, particularly in 'Cascade', by accelerating early-stage plant growth. This suggests that mulching can influence not just above-ground growth but also the long-term sustainability of the crop through root system development.

On the irrigation front, subsurface drip irrigation (SSDI) proved to be highly effective in improving hop performance under the challenging conditions of the Mediterranean climate. SSDI increased cone yield by 29% compared to traditional surface drip irrigation (SDI) by providing better water availability during periods of thermal stress. It also helped maintain better chlorophyll function and water use efficiency (WUE), ensuring plants remained physiologically healthier during extreme heat. Together, these findings indicate that successful hop cultivation in Mediterranean regions depends on a combination of well-adapted genotypes, effective mulching strategies, and advanced irrigation techniques like SSDI. These approaches collectively enhance the resilience of hops to environmental stressors and support sustainable production in drought-prone areas.

3. CHAPTER III

This chapter investigates the effects of agronomic treatments, environmental conditions, and genetic factors on hop growth, yield, and quality within Mediterranean agroecosystems. It focuses on the performance of "Cascade" and "Chinook" hop varieties under varying trellis heights and environmental stressors in Sicily's semi-arid climate. Key aspects such as trellis height and Extreme Degree Days (EDD) are analyzed to understand their impact on hop productivity. The findings provide guidance on optimizing cultivation practices to achieve high-quality hop production in challenging climates, emphasizing the importance of variety selection and agronomic adaptation to enhance resilience and yield amid rising global temperatures and climate variability.

The first study, published in *Horticulturae* 57(11), 1409-1415; https://doi.org/10.21273/HORTSCI16754-22, highlights the significant impact of trellis height on hop performance, particularly noting Cascade's exceptional adaptability to low-trellis systems. Evaluations at different trellis heights (2.60, 3.60, and 4.60 meters) revealed that key factors such as stem and leaf weight are crucial for cone yield. The distinct responses of Cascade and Chinook to these configurations emphasize the need for tailored cultivation strategies to optimize hop production in semi-arid conditions.

In addition, the second research (submitted to the *Journal of Agricultural and Food Chemistry*) assesses the influence of extreme heat events, quantified as Extreme Degree Days (EDD), on hop cultivation. By analyzing microclimatic data from Sicilian weather stations during the 2023 growing season, the study underscores the critical role of environmental conditions and genetic selection in improving hop yield and quality. The findings reveal that effective management of environmental stressors, alongside appropriate farming practices and the selection of resilient hop varieties, is essential for expanding hop production into non-traditional, semi-arid regions.

3.1. Adapting American Hop (*Humulus lupulus* L.) Varieties to Mediterranean Sustainable Agriculture: A Trellis Height Exploration

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Article

Adapting American Hop (*Humulus lupulus* L.) Varieties to Mediterranean Sustainable Agriculture: A Trellis Height Exploration

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Abstract: In recent years, Italy's craft beer industry has seen remarkable growth, fostering the local production of key ingredients, notably hops. However, a research gap exists in exploring open-field hop productivity in typical Mediterranean climates using low-trellis systems. This study addressed this gap by evaluating the productive performances of "Cascade" and "Chinook" hop varieties on "V" trellis systems at different heights (2.60, 3.60, and 4.60 m above ground) in inner Sicily's Mediterranean climate and soil conditions. The results highlighted the significant impact of trellis height on various parameters, with Cascade displaying exceptional adaptability to low-trellis farming. Key factors like stem and leaf weight emerged as crucial drivers of cone yield, emphasizing their significance in hop cultivation. The distinct responses of Cascade and Chinook varieties to varying trellis heights underscored the need for tailored approaches, offering valuable insights for optimizing hop cultivation practices in semi-arid climates.

Keywords: *Humulus lupulus*; low trellis; semi-arid environment; cone yield

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1. Introduction

Hop cones are the female inflorescences of the hop plant (*Humulus lupulus* L.) and represent a key component of the brewing process [1]. Hops are responsible for the distinctive bitterness and aroma, as well as giving antimicrobial properties to the finished product [2].

For several years in Italy, the entire craft beer industry has been growing, which has led to the enhancement of local raw ingredient production, including hops [3,4]. This movement has triggered intensive research focusing on the adaptability of hops species to different cultivation environments in the peninsula [5–11]. Indeed, several studies conducted in Mediterranean climate environments have highlighted the excellent adaptability of various American varieties, both in terms of production [12–14] and quality (i.e., content in alpha acids and aromatic oils) [15–17]. This suggests that the Italian hop industry would have nothing to envy about other production markets abroad.

However, in terms of farming management, there are still relevant knowledge gaps, as hops are a new crop for Mediterranean agroecosystems. To our knowledge, no research has investigated, so far, the open-field productivity of hops under typical Mediterranean climates when employing low-trellis systems. Several studies have demonstrated that low-height systems offer benefits in terms of sustainability and environmental protection [12,18]. Besides requiring lower technical inputs (such as water and fertilizers), they are also less demanding in terms of crop management and initial expenses. Furthermore, in low-trellis systems, plant protection treatments can be carried out more efficiently and with less dispersion due to increased plant wall density and reduced vertical development [18–20]. This allows for more effective biological control of key insects and phytophagous mites [21].

Thus, utilizing the most suitable hop varieties in "low-trellis" systems could present a highly advantageous opportunity to embrace organic farming practices in Mediterranean environments, enabling local farmers to harmonize both productive and economic objectives. The objective of this work was to assess the performance of two American hop varieties, namely "Cascade" and "Chinook", in combination with "V" trellis systems at three different heights within the typical Mediterranean climate and soil conditions of inner Sicily.

2. Materials and Methods

2.1. Experimental Site and Design

The trial took place at the "Sparacia" experimental farm, belonging to the Department of Agricultural, Food, and Forestry Sciences of the University of Palermo, in two growing seasons, namely 2022 and 2023 (Figure 1). The farm, located in the territory of Cammarata (Agrigento, AG, Sicily, Italy; (37°63′ N, 13°76′ E; 600 m asl), is predominantly hilly and is characterized by a semi-arid climate [22], with warm, mainly dry summers and mild, rainy winters.

Figure 1. Satellite image via Google Earth (accessed on 5 February 2024), showing the location of the experimental hopyard in the "Sparacia" farm.

Throughout the trial, climatic conditions were marked by scarce rain events and high summer temperatures, with maximum values exceeding 30 °C (Figure 2).

The soil was a sub-alkaline Eutric Vertisol [23]; representative soil samples collected before the hop implant exhibited a clayey texture, richness in limestone, and a good mineral endowment (Table 1).

For the experiment, two American hop varieties that had already proven good adaptability to Mediterranean environments with semi-arid climates [9,12], i.e., Cascade and Chinook, were selected. The variety Cascade, developed in the USDA breeding program at Oregon State University and released in 1972, is characterized by a dark green elongated cone with a medium-strength aroma, often described as a distinctive floral, grapefruit-like character [24]. The variety Chinook was developed by the USDA breeding program in Washington State and released in 1985 as a high alpha-acid variety, also having a robust spicy, citrus aroma [25]. The trial involved micropropagated plants obtained from the company MrHops® (San Martino di Lupari, PD, Italy), which at transplant time were about 10 cm high (4–6 true leaves). On 5 March 2022, 108 plantlets were transferred into a properly designed planting with "V" trellis systems set up at different heights (2.60, 3.60, and 4.60 m above ground). Plant distances of 3.0 m between rows and 0.7 m in the row were adopted. A "split-plot" experimental layout was arranged, with the "trellis height" as the main experimental factor (I order) and the "variety" (II order) randomized within this. Each treatment was replicated three times, with blocks consisting of six plants each.

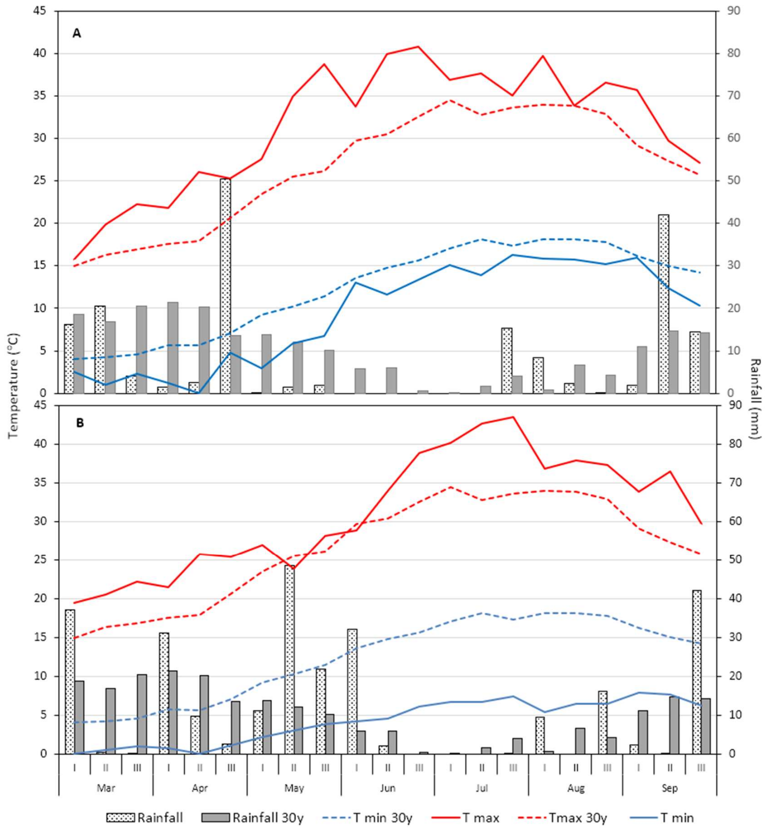

Figure 2. Ten-day values of temperatures and rainfall recorded at the "Sparacia" farm (Cammarata, AG, Sicily, Italy) throughout the 2022 (**A**) and 2023 (**B**) hop growing seasons, compared with the 30-year (1992–2021) mean values.

Table 1. Main chemical and physical characters of the soil used for hop cultivation at the "Sparacia" farm (Cammarata, AG, Italy) in the 2022 and 2023 growing seasons.

Parameter	Value
Clay (%)	37.1
Silt (%)	25.0
Sand (%)	37.9
pH (in water 1:2.5)	8.67
$EC_{1:2.5}$ (mS cm^{-1})	0.388
Total $CaCO_3$ (%)	24.5
Act. $CaCO_3$ (%)	3.25
Organic C (%)	0.84
N (‰)	0.98
P (Olsen; mg g^{-1})	0.037
K (mg g^{-1})	0.031

2.2. Soil and Plant Management

A double-layer pre-planting tillage (30 cm plowing and 50 cm subsoiling) was carried out, followed by subsequent tillering to break down large clumps. Mechanical weeding was performed during the summer in both years (two operations in 2022 and three in 2023, respectively). After the transplant, an irrigation system and plastic mulch (PE film) were positioned. Fertilization was made using the following commercial products:

- Kelpstar® (Mugavero fertilizers, Palermo, Italy), a liquid extract of *Ecklonia maxima*, containing organic nitrogen (1%), organic carbon (10%), phytohormones, mainly auxin (11 mg L^{-1}) and cytokinin (0.03 mg L^{-1}), and organic substances with nominal molecular weights < 50 kDa (30%).
- Siapton® (Isagro spa, Milano, Italy), a liquid formulation containing organic matter (567–720 g L^{-1}), composed of organic nitrogen (8.7–9.1%), organic carbon (26%) and free amino acids (7.9–10%).
- YaraTera Deltaspray 20-20-20 +ME (Yara Italia, Milano, Italy), a highly soluble compound fertilizer ($N:P_2O_5:K_2O$ = 20:20:20), also containing microelements (Mo, B, Fe, Zn, Cu, Mn).

All three fertilizers and biostimulants were dosed and supplied in a single foliar treatment (Table 2) both in 2022 (5 July) and 2023 (13 July), i.e., when all plants had fully emitted the secondary shoots (stage 2.9 of the BBCH scale) [25]. The operation was performed using a shoulder sprayer pump with a capacity of 20 L (Record®, Volpi Originale, Casalromano, Italy).

Table 2. Doses of fertilizers and biostimulants applied on hop in 2022 and 2023 at the "Sparacia" experimental farm.

Growing Season	Fertilizers and Biostimulants Used		
	KelpStar ® ($mL\ ha^{-1}$)	Siapton® ($mL\ ha^{-1}$)	20.20.20 NPK ($kg\ ha^{-1}$)
2022	150	150	-
2023	150	150	15

The localized irrigation system included two dripping lines for each row, with in-line and self-compensating dispensers (2 L h^{-1}). Each block was equipped with a closing valve and a pressure gauge to control the working pressure. Irrigation was administered with frequent shifts and reduced delivery intensities in order to maintain a satisfactory available water content without limiting the growth of the species. Water supply per intervention was kept equal for all treatments, totaling a seasonal amount of about 2000 $m^3\ ha^{-1}$ (202 mm and 189 mm for 2022 and 2023, respectively). The trial did not require control interventions for parasites and pathogens in both years.

2.3. Field Measurements

In both growing seasons, field measurements encompassed phenological phases, crop growth rates, and biomass yields. Phenological measurements were carried out weekly following the BBCH model with a centesimal scale [26]. Crop growth rates (GR) were determined according to the following formula:

$$\mathbf{GR} = \mathbf{H_t} / \sum_{i=1}^{k} \mathbf{dds} \quad (1)$$

where H_t is the height value reached on the wire by the plants, $\sum_{i=1}^{k}$ dds represents the corresponding time interval between measurements, and i and k represent the starting and ending dates of each vertical growth interval.

Vertical development and increase in the number of nodes of the plants were monitored weekly by graphic processing of the photographic material collected using the

ImageJ software (US National Institutes of Health, Bethesda, MD, USA), version 1.52 t.01.30.2020. Likewise, relative chlorophyll content was estimated weekly by recording the SPAD (Soil Plant Analysis Development) values using a SPAD-502 device (Minolta Camera Co., Osaka, Japan).

Harvest time was identified by evaluating cone dry matter content, with collection occurring once a 20% dry matter value was reached [27]. Representative cone samples were taken from each plot and oven-dried at 104 °C for 24 h, to determine their final dry matter weight (DM). Total biomass yields, sorted by stems and leaves, were recorded at the end of each growing season as fresh matter (FM).

2.4. Statistical Analysis

Statistical analysis was performed using the GLM procedure in Minitab® (Minitab LLC, State College, PA, USA), version 19.2.0.0; and Systat® (Systat Software Inc., San Jose, CA, USA), version 13.2. To ensure compliance with the ANOVA assumptions, all data were tested for normality and variance homogeneity through the Ryan–Joiner test (α = 0.05) and the Levene's test (α = 0.05), respectively [28]. When the ANOVA yielded significant results, the differences between mean values were appreciated through Tukey's test ($p \leq 0.05$) [28].

A stepwise regression analysis (backward elimination procedure) between cone yield (g DM plant^{-1}; dependent variable) and all the other recorded plant traits (independent variables) was carried out to cope with multicollinearity and to reduce the number of parameters associated to all observations. Eventually, linear regression models were employed to establish associations between cone yield (g DM plant^{-1}; dependent variable) and weight of stems and leaves (g FM plant^{-1}; independent variable).

Repeated-measures ANOVA was applied to analyze separately over time the effect of variety, trellis height, and their interaction on plant heights, SPAD values, and vertical growth rates (GR). Time (expressed as DOY, day of the year), treatments, and their interactions were also used as explanatory variables in the analyses. In all cases, model assumptions, i.e., normality and homoscedasticity of residuals, were checked, and Tukey's HSD test was used for comparisons of means, unless stated otherwise. The trend over time of the aforementioned variables (plant height, SPAD values, and GRs) was graphically assessed by means of linear and nonlinear regression models, according to the best fitting of each dataset (Table S1, Supplementary Materials).

3. Results and Discussion

3.1. Biomass Yields

The results of the ANOVA on the yield data collected in 2022 and 2023 are shown in Table 3.

For total biomass production, the interaction between "Variety" and "Trellis height" proved significant in both growing seasons ($p < 0.05$; Table 3). Specifically, Cascade plants in the L-Trellis system exhibited a significantly higher biomass yield, producing, on average, 44% and 40% more than all other treatments in 2022 and 2023, respectively. However, in 2023, yield responses were more heterogeneous, with a general leveling of biomass yields for the Cascade variety, which remained significantly higher than that of Chinook. A slight difference emerged among Chinook plants in the three trellis systems, although statistically not appreciable, given their belonging to the same grouping "b". The yield in stems and leaves in 2022 was influenced by the "Trellis height" factor, with L-Trellis recording, contrary to expectations, a production almost double that of the M and H-trellis systems ($p < 0.01$). In 2023, the significant interaction between "Variety" and "Trellis height" confirmed what was observed for total biomass, with a clearly distinct response between the two varieties. According to the results, Cascade plants, regardless of the trellis height adopted, produced more leaves and stems than Chinook. For the latter, the treatments at different heights responded with productions of leaves and stems statistically indistinguishable from each other (same grouping "b"). The cone yield in 2022 varied according to the interaction of both "Variety" and "Trellis height" factors, with highest yields obtained from L-Trellis treatments of 0.29 and 0.21 t ha^{-1} for Cascade and Chinook, respectively. In 2023, however, yields between all treatments seemed to level, while the difference at the varietal level was statistically significant ($p < 0.05$), indicating the clear production superiority of Cascade in the semi-arid environment, with cone yield on average 14% higher than Chinook in the same field conditions.

Table 3. Mean values of total biomass, weight of stems and leaves, and cone yield obtained in 2022 and 2023.

Treatments	2022			2023		
	Total Biomass (t ha^{-1} FM)	**Stems and Leaves (t ha^{-1} FM)**	**Cone Yield (t ha^{-1} DM)**	**Total Biomass (t ha^{-1} FM)**	**Stems and Leaves (t ha^{-1} FM)**	**Cone Yield (t ha^{-1} DM)**
Variety (V)						
Cascade (CA)	1.83	0.96	0.17	5.53	4.41	0.28 a
Chinook (CH)	2.08	1.22	0.17	2.58	1.73	0.21 b
Significance	NS	NS	NS	***	***	*
Trellis height (TH)						
2.60 m (L)	2.74	1.52 a	0.25	4.02	3.02	0.24
3.60 m (M)	1.45	0.83 b	0.12	4.22	3.12	0.27
4.60 m (H)	1.67	0.93 b	0.14	3.92	3.06	0.21
Significance	***	**	***	NS	NS	NS
V × TH						
CA × L	2.88 a	1.45	0.29 a	5.68 a	4.48 a	0.29
CA × M	1.45 bc	0.82	0.13 bc	5.21 a	4.07 a	0.29
CA × H	1.16 c	0.62	0.11 c	5.71 a	4.67 a	0.25
CH × L	2.60 ab	1.57	0.21 ab	2.37 b	1.56 b	0.19
CH × M	1.46 bc	0.85	0.12 c	3.25 ab	2.17 b	0.26
CH × H	2.18 ac	1.25	0.18 bc	2.12 b	1.45 b	0.17
Significance	*	NS	**	*	*	NS

*** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; * significant at $0.01 < p \leq 0.05$; NS: not significant. Values with different letters (including not reported intermediates) are statistically dissimilar according to the Tukey's HSD test at $p \leq 0.05$.

These initial outcomes, derived from this 2-year trial, affirm the suitability of Cascade for cultivation in semi-arid environments. Additionally, hop appears well-suited for low-trellis farming in Mediterranean climates, and this is consistent with the findings of several recent studies [12,13,15]. It is not in doubt that further investigations into other varieties or management systems could significantly contribute to improving our understanding of hop species behavior in new cultural areas, such as the regions of southern Italy.

The productive performance of hop plants was further investigated through stepwise regression analysis, allowing the determination of the most significant predictors of cone yields for each growing season. As shown in Table 4, already since step 1, all the traits included (i.e., stems and leaves, vegetative stages' length, $Spad_{harv}$, and $Height_{max}$) explained about 68.4% and 34.2% of the total regression effect for 2022 and 2023, respectively.

The backward elimination of regressors, conducted in the following steps, revealed that the regression coefficients for stems and leaves remained significant, leading to minor changes in the R^2 value. Remarkably, even after removing all other regressors from the regression models for both 2022 and 2023, "stems and leaves" emerged as the most significant trait, underscoring its crucial importance in determining hop cone yields in a Mediterranean semi-arid environment.

Table 4. Stepwise regression (backward elimination procedure; α to remove = 0.01) between hops cone yields (g DM plant^{-1}; dependent variable) and selected plant traits (independent variables) in 2022 and 2023.

Inserted Variable	**Step 1**		**Step 2**		**Step 3**		**Step 4**	
	Coeff	***p***	**Coeff**	***p***	**Coeff**	***p***	**Coeff**	***p***
2022								
Stems and leaves (g FM)	0.1423	***	0.1423	***	0.1424	***	0.1423	***
Vegetative stages' lenght (dd)	−0.0229	NS	0.02293	NS	0.2110	NS		
$SPAD_{harv}$	0.2130	NS	−0.0249	NS				
$Height_{max}$ (cm)	0.0015	NS						
Partial R^2	0.6840		0.0030		0.0030		0.0040	
Final R^2	**0.6840**	***	**0.6806**	***	**0.6742**	***	**0.6778**	***
2023								
Stems and leaves (g FM)	0.03924	***	0.03883	***	0.03886	***	0.03830	***
Vegetative stages' lenght (dd)	1.0880	NS	1.0030	NS	0.976	NS		
$Spad_{harv}$	0.3010	NS	0.3370	NS				
$Height_{max}$ (cm)	0.0169	NS						
Partial R^2	0.3416		0.0030		0.0020		0.0060	
Final R^2	**0.3416**	***	**0.3391**	***	**0.3369**	***	**0.3309**	***

***: b coefficient significant of at $p \leq 0.001$; NS: not significant. Coeff: b coefficient of the regressors in each step; R^2 = coefficient of determination (partial and final) for the regression model in each step.

Subsequent linear regression analysis, carried out between cone yield (g DM plant^{-1}; dependent variable) and the weight of stems and leaves (g FM plant-1; independent variable), confirmed that positive correlations occurred for both 2022 (Figure 3) and 2023 (Figure 4) growing seasons.

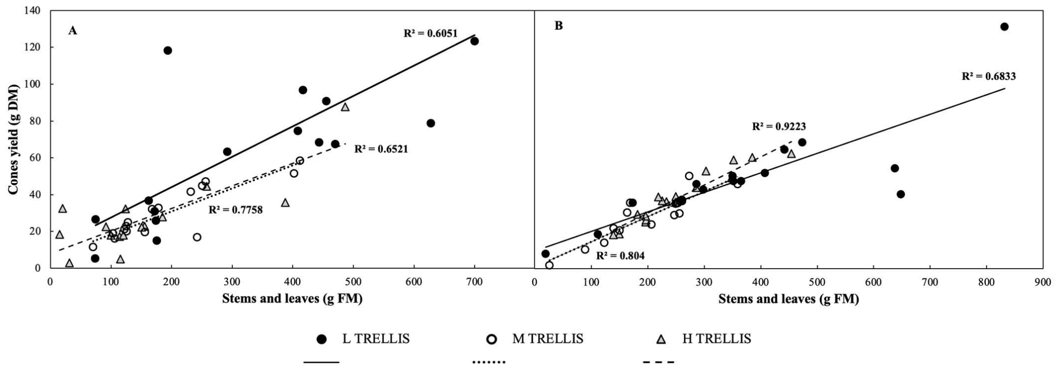

Figure 3. Regression lines, according to trellis height (low, medium, and high) between cone yield (g DM plant^{-1}; dependent variable) and weight of stems and leaves (g FM plant^{-1}; independent variable) in 'Cascade' (**A**) and 'Chinook' (**B**) hops in 2022.

In 2022, the response of Cascade plants was notably uniform, given the general orientation of the regression lines and the significance ($p < 0.001$) of the R^2 values (0.605, 0.776, and 0.652 for L, M, and H-trellis, respectively) (Figure 3). The R^2 value of Chinook plants in L-Trellis (0.683), although statistically significant ($p < 0.001$), in 2022 was lower than the M and H-Trellis treatments, which showed values of 0.804 and 0.922, respectively. In 2023, the R^2 values were significant for all varieties and treatments, except for Chinook plants in the H-Trellis system. For Cascade plants, those cultivated at reduced height showed the highest value (0.737), followed by M (0.679) and H-Trellis treatments (0.594) (Figure 4). This further confirms the excellent attitude of this variety to a low-trellis system.

In H-trellis, Chinook exhibited a statistically non-significant determination coefficient, notably lower (0.164) than the other treatments (i.e., 0.826 and 0.770 for L and M-Trellis, respectively) (Figure 4). The M-trellis is confirmed as a preferable system for this variety, given its greater propensity for production in terms of canopy and cone yield between years (Table 3). In contrast, the L-trellis appeared to have a dampening effect on the yield potential of Chinook plants in cultivation in a Mediterranean environment.

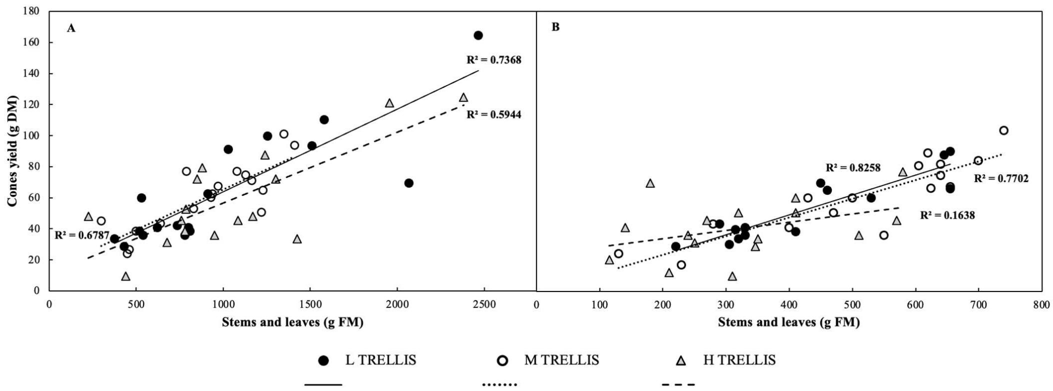

Figure 4. Regression lines, according to trellis height (low, medium, and high) between cone yield (g DM plant^{-1}; dependent variable) and weight of stems and leaves (g FM plant^{-1}; independent variable) in 'Cascade' (**A**) and 'Chinook' (**B**) hops in 2023.

3.2. Plant Height, SPAD Values, and Growth Trend

In 2022, the mean plant height was significantly influenced by all explanatory variables, including the interaction Time × V × TH (Table 5).

Table 5. *F*-values *(within subjects)* for treatments (variety and trellis height) and interactions from repeated-measures analysis of variance over time for plant height, SPAD values, and growth rates in 2022 and 2023.

2022												
Source of Variation Within Subjects	**Plants' Height SS**	**DF**	**MS**	**F-Ratio**	**SPAD Values SS**	**DF**	**MS**	**F-Ratio**	**Growth Rates SS**	**DF**	**MS**	**F-Ratio**
Time (T)	7,833,048.7	10	783,304.9	755.5 ***	20,327.4	8	2540.9	2.7 **	3953.1	8	494.1	53.8 ***
T × Variety (V)	147,821.7	10	14,782.2	14.3 ***	2667.7	8	333.5	<1 NS	1838.6	8	229.8	25.0 ***
T × Trellis height (TH)	167,898.2	20	8394.9	8.1 ***	19,129.1	16	1195.6	1.3 NS	567.3	16	35.5	3.9 ***
T × V × TH	89,457.8	20	4472.9	4.3 ***	6114.1	16	382.1	<1 NS	508.2	16	31.8	3.5 ***
Error	995,345.7	960	9,601,036.82		667,890.8	720	927.6		6974.8	760	9.2	
2023												
Source of Variation Within Subjects	**Plants' Height SS**	**DF**	**MS**	**F-Ratio**	**SPAD Values SS**	**DF**	**MS**	**F-Ratio**	**Growth Rates SS**	**DF**	**MS**	**F-Ratio**
Time (T)	7,625,289.6	8	95,3161.2	649.3 ***	418.4	5	83.7	6.4 ***	4709.0	7	672.7	54.4 ***
T × Variety (V)	42,917.2	8	5364.7	3.7 ***	268.3	5	53.7	4.1 ***	105.0	7	15.0	1.2 NS
T × Trellis height (TH)	334,357.7	16	20,897.4	14.2 ***	216.0	10	21.6	1.7 NS	1427.1	14	101.9	8.2 ***
T × V × TH	35,036.0	16	2189.8	1.5 NS	795.0	10	79.5	6.1 ***	296.3	14	21.2	1.7 *
Error	1,103,897.4	752	1468.0		5648.6	435	13.0		8229.5	665	12.4	

*** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; * significant at $0.01 < p \leq 0.05$; NS: not significant.

Otherwise, in 2023, plant height exhibited variations based on the interactions Time × V ($p < 0.001$) and Time × TH ($p < 0.001$). Cascade plants, under L-trellis systems, displayed accelerated growth, swiftly reaching the top of the wire, and differentiating secondary shoots before the other treatments in both growing seasons (Figure S1, Supplementary Material).

Chinook plants exhibited a later but more vigorous growth compared to Cascade, irrespective of the trellis height adopted in both years.

An analysis of the mean SPAD values indicated significant time-dependent differences in 2022 ($p < 0.05$), reasonably attributable to the typical physiological responses of plants cultivated in open fields. No other influences were identified from the remaining explanatory variables (Table 5). In 2023, the interaction time × V × TH significantly affected SPAD values, except for Chinook plants in the M and H-Trellis systems (Figure S2, Supplementary Material). Nevertheless, the Chinook variety consistently achieved the highest average SPAD values per plant, specifically in the H-Trellis system in 2022 (58.9) and the L-trellis in 2023 (49.3).

Hop growth rates (GR) were ultimately significantly influenced by the interaction T × V × TH in both 2022 ($p < 0.001$) and 2023 ($p < 0.05$) (Table 5). Specifically, in 2022, Cascade plants in M-Trellis exhibited the highest GR value (11.32 cm dd^{-1}) around the 203rd day of the year (DOY). The mean GR values of plants on H-Trellis also demonstrated a good fit with a Gaussian distribution pattern, reaching growth rates consistently higher than other treatments, beginning from the 196th DOY (10.85 cm dd^{-1}) and gradually decreasing to zero around the 224th DOY (Figure S3, Supplementary Material).

Chinook plants exhibited in 2022 markedly different responses in growth rates to varying trellis heights. Plants in H-Trellis displayed the highest GR value (11.52 cm dd^{-1}) around the 196th DOY, following a trend similar to that of plants in M-Trellis. The averages of GRs for the L-Trellis treatment were considerably smaller, as depicted by the Gaussian curve in Figure S3 (Supplementary Material). Likewise, in 2022, Cascade plants on M-Trellis showed the highest GR value (11.44 cm dd^{-1}) around the 181st DOY, with a slightly forward translation of the curve, possibly indicating a later growth compared to L and H-Trellis treatments. In 2023, Chinook plants on M and H-Trellis exhibited the highest GR values, recording 13.02 cm dd^{-1} on the 153rd DOY and 13.24 cm dd^{-1} on the 160th DOY, respectively. Conversely, the response of the L-Trellis treatment was modest, as evident from the specific trend of the regression.

4. Conclusions

The results underscored the substantial influence of trellis height on multiple parameters, particularly with Cascade exhibiting exceptional adaptability to low-trellis farming. The pivotal role of stem and leaf weight in determining cone yield highlighted its significance in hop cultivation.

Plant height, SPAD values, and growth rates further emphasized the distinct reactions of Cascade and Chinook to different trellis heights. These findings provide valuable insights for optimizing hop cultivation practices in semi-arid climates, underscoring the need for tailored approaches based on specific hop varieties and trellis systems. Exploring additional varieties and management strategies will be our future pursuits to enhance our comprehension of hop species behavior in diverse cropping environments. Furthermore, the study of biochemical attributes, like enzymatic activity or hormonal interaction, will represent a crucial tool for confirming the adaptation mechanisms of the species.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10020181/s1.

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3.2. Understanding the Impact of Environmental Factors, Farming Practices, and Genetic Diversity on Hop Yield and Quality

Submitted to *Journal of Agricultural and Food Chemistry*

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ABSTRACT

This study explores the effect of environmental factors, farming practices, and genetic differences on the growth, yield, and quality of hop plants (*Humulus lupulus* L.) in semi-arid regions of the southern Mediterranean basin. It emphasizes the importance of selecting suitable hop varieties and optimizing cultivation techniques for better productivity in challenging climates. The research focuses on the performance of Cascade and Chinook hop varieties under semi-arid conditions. Microclimatic data from Sicilian weather stations during the 2023 growing season were analyzed to assess the impact of extreme heat events, using Extra Degree Days (EDD). The findings highlight the necessity of tailored cultivation strategies to improve hop yield and quality, showing the crucial roles of environmental conditions, farming practices, and genetic selection. This research provides insights into expanding hop production into non-traditional regions through strategic agronomic interventions, demonstrating the potential of semi-arid Mediterranean climates to support high-quality hop cultivation. It underscores the need to understand and manage the interactions between environmental factors, farming practices, and genetic differences to maximize hop production, especially with the increasing global temperatures and climate variability.

INTRODUCTION

The cultivation of hops (*Humulus lupulus* L.), an essential ingredient in beer production and increasingly utilized in medical and human health applications[1,2], is significantly influenced by environmental factors, farming practices, and genetic variation among hop varieties[3,4]. These factors collectively impact the growth, yield, and quality of hop plants. Environmental conditions, including temperature, photoperiod, and soil composition, play critical roles in determining the productivity and chemical composition of hops[5]. Additionally, optimized farming practices, such as irrigation, fertilization, and pest management, are crucial for enhancing hop yield and quality, especially in challenging climates[6,7,8].

Indeed, the genetic diversity among hop varieties further influences their adaptability to different environments, their yield performance and brewing quality[9,10,11]. Extensive research has been conducted in regions outside the traditional hop cultivation areas, including the USA[12,13,14] and Brazil[15,16,17,18], as well as in Italy, and France[19,20,21,22]. Other countries, such as the Russian Federation, are also working to restore and innovate their hop cultivation systems after significant declines[23].

The hop-growing sector is currently expanding, fueled by the rising demand for craft beer production. In Italy, the cultivated area has steadily increased since 2020[24], now reaching 97.5 hectares dedicated to hop cultivation nationwide[25]. In southern regions, various cultivation trials have demonstrated that modern hop varieties are well-suited for open-field production[26,20]. Studies conducted in central and southern Italy, including the main islands, have shown the high adaptability of American hop varieties such as Cascade, Chinook, Comet, and Nugget, with favorable yield and quality parameters[27]. In semi-arid regions like those in the southern Mediterranean basin, adopting tailored cultivation strategies is crucial to optimizing hop production. These regions face unique challenges, such as limited water availability and high temperatures, which can significantly impact hop growth and yield[28,29]. Understanding the interactions between environmental factors, farming practices, and genetic differences is essential for developing effective hop cultivation strategies in these areas.

This paper aims to explore these interactions and provide insights into maximizing hop production in semi-arid Mediterranean climates. Specifically, this work focuses on two hop varieties particularly suited for semi-arid Mediterranean environments: Cascade and Chinook[30,31,32]. Considering the increasing prevalence of hot and dry weather globally[33], this study also provides a preliminary estimate of the effect of extreme heat events, measured through the calculation of Extra Degree Days[34] (EDD), on hop farming. Detailed microclimatic observations were collected from weather stations operated by the Sicilian Government (SIAS) in the Sicilian hinterland during the 2023 growing season. This includes assessing their potential impact on the productive and qualitative performance of hops in a Mediterranean environment, considering variations in genotype, trellis design, and farming practices.

MATERIALS AND METHODS

Study area and experimental design

The experiment considered two locations in inland Sicily, characterized by a semi-arid climate, with warm, predominantly dry summers, and mild, rainy winters. The sites included the "Sparacia" experimental farm (37°63' N, 13° 76' E; 600 m above sea level), in the Cammarata countryside (Agrigento, AG, Sicily, Italy), and a private farm (37°42’ N, 14°17’ E; 470 m above sea level) in the countryside of Pietraperzia (Enna, EN, Sicily, Italy). Throughout the hop cultivation cycle, both locations experienced infrequent rain events and elevated summer temperatures, with maximum values exceeding 30 °C[35] (Figure 1).

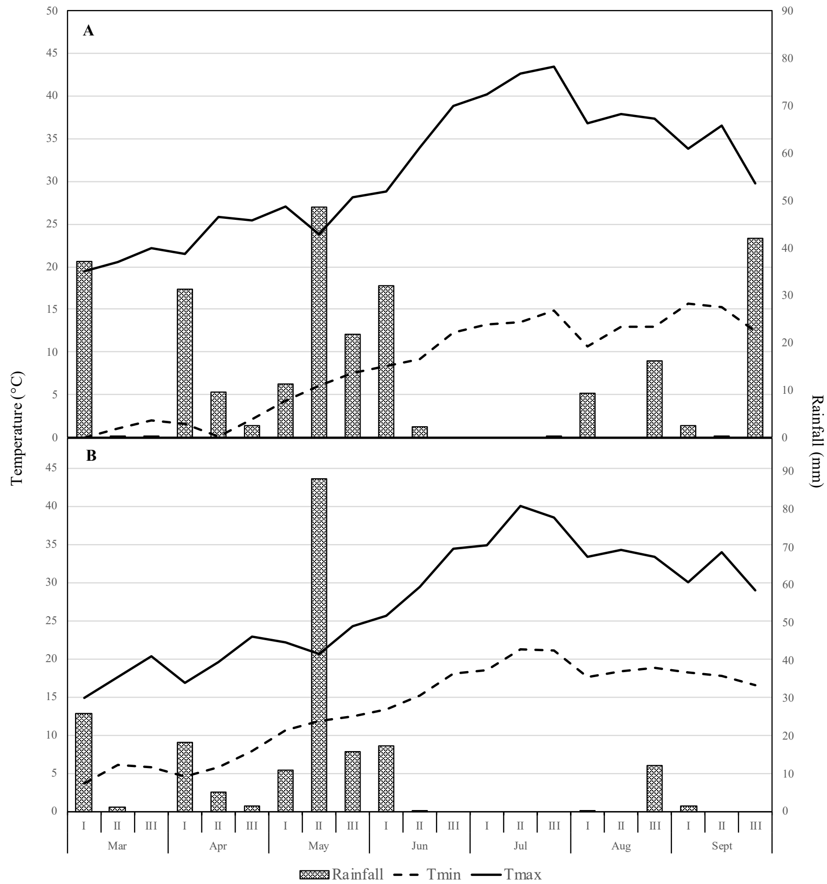

Figure 1. Ten-day values of rainfall and temperatures recorded at Sparacia (A) (Agrigento, AG, Sicily, Italy) and Pietraperzia (B) (Enna, EN, Sicily, Italy) during the 2023 growing season.

The hopyard at the "Sparacia" farm was established in 2021, while the one in Pietraperzia was set up in 2020. Both orchards were organized with rows spaced 3.0 meters apart and 0.7 meters between hop plants within the rows. The experimentation aimed to explore the productive and qualitative responses of two hop genotypes (Cascade and Chinook) subjected to different management practices, including trellis design (S-trellis and V-trellis), height of poles (2.6, 3.6, and 4.6 m), and mulching (no mulch or plastic mulch). This resulted in five different “Experimental Settings” (ES) with the factor “Variety” nested within them. Each elementary treatment had an equal number of repetitions (n=3), with each repetition consisting of 6 plants. Table 1 summarizes the experimental settings. Within each ES, plants were cultivated following the standard agronomic practices for Mediterranean environments[36,26].

Table 2. Details of Farming practices adopted in 2023 at Sparacia (Agrigento, AG, Sicily, Italy) and Pietraperzia (Enna, EN, Sicily, Italy).

Farming Practice codification	**Description**
ES1	S-trellis design; 4.6 m-high; no mulch; Pietraperzia (EN).
ES2	S-trellis design; 4.6 m-high; plastic mulch (PE film); Pietraperzia (EN).
ES3	V-trellis design; 4.6 m-high; plastic mulch (PE film); Cammarata (AG).
ES4	V-trellis design; 2.6 m-high; plastic mulch (PE film); Cammarata (AG).
ES5	V-trellis design; 3.6 m-high; plastic mulch (PE film); Cammarata (AG).

Field measurements

In both growing environments, hop plants were periodically monitored for their phytosanitary and developmental conditions. The BBCH (Biologische Bundesanstalt, Bundessortenamt, and CHemical industry) scale37 was employed to evaluate the main phenological phases, determining the starting and ending dates of each developmental stage when at least 50% of plants had reached the respective phase. This approach allowed for calculating the average duration of each stage for every replication in Growing Degree Days (GDDs; °C), computed from the day of shoot emergence (BBCH phase 0.9) using the formula:

$$\mathrm{GDD} = \sum_{i=1}^{k} (\mathrm{T_{avg}} - \mathrm{T_{base}})_{\mathrm{i}},$$

where *i* and *k* indicate the starting and ending date of each phenological stage (i.e., first and last days of measurement, respectively), T_{avg} is the daily average temperature (obtained by fitting a sine curve to daily T_{min} and T_{max}), and T_{base} is the base temperature (i.e., the temperature value below which plant growth is assumed to be zero). A T_{base} of 0 °C was adopted for the vegetative stages[38], whereas a threshold of 5 °C was selected for the reproductive phases[39].

To assess crop responses to extremely high temperatures, the accumulation of degree days over 30 °C (EDD) was computed according to Lobell et al.[34]. Hourly temperatures were derived from daily minimum and maximum temperature values using climatic data collected from local weather stations, and extreme degree days were calculated as:

$$\mathrm{EDD} = \sum_{t=1}^{N} \mathrm{DD}_{30+,t} \quad \mathrm{DD}_{30+,t} = \begin{Bmatrix} 0 & \text{if } T_t < 30\,^\circ\mathrm{C} \\ (T_t - 30)/24 & \text{if } T_t \geq 30\,^\circ\mathrm{C} \end{Bmatrix},$$

where $DD_{30+,t}$ is the EDD for hour *t*, with *t* spanning from 27 May to 29 August 2023 for a total of 2,304 hours.

All calculations were performed separately for the vegetative (VGD) and the reproductive (RGD) growth stages, with BBCH stage 5.1 (inflorescence bud visible) marking the transition between phases. The harvest time was based on a cone dry matter content of 20%[40]. All botanical fractions were harvested, weighed, and representative cone samples were sorted and stored under vacuum at -25 °C before analysis.

Chemical analyses

In all 5 experimental settings, the quality of hops cones was tested following the standard methods of Analytica European Brewery Convention (A-EBC) 7.2 for moisture content, 7.7 for α- and β-acids (%)[41].

In ES2 and ES3, essential oil (EO) content and composition were assessed through A-EBC methods 7.10 and 7.12 (32). EOs were obtained through steam distillation using a 20 L Albrigi Luigi EO extractor (Albrigi Luigi SRL, Verona, Italy). The bottom of the extractor was filled with tap water and fresh plant material was positioned on top of a grill placed above the water. The extractor was hermetically sealed, and a 2000W electric stove heater was employed to heat the apparatus, generating steam. This steam, passing through the plant material, transported the volatile components released from the raw material. The vapor was condensed as it passed through the cooler, and then collected in a graduate tube, where EO and residual waters (hydrolates) were separated. The process was sustained for a minimum of 3 hours, concluding when no additional EO was extracted for 30 minutes. EO yields were expressed in mL per 100 g, and all EOs were stored at 4 °C until analysis.

The constituents of the EOs were quantified using gas chromatography (GC) on a Clarus 500GC Perkin–Elmer apparatus with a Flame Ionization Detector (FID) and a capillary column ZB-5 (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The injection volume was 1 μL. The GC oven temperature was initially set at 60 °C for 5 minutes, increased by 3 °C per minute to 180 °C, and further increased by 20 °C per minute to 280 °C, maintaining this temperature for 10 minutes. Helium served as the carrier gas (1.2 mL/min). Injector and detector temperatures were both set at 250 °C. The percentage composition of the EO was calculated from GC peak areas without correction factors, using the Total Chrom 6.2 software (Perkin-Elmer Inc., Wellesley, PA, USA). To identify the compounds, gas chromatography coupled to mass spectrometry (GC–MS) was conducted using a Clarus 500 GC–MS apparatus from Perkin-Elmer Inc. equipped with the same capillary column, carrier gas, and operating conditions as described for GC analysis. The ionization source temperature was set at 200 °C, with an electron impact mode of 70 eV. Mass spectra were obtained through the total ion scan (TIC) mode within a mass range of m/z 45–500 uma. The total ion chromatograms and mass spectra underwent processing using Turbomass 5.4 software (Perkin-Elmer Inc., Waltham, MA, USA). Retention indices were established by injecting a standard solution of C8–C32 n-alkanes under identical conditions. Identification of the EO components was accomplished by comparing their mass spectra with those in the NIST MS Search 2.0 computer library and referencing available data in the literature[42].

Statistical analysis

Statistical analysis was performed using Minitab® (Minitab LLC, State College, PA, USA), version 19.2.0.0, and Sigmaplot® (Grafiti LLC, Palo Alto, CA, USA), version 12.0. The GLM procedure was used, setting the durations of the main growing phases and all qualitative features of hop cones (α- and β-acids, EO yields and composition) as dependent variables. The experimental scheme was a two-factor fixed nested design with three replications, with the factor 'Variety' nested within the 'Experimental Setting' (ES) factor.

To validate adherence to the ANOVA assumptions, normality and variance homogeneity of all data were assessed using the Ryan–Joiner test ($\alpha = 0.05$) and Levene's test ($\alpha = 0.05$) respectively, according to Gomez and Gomez[43]. Significant ANOVA results were further analyzed using Tukey's test[43] ($p \leq 0.05$).

Principal component analysis (PCA) was also performed using MATLAB Version 7.6.0 (The MathWorks Inc., Natick, MA, USA) to understand the relationships between experimental settings and quality parameters of each hop variety.

RESULTS

Growing duration phases, hop biomass and cone yields, and quality

No significant differences were observed for the VGD and RGD parameters (expressed in EDD) as a function of the experimental setting (ES) factor. However, significant differences in GDD requirements during the vegetative stage were observed depending on the ES considered ($p < 0.01$; Table 2). No statistical differences were detected for RGD (expressed in GDDs) based on the ES factor alone (Table 2). However, considering the varieties nested within the ES factor, significant differences between Cascade and Chinook were observed only within ES1 for the RGD parameter expressed in GDDs ($p < 0.05$; Table 2), with Chinook plants maturing earlier than Cascade (620.5 and 829.8 °C dd^{-1}, respectively). With this only exception, no statistical differences were observed for the accumulation of GDDs or EDD during the vegetative and reproductive development phases for all the sources of variation introduced.

Table 2. Averages + SEM of VGD and RGD (expressed in GDDs and EDDs) of Cascade and Chinook hops grown in 2023 in two Sicilian environments, each according to different ESs (n=3).

Source	DF	VGD (gdd)	VGD (edd)	RGD (gdd)	RGD (edd)
Experimental setting (ES)	**4**				
ES1		618.4 ± 137.2^{a}	24.91 ± 13.57	725.1±177.9	78.3±13.6
ES2		594.4± 140.7^{ab}	22.65 ± 14.56	749.1±204.5	80.5± 14.6
ES3		406.8 ± 99.3^{bc}	13.07 ± 9.09	869.9 ± 99.3	84.1±9.1
ES4		447.3 ±125.6^{ac}	16.78 ± 11.50	829.3±125.6	80.4±11.5
ES5		366.2 ± 0.01^{c}	9.36 ± 0.01	910.4 ± 0.01	87.8± 0.01
Significance of *p*-value		**	n.s.	n.s.	n.s.
Variety within ES	**5**				
var within ES1	*1*				
CA x ES1		602.4± 215.1	26.86 ± 21.19	829.8± 215.1	76.3± 21.19
CH x ES1		634.5± 0.00	22.96 ± 0.00	620.5± 0.00	80.2± 0.00
Significance of *p*-value		n.s.	n.s.	*	n.s.
var within ES2	*1*				
CA x ES2		535.2± 141.6	16.84 ± 7.91	897.0± 141.6	86.4± 7.91
CH x ES2		653.7± 137.4	28.46 ± 19.13	601.3± 137.4	74.7± 19.13
Significance of *p*-value		n.s.	n.s.	n.s.	n.s.

var within ES3	*1*				
CA x ES3		366.2± 0.00	9.36 ± 0.00	910.4± 0.00	87.8± 0.00
CH x ES3		447.3± 140.4	16.78 ± 12.85	829.4± 140.4	80.4± 12.85
Significance of *p*-value		n.s.	n.s.	n.s.	n.s.
var within ES4	*1*				
CA x ES4		447.3± 140.4	16.78 ± 12.85	829.4± 140.4	80.4± 12.85
CH x ES4		447.3± 140.4	16.78 ± 12.85	829.4± 140.4	80.4± 12.85
Significance of *p*-value		n.s.	n.s.	n.s.	n.s.
var within ES5	*1*				
CA x ES5		366.2± 0.00	9.36 ± 0.00	910.4± 0.00	87.8± 0.00
CH x ES5		366.2± 0.00	9.36 ± 0.00	910.4± 0.00	87.8± 0.00
Significance of *p*-value		n.s.	n.s.	n.s.	n.s.
Error	***20***				
Total	***29***				

VGD = vegetative growth duration; RGD = reproductive growth duration; GDD =growing degree days; EDD = extreme degree days.

Significance of the calculated F values: *** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; * significant at $0.01 < p \leq 0.05$; n.s. not significant. For the Experimental Settings (ES): means with the same letter within a column are significantly not different according to Tukey's Test ($p < 0.05$).

The biomass of hop plant stems and leaves varied significantly depending on the ES ($p < 0.001$) as well as on the variety nested within the ES factor (Figure 2). Except for ES2, where no statistical difference between the means was detected, Cascade plants consistently exhibited greater stems and leaves production, always exceeding the average values calculated among all treatment combinations (i.e., 2.89 t ha^{-1}). Specifically, within the different ESs, Cascade showed yields approximately +96%, +220%, +186%, and +87% higher compared to the average of Chinook plants within the ES1, ES3, ES4, and ES5, respectively (Figure 2). In most cases, no statistical differences were observed between cone yields at the ES factor level; only within ES1, Cascade plants showed statistically higher productivity than Chinook, with average yields of 1.87 t ha^{-1} and 0.56 t ha^{-1}, respectively ($p < 0.05$; Figure 2).

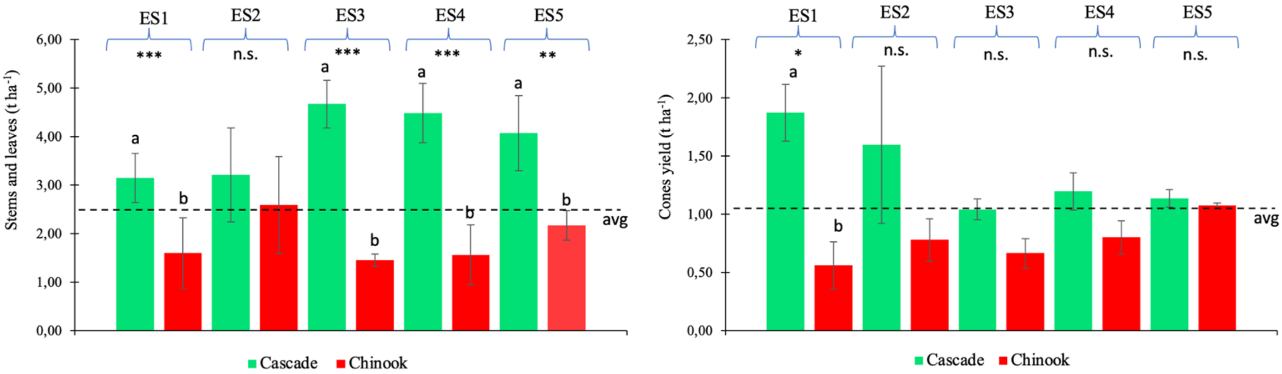

Figure 2. Mean values ± SEM and results of nested ANOVA of stems and leaves biomass (on the left) and cones production (on the right) of Cascade and Chinook hops grown in 2023 in 5 experimental settings (ES) in Sicily. In both graphs, horizontal dashed lines represent the mean value across varieties and ES. Significance of the calculated F values within each ES: *** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; * significant at $0.01 < p \leq 0.05$; n.s. not significant. For varieties within ES: different letters on top of the two bars indicate significant difference according to Tukey's Test ($p < 0.05$).

Regarding the qualitative aspects of hop cones (i.e., α-acids, β–acids, and their investigated components), the results for the main parameters determined are reported in Table 3.

Table 3 Averages + SEM of α acids, β acids, and their components from Cascade and Chinook hops grown in 2023 in two Sicilian environments, each according to different ESs (n=3).

Source	DF	**α-acids (% DM)**	**co-hum (% DM)**	**n+ad-hum (% DM)**	**β-acids (% DM)**	**co-lup (% DM)**	**n+ad-lup (% DM)**	**α/β acids ratio**	**co-hum/α-ac (%)**
Experimental setting (ES)	**4**								
ES1		8.19 ± 1.56^{ab}	2.29 ± 0.45^{ab}	5.90 ± 1.11^{ab}	4.31 ± 0.78^{a}	2.15 ± 0.39^{a}	2.16 ± 0.40^{a}	1.93 ± 0.35^{b}	27.91 ± 0.65
ES2		10.05 ± 2.04^{a}	2.58 ± 0.57^{a}	7.48 ± 1.64^{a}	3.80 ± 0.81^{ab}	1.88 ± 0.43^{ab}	1.92 ± 0.42^{ab}	2.86 ± 1.25^{ab}	25.82 ± 3.75
ES3		6.84 ± 0.63^{b}	1.78 ± 0.16^{bc}	5.06 ± 0.47^{b}	2.22 ± 0.81^{c}	1.12 ± 0.34^{c}	1.10 ± 0.47^{c}	3.50 ± 1.38^{a}	26.07 ± 0.61
ES4		7.92 ± 1.88^{ab}	2.05 ± 0.56^{ac}	5.87 ± 1.31^{ab}	2.66 ± 0.91^{bc}	1.33 ± 0.38^{bc}	1.33 ± 0.53^{bc}	3.34 ±1.52^{ab}	25.63 ± 1.26
ES5		6.24 ± 1.27^{b}	1.59 ± 0.33^{c}	4.65 ± 0.94^{b}	2.11 ± 0.96^{c}	1.06 ± 0.42^{c}	1.05 ± 0.53^{c}	3.48 ± 1.64^{a}	25.39 ± 0.88
Significance of *p*-value		***	***	***	***	***	***	*	n.s.
Variety within ES	**5**								
Var within ES1	*1*								
CA x ES1		6.91 ± 0.93^{b}	1.92 ± 0.30^{b}	4.99 ± 0.63^{b}	3.88 ± 0.96	1.91 ± 0.43	1.97 ± 0.54	1.86 ± 0.54	27.76 ± 0.92
CH x ES1		9.46 ± 0.61^{a}	2.65 ± 0.15^{a}	6.81 ± 0.46^{a}	4.74 ± 0.23	2.39 ± 0.11	2.35 ± 0.12	2.00 ± 0.06	28.06 ± 0.40
Significance of *p*-value		*	*	*	n.s.	n.s.	n.s.	n.s.	n.s.
var within ES2	*1*								
CA x ES2		11.02 ± 2.60	2.90 ± 0.64a	8.13 ± 2.06	3.43 ± 1.00	1.75 ± 0.52	1.69 ± 0.48	3.51 ± 1.58	26.54 ± 3.31
CH x ES2		9.09 ± 0.94	2.26 ± 0.32b	6.83 ± 1.08	4.16 ± 0.51	2.01 ± 0.36	2.14 ± 0.21	2.21 ± 0.36	25.10 ± 4.75
Significance of *p*-value		n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
var within ES3	*1*								
CA x ES3		6.89 ± 0.85	1.79 ± 0.23	5.10 ± 0.64	2.55 ± 0.94	1.24 ± 0.40	1.31 ± 0.55	3.12 ± 1.68	25.91 ± 0.92
CH x ES3		6.79 ± 0.50	1.78 ± 0.13	5.01 ± 0.37	1.88 ± 0.64	1.00 ± 0.30	0.89 ± 0.34	3.88 ± 1.21	26.22 ± 0.09
Significance of *p*-value		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
var within ES4	*1*								
CA x ES4		6.58 ± 1.46^{b}	1.65 ± 0.44^{b}	4.93 ± 1.02^{b}	3.36 ± 0.73^{a}	1.60 ± 0.36	1.76 ± 0.37^{a}	1.96 ± 0.01^{b}	24.92 ± 1.38
CH x ES4		9.25 ± 1.16^{a}	2.44 ± 0.37^{a}	6.81 ± 0.79^{a}	1.96 ± 0.20^{b}	1.06 ± 0.11	0.90 ± 0.09^{b}	4.72 ± 0.27^{a}	26.34 ± 0.74

Significance of *p*-value		*	*	*	*	n.s.	**	***	n.s.
var within ES5	*1*								
CA x ES5		5.65 ± 1.70	1.47 ± 0.48	4.18 ± 1.21	2.83 ± 0.82a	1.36 ± 0.42^{a}	1.47 ± 0.40^{a}	1.99 ± 0.05^{b}	25.84 ± 0.94
CH x ES5		6.83 ± 0.34	1.70 ± 0.08	5.13 ± 0.27	1.38 ± 0.12b	0.76 ± 0.06^{b}	0.62 ± 0.06^{b}	4.97 ± 0.20^{a}	24.93 ± 0.64
Significance of *p*-value		n.s.	n.s.	n.s.	*	*	**	***	n.s.
Error	***20***								
Total	***29***								

co-hum = co-humulone; n+ad-hum =n+ad-humulone; co-lup = co-lupulone; n+ad-lup =n+ad-lupolne; α/β acids = α to β acid ratio; co-hum/α-ac = co-humulone to α-acids % ratio.

Significance of the calculated F values: *** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; * significant at $0.01 < p \leq 0.05$; n.s. not significant. For the Experimental Settings (ES): means with the same superscript letter within a column are significantly not different according to Tukey's Test ($p < 0.05$).

Except for the percentage of cohumulone to total α-acids, all qualitative parameters were significantly influenced by the ES factor. The yields of total α-acids ($p < 0.001$), cohumulone ($p < 0.001$), and n+ad-cohumulone ($p < 0.001$) were significantly higher in ES2, with average values of approximately 28%, 25%, and 29% higher compared to the average of the other treatments (Table 3). For β-acids and their components (i.e., lupulone and n+ad-lupulone), significant differences were also observed at the ES level, with ES1 boosting the yield of these compounds. The α/β-acids ratio also varied significantly due to the specific ES ($p < 0.05$; Table 3), with ES5 showing the highest value, likely due to the lowest β-acid yields recorded for this treatment. Considering the nested factors shown in Table 3 (i.e., variety within ES), the highest values for α-acids, cohumulone, and n+ad-cohumulone were observed in Cascade plants within ES2, although these were not statistically different from Chinook within the same ES. For α-acids, significant differences were identified between Cascade and Chinook in ES1 ($p < 0.05$) and ES4 ($p < 0.05$), with significantly higher values showed by Cascade plants in the first case, and by Chinook in the second case. The highest average yields in absolute terms for β-acids were observed in Chinook in ES1, although these were not statistically different from Cascade within the same experimental treatment. Significant differences in β-acid yields were recorded in ES4 ($p < 0.05$) and ES5 ($p < 0.05$), with Cascade showing 71% and 106% higher values than Chinook in ES4 and ES5, respectively. Similar to the β-acid yields, and clearly correlated, significant differences in lupulone content were observed within the ES5 treatment ($p < 0.05$). For n+ad-lupulone, significant differences ($p < 0.01$) were noted in both ES4 and ES5, with Cascade showing significantly higher values compared to Chinook (Table 3).

For EO yield, statistical differences were spotted between the experimental settings, with superior yields in ES2 ($p < 0.05$; Table 4). Significant differences between varieties emerged only within ES2 ($p < 0.05$), with Cascade showing EO yields approximately 42% higher compared to Chinook under the same experimental conditions. The detected amounts of the three main EO components—myrcene, humulene, and caryophyllene—were significantly affected by both the genotype and the experimental setting. The concentration of myrcene varied significantly with the experimental setting ($p < 0.001$; Table 4). The highest yields were found in ES3, with production levels 25% higher than the other setting. Significant differences ($p < 0.05$) were also observed between varieties, with Cascade EO containing more myrcene than Chinook in both ES2 and ES3. The highest humulene content was recorded in ES2, with concentrations approximately double than those in the other setting (p

< 0.001; Table 4). Within these ES, Chinook consistently showed the highest humulene values ($p < 0.001$). Caryophyllene concentrations varied significantly depending on the ES ($p < 0.001$) and on the variety within either ES2 ($p < 0.001$) and ES3 ($p < 0.001$). Chinook EO was the most enriched in caryophyllene ($p < 0.001$; Table 4). For other compounds in hop EOs, significant differences were observed both at the ES level and among varieties within specific ES treatments ($p < 0.001$; Table 4).

Table 4 Averages + SEM of EOs yields and main compounds from Cascade and Chinook hops, obtained from cones harvested in 2023 in two experimental settings in Sicily.

Source	DF	EOs (mL 100g^{-1} DM)	Myrcene (%)	Humulene (%)	Caryophyllene (%)	Others (%)
Experimental setting (ES)	**1**					
ES2		1.62 ± 0.33^{a}	55.29 ±0.13^{b}	23.57 ± 0.10^{a}	11.65 ± 0.06^{a}	9.43 ± 0.03^{b}
ES3		1.34 ± 0.52^{b}	69.54 ± 0.09^{a}	12.13 ± 0.01^{b}	5.40 ± 0.01^{b}	12.92 ± 0.06^{a}
Significance of *p*-value		*	***	***	***	***
Variety within ES	2					
var within ES2	*1*					
CA x ES2		1.91 ± 0.09^{a}	67.23 ± 0.01^{a}	14.55 ± 0.01^{b}	5.72 ± 0.00^{b}	12.50 ± 0.00^{a}
CH x ES2		1.34 ± 0.15^{b}	43.37 ±0.03^{b}	32.59 ± 0.03^{a}	17.59 ± 0.02^{a}	6.36 ± 0.01^{b}
Significance of *p*-value		*	*	***	***	***
var within ES3	*1*					
CA x ES3		1.21 ± 0.61	77.89 ± 0.01^{a}	10.89 ± 0.00^{b}	4.27 ± 0.00^{b}	6.94 ± 0.01^{b}
CH x ES3		1.53 ± 0.40	61.18 ±0.01^{b}	13.38 ± 0.00^{a}	6.54 ± 0.01^{a}	18.90 ± 0.00^{a}
Error	***8***	n.s.	*	***	***	***
Total	***11***					

EOs = essential oils; Others = other compounds.

Note: for each variable, means within the same column with the same superscript letter are significantly not different according to Tukey's Test ($p \leq 0.05$); *** significant at $p \leq 0.001$; ** significant at $0.001 < p \leq 0.01$; * significant at $0.01 < p \leq 0.05$; n.s. not significant.

Association between growing duration phases and hop quality characteristics (PCA)

A PCA was conducted to explore the possible association between thermal requirements, durations of vegetative (VGD) and reproductive (RGD) phenological phases, biomass production, and the main qualitative characteristics of hop plants, including the content of α- and β–acids, α- to β–acids ratio, and co-humulone to total α-acids ratio.

A biplot of the scores and loadings of the PCA is shown in Fig 3. Two principal components were individuated, explaining 79.9% of the total variance, (54.0% and 25.9% the first (PC1) and the second (PC2) principal components, respectively). The first PC was strongly associated with the durations of phenological stages, which appear well separated and positioned into opposite quadrants. The second PC was otherwise more associated with biomass yield and, to a lesser extent, with the α/β–acids ratio. The PC1 spread the sample scores according to the growth location; irrespective to the variety, all samples subjected to ES1 and ES2 (i.e., obtained from the Pietraperzia farm) were plotted on the positive side of the PC1, whereas all samples from ES3, ES4 and ES5 (i.e., from the Sparacia farm) were on the left side of the same PC. The first group of samples (ES1 and ES2) were characterized by a prolonged vegetative phase (VGD) and higher α and β acids content. Contrastingly, the second group of samples (ES3, ES4, and ES5) showed a prolonged reproductive phase (RGD). The PC2 spread the samples according mainly to genotype, showing most Chinook samples (4 out of 5) in the positive side and the majority of Cascade's (4 out of 5) in the lower part of the biplot. On the positive side of PC2 the samples showing the highest α- to β– acids ratio took place, while on the negative side were found the samples that showed higher biomass production, two genotype-dependent characteristics (Table 3).

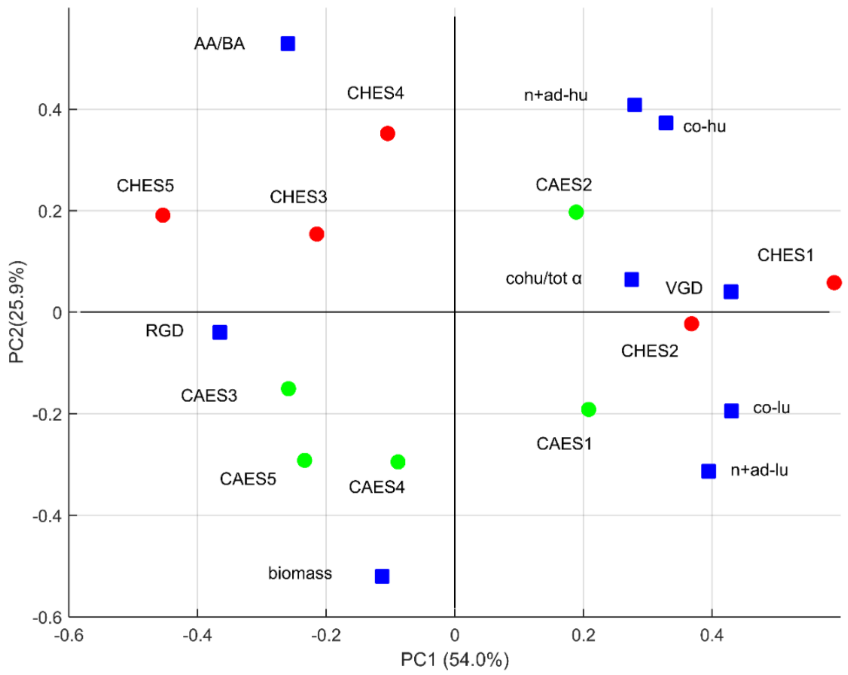

Figure 3. Biplot of scores and loadings (blue squares) of the PCA carried out on the duration of vegetative (VGD) and reproductive (RGD) growth stages, biomass yields, and analytical features (co-hu = cohumulone; n+ad-hu = n+ad humulone; cohu/tot α = cohumulone to total α acid ratio; co-lu = colupulone; n+ad-lu = n+ad lupulone;

AA/BA = α to β acids ratio) in Cascade (CA, green dots) and Chinook (CH, red dots) hop cultivars subjected to five experimental settings (ES 1 to 5). Each point is the average of 18 plants.

DISCUSSION

Our findings indicate that while the parameters VGD and RGD, when expressed in EDD, remain relatively stable across different experimental settings, the GDD requirements for VGD are influenced by these settings. This suggests that environmental or management factors can impact vegetative growth, exerting an effect even higher than that of genotype. The crucial role of the duration and heath accumulation in hop vegetative stage was already evidenced by previous research[20], showing that a shorter vegetative stage is generally associated with a better plant development. Contrastingly, in the reproductive phases, the consistent RGD values across experimental settings imply minimal effect due to the environmental conditions alone. However, significant differences in RGD between Cascade and Chinook within ES1—where Chinook showed earlier development—highlight important varietal differences in developmental timing. These differences could be crucial for cultivation strategies, especially in adapting to climate change, as supported by previous research[44,45].

Regarding biomass production, results show that hop plant biomass, particularly in stems and leaves, is significantly influenced by both experimental settings and variety. Cascade plants consistently produced more biomass than Chinook. Although cone yield did not vary significantly across settings, Cascade generally demonstrated higher productivity compared to Chinook, suggesting that while Cascade excels in biomass production, cone yield differences are more dependent on specific conditions.

Except for the percentages of cohumulone to total α-acids that were slightly low, most of the investigated qualitative parameters were in line with the genotype characteristics. In particular, our hop samples showed on average comparable levels of α-acids, and lower levels of β-acids when compared to the same hop cultivars grown in the Mediterranean areas[11]. In our tests the quality parameters were significantly affected by the interaction among genotype and experimental setting, with Cascade consistently showing the highest values. The α to β acids ratio was affected by the low levels of β acids and, for the investigated varieties, the values were slightly high. As reported in other studies, this trend could be due to the chemical properties of β acids that are more prone to oxidation when hops are stored as cones because of their higher surface area[46]. These findings align with the results of numerous field experiments conducted in Mediterranean environments by various research groups[47,20,22].

In terms of essential oil (EO) yields, significant differences based on the experimental setting were observed, with ES2 yielding the highest production of oil that were in line with the reference values for the investigated cultivars[48]. This underscores the importance of optimizing conditions to maximize EO yields. Cascade plants consistently produced higher EO yields than Chinook across different settings, indicating that some varieties may be better suited for specific conditions. The hop oil composition showed significant differences due to genotype and interaction between genotype and ES, but in any case, the composition was different compared to the reference values for the studied cultivars, with higher levels of myrcene for both hops[49]. This supports the notion that both environmental conditions and genetic factors interplay to influence EO production and storage in hop plants, aligning with findings from Ruggeri et al.[29], Gresta et al.[31], and Forteschi et al.[30]. The study also confirms the impact of terroir on hop cone quality, as even minor climatic variations significantly affect outcomes[50,51,52]. Future research should focus on optimizing conditions for higher yields and targeted compound production, benefiting both research and industry.

Finally, Principal Component Analysis (PCA) revealed that both experimental settings and genetic factors significantly affect hop plant phenology and quality. The Pietraperzia location, with or without mulch, showed higher α- and β-acids content and a longer vegetative phase, suggesting the existence of some physiological relationship between stages duration and cones quality features. Further investigations on a regional scale are needed to explore this aspect. Genetic traits also influenced samples based on the cohumulone to total α acids ratio and biomass production. PCA effectively demonstrated how location, farming practices, and genotype impact hop plant development and quality.

CONCLUSIONS

The research highlights the substantial influence of environmental conditions, agronomic practices, and genetic variability on hop plant growth, yield, and quality. Our findings indicate that hop vegetative growth is predominantly regulated by environmental factors rather than genetic differences, as evidenced by the significant variability in growth patterns observed across different environmental settings.

From a genotypic perspective, Cascade consistently exhibited superior performance compared to Chinook in terms of biomass and essential oil production, although these outcomes were contingent on specific environmental conditions. This underscores the critical need for genotype-specific and environment-adapted cultivation strategies.

Furthermore, the interaction between genotype and environment plays a pivotal role in determining hop cone quality, as demonstrated by the variations in essential oil profiles and acid content. These results emphasize the necessity of optimizing environmental conditions to maximize hop yield and quality, reinforcing the importance of targeted agronomic management for specific hop cultivars.

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3.3. Conclusions

In summary, both studies clearly underscore the substantial impact of environmental conditions, trellis height, and agronomic practices on the growth, yield, and quality of hop plants. The findings consistently highlight Cascade's superior adaptability and biomass production, particularly in low-trellis systems, reinforcing the importance of developing variety-specific and trellis-specific cultivation strategies.

Additionally, the genotype-environment interaction emerged as a crucial factor influencing cone quality, with significant variations in essential oil profiles and acid content further emphasizing the need for targeted, environment-adapted agronomic practices. Taken together, these results suggest that future research should not only explore a broader range of hop varieties but also delve deeper into biochemical attributes, such as enzymatic activity and hormonal responses, to refine cultivation techniques. This is especially important for optimizing hop production in diverse cropping environments, particularly semi-arid regions.

4. CHAPTER IV

This section of the thesis explores how hop species (*Humulus lupulus* L.) adapt to subtropical climate environments, focusing on the potential for expanding cultivation into non-traditional regions such as southwest Florida. The two papers presented above were based on experimental data collected during trials at the Gulf Coast Research and Education Center (Wimauma, FL, USA) at the University of Florida. These studies were conducted as part of my internship as a visiting student researcher, sponsored by the Fulbright Foundation.

The first study, submitted to the *Agronomy Journal* (Wiley), evaluated whether off-season cropping of hops, enabled through "multiple harvests," could promote sustainable and economically viable cultivation in these new areas. The findings suggest that implementing a double annual harvest can optimize hop production in subtropical climates, aligning with the increasing global interest in hop cultivation driven by the burgeoning craft beer industry. Methodologies for calculating growing degree days (GDDs) were assessed, highlighting that Method I offered superior stability for both vegetative and reproductive phases, although Method II provided a better overall fit. Corrections for maximum temperatures above 30 °C reduced variability and improved model accuracy, indicating distinct growth responses in subtropical versus temperate regions. The study underscores the necessity of appropriate post-harvest processing infrastructure to support local agricultural stakeholders and brewing industries.

The second study, submitted to *Agronomy* (MDPI), adopted an integrated approach to explore the relationship between vertical growth rates and vegetative development in the Cascade hop cultivar across two distinct environments: the semi-arid region of Sicily, Italy, and the subtropical climate of Florida, USA. Utilizing the BBCH scale, this research demonstrated consistent correlations between growth and developmental stages, offering insights into the dynamic interactions between plant development and external factors such as pests and diseases. The study underscores the importance of understanding growth characteristics associated with developmental stages to inform targeted interventions aimed at improving crop health and yield. This integrated perspective provides a valuable tool for comprehending hop cultivation dynamics, suggesting that insights from developmental observations can be used to predict growth dynamics at any point during the vegetative phase. Such knowledge is crucial for optimizing hop production and addressing the challenges associated with cultivating hops in new, diverse climatic zones.

The combined findings from both studies highlight the adaptability of hop cultivation in subtropical and semi-arid climates. By employing innovative cultivation strategies such as multiple harvests and understanding the interactions between growth and development stages, it is possible to enhance hop production outside traditional temperate zones. These studies reveal that subtropical regions can support economically viable hop production, provided that adequate infrastructure is in place. Moreover, integrating growth and developmental insights offers a strategic advantage in managing environmental challenges and optimizing crop yield and quality. This research not only advances the scientific understanding of hop cultivation in diverse climates but also provides practical recommendations for expanding hop production to new regions, supporting local agriculture, and meeting the demands of the global brewing industry.

4.1. Growing Hops (*Humulus lupulus* L.) in a Sub-Tropical Environment: Climatic Patterns Affect Hop Phenological Stages' Lenght, and Crop Performance Within Years and Growing Seasons

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Abbreviations: AC = a-acids; AC/BC = a- and b-acids ratio; ANOVA = analysis of variance; BC = b-acids; Car = caryophyllene; CQV = coefficients of quartile variation; CV = coefficient of variation; DM = dry matter; EOs = essential oils; Far = farnesene; FM = fresh matter; GDD = growing degree day; Ger = geraniol; GLM = general linear model; Hum = humulene; Lin = linalool; Myr = myrcene;

ABSTRACT

Crop diversification has emerged as a crucial strategy for advancing agricultural sustainability and mitigating climate change impacts, while also presenting novel economic opportunities in emerging climatic zones. The burgeoning global craft beer industry has intensified interest in cultivating hops (*Humulus lupulus* L.) in non-traditional regions, including Brazil, the Southeastern United States, and the Mediterranean. Traditionally adapted to temperate climates, hops must be acclimated to local conditions for successful cultivation in new environments. This study assessed various methodologies for calculating growing degree days (GDDs) and found that Method I demonstrated superior stability for both vegetative and reproductive phases, though Method II exhibited a better overall fit. While the application of Tmax > 30 °C corrections reduced variability and enhanced R^2 values, no single method proved definitively superior. Analysis indicated elevated GDD requirements during vegetative stages, attributable to increased thermal averages across the two-year study period. Positive correlations between GDDs and biometric data suggest distinctive growth responses in sub-tropical environments compared to temperate regions. Quality evaluations revealed significant variability in bittering and aromatic compounds, with spring 2021 showing higher

overall quality. These findings advocate for the viability of a double annual harvest as a strategic approach to optimizing hop production in sub-tropical climates. The results underscore the necessity for appropriate infrastructure to support post-harvest processing and provide valuable insights for local agricultural stakeholders and brewing industries.

1. INTRODUCTION

Since the late ’90s, the introduction of new crops has been identified as a means of promoting the sustainability of modern agriculture (Heywood, 1999). Indeed, crop diversification is emerging as a promising strategy for the climate resilience of agricultural areas, contributing to not only mitigating the negative impact of climate change (Ahmed and Stepp, 2016), but also providing novel economic and market opportunities in new climatic zones (Lakhran et al., 2017).

In recent years, rapid growth in the global craft beer market (Barth-Hass report, 2022) has increased interest in growing hops (*Humulus lupulus* L.) in non-traditional production regions, such as Brazil, Southeastern United States, and the Mediterranean region (Acosta-Rangel et al., 2021; Alves et al., 2019; Acosta-Rangel, et al., 2024 Jastrombek et al., 2022; Marceddu et al., 2022a, 2024; Rossini et al., 2016; Ruggeri et al., 2023). Hops are a short-day species, typically grown in temperate climates (Neve, 1991). Therefore, the identification of adaptive cultivars to local climatic conditions is among the top prerequisites for the development of hops cultivation in new growing areas, especially located at non-optimal latitudes.

Growth and development rates of hops are highly influenced by both biotic and abiotic stressors (Smith et al., 2021; Marceddu et al., 2022b). In general, temperature is one of the major environmental variables influencing growth, development, yield, and quality of many commercial crops (Menzel et al., 2006; Luo, 2011). Plants have basic temperature requirements to complete specific phenological phases (Hunter & Lechowitz, 1992; Galàn et al., 2001). Phenological dynamics is further influenced by complex genetic by environmental interactions (Ruml & Vulic, 2005). Therefore, knowledge of crop phenology is very important, especially for optimal crop yield in a new environment. Such information is needed to develop better cropping systems so that high and/or stable productivity can be achieved (Ong, 1985). Moreover, the crops’ growth and developmental rate are accelerating in many climatic conditions due to the increasing warming trend caused by climate change (Cleland et al., 2007). In vulnerable environments, a deeper knowledge of crops’ phenological responses would enable to develop more effective adaptation strategies, which might compensate for the harmful impact of these phenomena (Anwar et al., 2015; Hoffmann & Sgrò, 2011). In hop, the photoperiod was assessed as one of the main factors determining hop growth and floral induction (Thomas & Schwabe, 1969). Recent studies showed high phenological plasticity and acclimation of hops to new growing environments and photoperiod manipulation (Jastrombek et al., 2022; Fortuna et al., 2023; Acosta-Rangel et al., 2024). As stressed by these authors, photoperiod manipulation using supplemental lighting allowed to have more than one production cycle per year, by inducing vegetative bine growth and preventing early flowering during the growing season. In fact, since hops need >15 hr of daylight during the vegetative growth and <15 hr in the reproductive growth stages (Jastrombek et al., 2022), supplemental lighting in the early season allowed the crop to define its real cultivation potential both in term of yield and quality in those new environments.

Although several studies already demonstrated that hops can be successfully grown in tropical and sub-tropical environments (Jastrombek et al., 2022; Acosta-Rangel et al., 2021, 2024; Agehara, 2020a, 2020b, 2021, 2022), there is still a lack of knowledge on the effects of temperature on the phenology of hops in such environments. High air temperatures have been already reported to affect hop crop phenology, including the start and duration of development stages, with significant implications for hop cone yield and quality (Mozny et al., 2009).

Hence, to fill the existing gap in the knowledge, a trial was carried out in two subsequent growing seasons (2020 and 2021) in west-central Florida. The experiment involved the setting of an experimental hopyard (4.6-meter-high trellis) with supplemental lighting for photoperiod manipulation (Agehara et al., 2020a). This work aims to deepen the effectiveness of this novel cropping management on the development stages of hops in a double-season production system, as well as its impact on productive and qualitative crop responses. The outcomes will be of support in identifying effective adaptation options to deal with a challenging temperate-climate crop in a new growing area, taking into special account the peculiar climatic features of a sub-tropical environment.

2. MATERIALS AND METHODS

2.1 Experimental site and trial design

The experiment took place at the University of Florida's Gulf Coast Research and Education Center in west-central Florida (27.76°N; 82.23°W; 40 m a.s.l.), over two years (2020 and 2021) with two growing seasons per year (Spring and Fall).

The site is reported to have a humid subtropical climate with hot and humid summers and mild winters. The mean annual precipitation is 1350 mm (over 10-year assessment), mainly distributed from June to August. The daily temperatures range from 22.1 to 33.5 °C during the hottest months (from June to September) and from 8.7 to 26.2 °C during the coldest months (FAWN, 2022). The relative humidity ranges from 43 to 95% (FAWN, 2022). The soil was classified as an Hyperthermic Oxyaquic Alaquods (USDA-NRCS), with a pH of 6.5, 1% organic matter content and a cation exchange capacity of 4.1 meq/100 g (at 0 to 15 cm in depth). During the 2-year trial, both temperature and rainfall trends appeared to follow the above-mentioned climatic patterns at the experimental site.

2.2 Experimental design and crop management

Tissue-cultured cv 'Cascade' plantlets were purchased from a commercial nursery (Agri-Starts, Apopka, FL, USA). Hop plants were transplanted in the field in February 2020. The trellis system was built using six-meter poles and 8 mm diameter galvanized cables; the rows were spaced 4.56 m apart, and oriented in North-South direction. Additional specifications of the trellis layout and configuration are provided by Agehara et al., 2020b. The experiment layout was a randomized block design, with four field replications (experimental units) distributed in a 78-meter row. Each plot consisted of 10 hills spaced 0.9 m apart, and each hill comprised two hop plants.

A sub-superficial drip irrigation system was provided, and irrigation was applied as needed (FAWN, 2022). The amounts of macronutrients applied through fertigation are provided in Table 1.

Table 3. Macro-elements distributed during each growing season through the fertigation system.

Growing season	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})
Spring 2020	190	33	252	53
Fall 2020	351	61	466	98
Spring 2021	318	56	422	89
Fall 2021	353	62	469	99

Weeds were chemically controlled both between and on plant rows. Commercial insecticide and fungicides were sprayed as foliar treatments to prevent or limit the spread of spider mites, aphids, and fungal diseases.

To prevent early flowering (an expected effect of reduced photoperiod in tropics; Acosta-Rangel et al., 2021), a supplemental lighting system was added to the trellis and programmed following the method described by Agehara et al. (2020a). Day length was extended to at least 16.5 hours using supplemental lighting from 6 pm to midnight. Supplemental lighting was discontinued on May 1 in Year 1 Spring, September 24 in Year 1 Fall, May 1 in Year 2 Fall, and September 7 in Year 2 Fall, after all plants reached the top trellis cable. Moreover, the effectiveness of LED light on flowering prevention was checked by a biweekly assessment.

2.3 Field measurements and phenology evaluation

Phenological observations were used to determine major developmental stages according to the BBCH scale (Rossbauer et al., 1995). The start and end dates when reaching a certain development stage by at least 50% of plants were recorded. The duration of each developmental stage was measured in both days (dd) and Growing Degree Days (GDDs; °C). GDDs were calculated starting on the day of shoot emergence, corresponding to the BBCH phase 0.9. All calculations were performed according to the general formula as follows:

$$GDD = \sum_{i=1}^{k}(T_{avg} - T_{base})_i \qquad \text{Eqn. 1}$$

where *i* and *k* indicate the start and end dates of each growth stage, T_{avg} is the daily average temperature, and T_{base} is the base temperature (i.e., the temperature value below which plant growth is assumed to be zero). T_{base} was chosen by referring to recent hop phenological studies (Carrubba et al., 2022; Marceddu et al., 2020). Specifically, Marceddu et al. (2020) discussed the hypothesis of differentiating the T_{base} according the phenological crop phases. T_{base} = 0 °C was adopted in the vegetative phases, whereas, after the phase BBCH 2.9, a T_{base} = 5 °C (Srečec et al., 2008) was used for GDD calculations. T_{avg} was calculated based on daily temperature measurements, as follows:

$$T_{avg} = \frac{(T_{max}+T_{min})}{2} \qquad \text{Eqn.2}$$

where T_{min} is the minimum day temperature; when $T_{min} < T_{base}$, then T_{base} should be used for calculation. This, however, never happened in both trial growing seasons.

To assess the influence of heat accumulation on hops growth in a sub-tropical environment, three different criteria for T_{max} were used to calculate GDDs. In Method I, raw T_{max} values were used regardless of the values. In Method II, a ceiling value of 30 °C was used for T_{max} when T_{max} exceeded 30 °C. In Method III, the value T = 30 – (T_{max}-30) was used when T_{max} exceeded 30 °C. The tested GDDs calculation methods are summarized in Table 2.

Table 2. GDD's calculation methods adopted in the trials. Tbase: base temperature; Tmax: maximum daily temperature.

Phenological phase	**Method**	**T_{base}**	**T_{max}**
Vegetative growth (0.9- 2.9 BBCH)	I	0 °C	T_{max}
	II	0 °C	If $T_{max} \leq 30$ °C, then T_{max}; if $T_{max} > 30$ °C, then 30 °C
	III	0 °C	If $T_{max} \leq 30$ °C, then T_{max}; if $T_{max} > 30$ °C, then 30 °C – (T_{max} – 30 °C)
Reproductive phase (5.1 – 8.9 BBCH)	I	5 °C	T_{max}
	II	5 °C	If $T_{max} \leq 30$ °C, then T_{max}; if $T_{max} > 30$ °C, then 30 °C
	III	5 °C	If $T_{max} \leq 30$ °C, then T_{max}; if $T_{max} > 30$ °C, then 30 °C – (T_{max} – 30 °C)

Harvesting was performed using a hop harvester (Hopster 5P; HopsHarvester LLC, Honeoye Falls, NY, USA) following the methods described by Agehara et al. (2022). Fresh cones were dried at 55 °C until 10% of moisture was reached, and dry cone yield was reported in tons of dry matter per hectare (t DM ha^{-1}). Four samples of dried cones (120 g) were then collected in each season and shipped for quality analysis (Alliance Analytical Laboratories Inc., MI, USA). All the other botanical fractions were harvested and weighted, and their values were reported in tons of fresh mater per hectare (t FM ha^{-1}).The α acids (%), β acids (%), and the α acid/β acid ratio were therefore determined, as well as the total essential oil content (mL 100 g^{-1}), and the content in the major aromatic compounds (i.e., myrcene, humulene, caryophyllene, farnesene, linalool and geraniol).

2.4 Statistical analysis

Statistical analysis was performed using the software Minitab® version 19.2.0.0 (Minitab Inc., State College, PA, USA, 2013). To ensure compliance with the ANOVA assumptions, all data were tested for normality and for variance homogeneity by means of the Ryan-Joiner test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$), respectively, and when necessary, the proper data transformations were applied (Gomez and Gomez, 1984). When data transformation was made, all statistical evaluations were performed on transformed data;

however, the mean values reported in tables and graphs have been transformed back into the original scale (Steel and Torrie, 1980).

The statistical analysis was performed following the GLM procedure. Since all replicate measurements were made on the same experimental unit, all the biometric data and the quality parameters (α–acids, β–acids, and essential oil content) were subjected to a nested ANOVA, according to the hierarchical classification model of Cochran (1967), including as explanatory variables the year and the harvest seasons within each trial year When the ANOVA offered statistically significant results, the differences between mean values were appreciated through Tukey's test ($p \leq 0.05$) (Gomez and Gomez, 1984).

The studies on GDDs were made on pooled data. To identify the GDD methods with the best fitting with the hop phenological data, coefficients of quartile variation (CQV%) and coefficients of variation (CV%) for each method were calculated. CQV% was calculated as shown in the following formula:

$$CQV\% = \frac{(Q3-Q1)}{(Q3+Q1)} x\ 100 \quad \text{Eqn.3}$$

where Q3 and Q1 are the values corresponding to the 3rd and the 1st quartile, respectively, of each dataset, being the GDD values calculated by means of each method representing one dataset (Bonett, 2006).

A Principal Component Analysis (PCA) was performed as first step before the regression analysis, with the aim to detect multicollinearity and to reduce the number of parameters relative to all the observations. Then, a simple correlation matrix was built up, by calculating Pearson's correlation coefficients between most representative biometric and quality data, by one side, and the GDD requirements for each main phenological stage of the crop cycle, by the other.

3. RESULTS

3.1 Phenological observations and GDDs' calculations

The different calculation methods of the GDDs allowed us to appreciate a wide variability within the values of heat accumulation (°C) (table 3; figures 1 and 2). The distinction made between the vegetative and reproductive phases was functional to a more in-depth study of the effect of temperature on the phenological development trends of the species in a sub-tropical environment. The coefficients of variation (CV%) and interquartile variation (CQV%) offered an interesting point of view in the analysis of the dataset (table 3).

Table 3. Coefficients of variation (CV%), coefficients of quartile variation (CQV%), angular coefficient (m) and coefficient of determination *(R^2)* of the regression line "GDD vs days", for the GDD datasets obtained using three methods (I to III) for the vegetative (0.9 to 2.9 BBCH) and reproductive (5.1 to 8.9 BBCH) stages in a 2-year cultivation trial of hop in central-west Florida.

Method	Vegetative stages (from 0.9 to 2.9 BBCH)				Reproductive stages (from 5.1 to 8.9 BBCH)			
	CV%	CQV%	m	R^2	CV%	CQV%	m	R^2
I	14.3	14.5	–33.8	0.288	23.4	12.8	11.3	0.057
II	12.3	12.4	–26.6	0.256	14.5	15.4	12.0	0.243
III	10.2	10.0	–21.4	0.258	15.8	14.2	12.5	0.239

For GDDs calculated in the vegetative phases, the CV% varied from 10.2% (III) to 14.3% (I), showing a lower variability within the calculation method with correction for high temperatures (i.e., deduction of the maximum temperatures exceeding 30 °C). Similarly, CQV% showed the same trend, with values of 14.5% 12.4%, and 10%, respectively for Methods I, II, and III. Considering the adoption of the same T_{base}, all the methods had a similar range of distribution as shown in the corresponding regression lines, all belonging to the same grouping (figure 2a). The angle of the regression lines was, however, diversified, showing higher angular coefficients *m* in absolute value for the method I (table 3). The determination coefficient R^2 for the method I (0.288) was also higher than the other ones obtained for methods II (0,256) and III (0,258), both with correction for T_{max}>30 (table 3).

In reproductive phases, CV% values were generally higher than vegetative (Table 3), showing the highest value for the method I (23.4%), followed by III (15.8%) and II (14.5%) calculation methods. On the other hand, the comparison between the different CQVs, being based on the median rather than the mean, showed a reduced variability for the method I, unlike the other two with correction for T_{max}>30 (15.4% and 14.2% for method II and III, respectively). In this case, though, all curves had positive intercepts, with a more moderate slope, as shown by the angular coefficients in table 2. However, the GDDs in reproductive stages showed a worse fit on observed data, compared to the vegetative ones.

Also, through nested ANOVA, variability was analyzed between the different GDDs calculation methods, separately for the vegetative phases (from 0.9 to 2.9 BBCH) (Figure 1) and the reproductive phases (from 5.1 to 8.9 BBCH) (Figure 2).

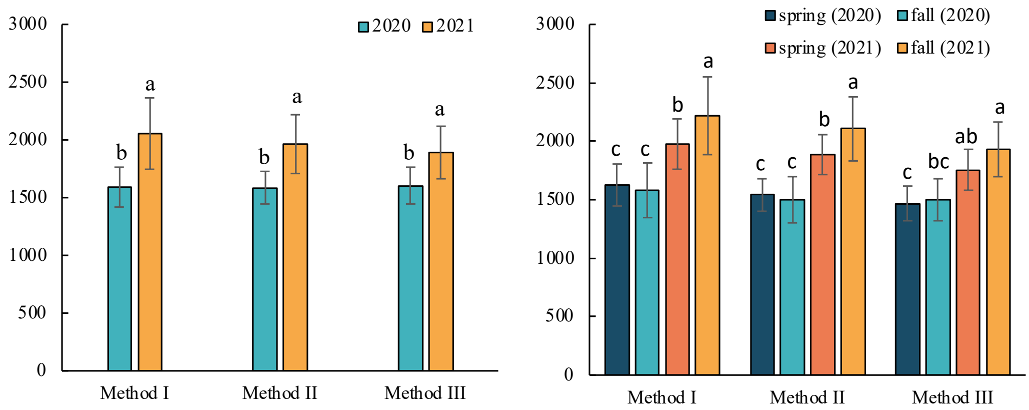

Figure 1. Cumulated values of GDDs calculated with three methods (I, II and III) for the vegetative stages (0.9–2.9 BBCH) obtained in a 2-year cultivation trial; mean values across years (A) and growing seasons within years (B). In each GDDs calculation method, bars marked by the same letter are not significantly different at P ≤ 0.05 (Tukey's test).

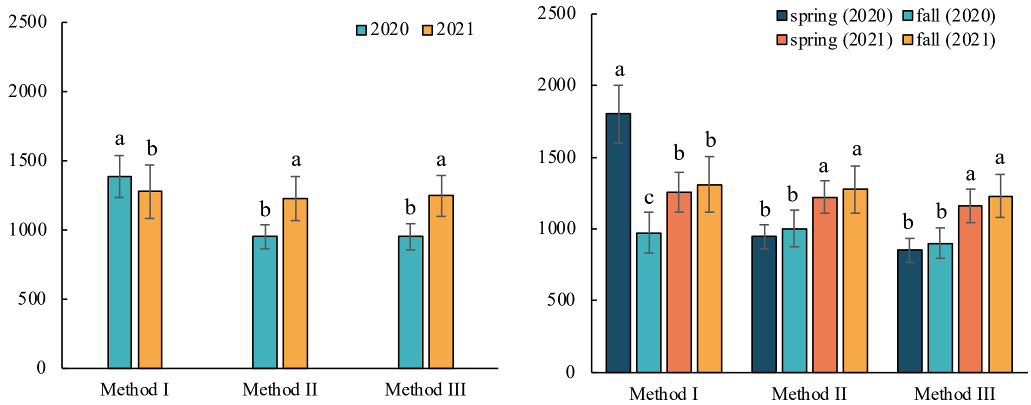

Figure 2. Cumulated values of GDDs calculated with 3 methods (I, II and III) for the reproductive stages (5.1–8.9 BBCH) obtained in a 2-year cultivation trial; mean values across years (A) and growing seasons within years (B). In each GDDs calculation method, bars marked by the same letter are not significantly different at P ≤0.05 (Tukey's test).

The "year" factor had a significant effect among all three calculation methods considered. For the vegetative phases, the GDDs in 2021 were significantly higher than in 2020, showing deviations from 2020's heat requirements of 463, 381, and 288 °C for the I, II, and III methods respectively. On the other hand, if we consider the nested factor "season(year)", there were clear differences in terms of needs in GDDs between the seasons for methods I and II, with the spring (2020) and fall (2020) statistically different from the spring (2021) and fall (2021) (Figure 1). Otherwise, in method III, there was a leveling of the averages, thus not allowing for appreciable net significant differences between the growing seasons. As regards the reproductive phases, the GDDs values were again statistically distinguishable according to the "year" factor (figure 2). Except for method I, GDDs in 2021 were significantly higher than in 2020, showing deviations of 278 and 296 °C. In method I there has always been a significant difference between average GDDs values between years, but in this case, 2020 was significantly higher than in 2021 ($p \leq 0.001$). The comparison of GDDs means values for the "season (year)" factor showed significant differences between all calculation methods considered. Except for method I, the other two again showed the same pattern, with spring (2021) and fall (2021) not statistically differentiable, but significantly higher than spring (2020) and fall (2020), also not dissimilar because of the same grouping b ($p \leq 0.05$) (figure 2). In method I, the GDDs value for spring (2020) was found on average to be significantly higher than in the other growing seasons of 1802, followed by 1311 and 1257 °C for fall (2021) and spring (2021) respectively (statistically not dissimilar; $p < 0.001$) and finally 974 °C for fall (2020).

In addition, to reduce the number of parameters relative to all the observations obtained during the 2-year trial, PCA was carried out before the analysis of correlation between the GDDs output and the most representative biometric and quality data (figure 3; table S4). According to PCA results for both vegetative and reproductive phases, it emerged that the first principal component adopted (i.e., GDDs requirements) had large associations with stems and leaves (t FM ha^{-1}), cones yield (t FM ha^{-1}), α-acids (%) and essential oils content (%).

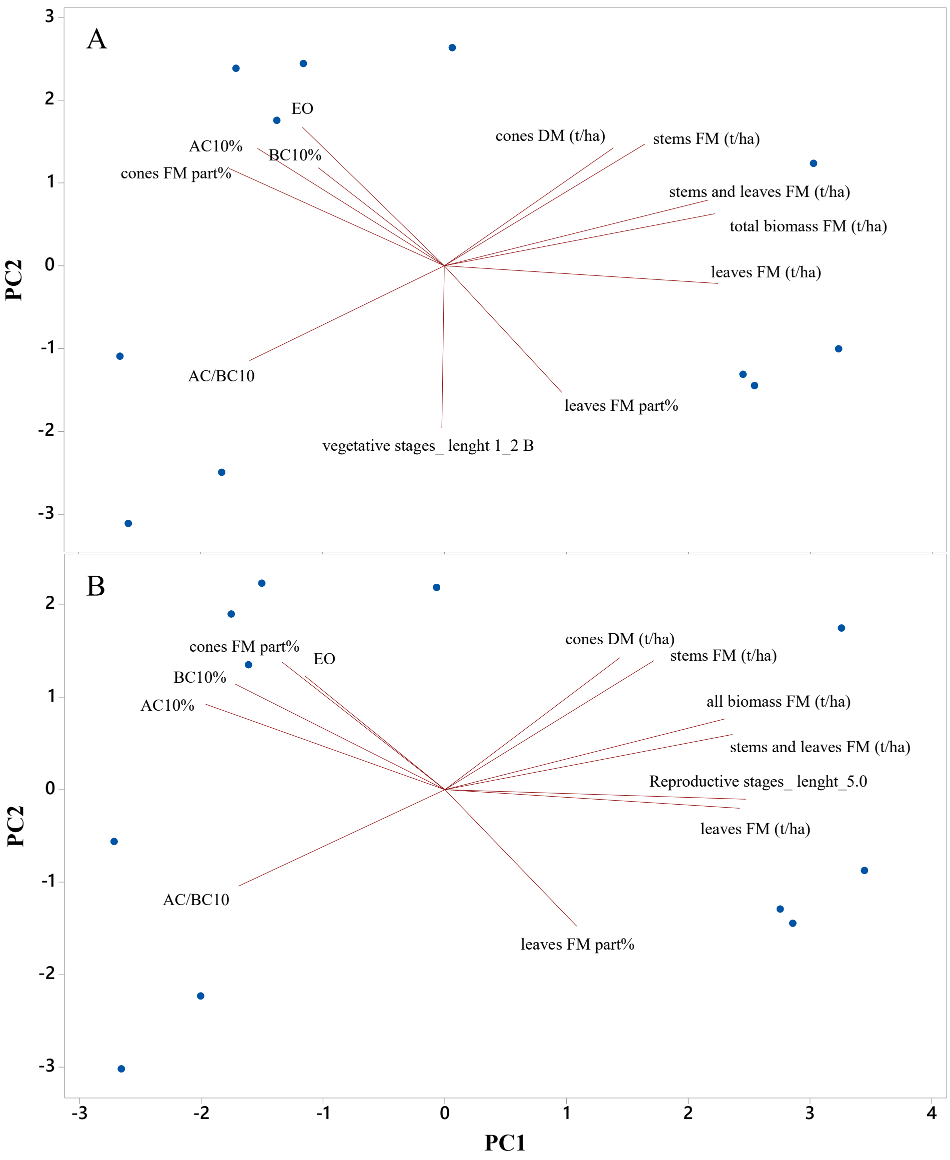

Figure 3. PCA biplot graph displaying the cascade plants' traits, such as biomass yield, quality parameters, and the durations of the vegetative (A) and reproductive (B) stages, represented along the two main principal components. This data was measured during a 2-year trial. The vectors' directions and lengths illustrate the extent and direction of each trait's contribution to the first two principal components in the PCA. The abbreviations used for the parameters are listed below. AC = α–acids; BC = β–acids; AC/BC = α– and β–acids ratio; DM = dry matter; EO = essential oils; FM = fresh matter.

Therefore, the analysis of correlation was finally carried out among GDDs and the parameters above mentioned (table 5).

Table 5. Pearson's correlation coefficients (r) between dry weight of cones per plant and unitary plant mass and calculated GDDs for the vegetative development stages, the reproductive development stages and the entire cycle length of hop.

Methods	Vegetative Stages (from 0.9 to 2.9 BBCH)				Reproductive Stages (from 5.1 to 8.9 BBCH)			
	Stems and leaves (t FM ha^{-1})	Cone yield (t DM ha^{-1})	a-acids (%)	Essential oils (%)	Stems and leaves (t FM ha^{-1})	Cone yield (t DM ha^{-1})	a-acids (%)	Essential oils (%)
I	0.812***	0.294	-0.362	-0.004	0.409	0.738***	-0.184	0.202
II	0.805***	0.287	-0.346	0.009	0.550	0.053	-0.308	0.088
III	0.607***	–0.067	-0.435	-0.157	0.548	0.041	-0.296	0.104

Note:Vegetative stages: n = 12; reproductive stages: n = 12.

*Significant at the .05 probability level. ***Significant at the .001 probability level.

For vegetative stages, GDDs requirements resulted positively correlated to stems and leaves (t FM ha^{-1}) for all the calculation methods adopted ($p \leq 0.001$), as well as cones yield (t FM ha^{-1}) resulted positively correlated to GDDs, even though just for the I ($p \leq 0.001$) and II calculation methods. A marked negative correlation resulted between GDDs requirements during the vegetative stages and a-acids content (%), whereas a very poor correlation linkage appeared to exist with essential oils content (table 5). Considering the reproductive phases, similar to what we have seen above, the GDDs requirements were positively correlated to stems and leaves (t FM ha^{-1}) for all the calculation methods adopted, albeit in a statistically insignificant way. On the other hand, except for Method I ($p \leq 0{,}001$), the cone yield appeared to be poorly correlated with the GDDs requirement during the reproductive stages. Also, a marked negative correlation resulted between reproductive GDDs requirement and α–acids content (%). The essential oils content (%) resulted in this case positively correlated with GDDs calculated with Methods I (0.202) and III (0.104), even though the correlation linkage appeared to be very poor and statistically not significant (table 5).

3.2 Biometric and cones quality data

According to the ANOVA outcomes (table 6), the total epigeal biomass (t FM ha^{-1}) significantly differed between the years ($p \leq 0.001$) and the harvesting seasons ($p \leq 0.001$). Specifically, in 2021 the crop produced more biomass showing an average increase of about 76% over 2020. The "season (year)" factor brought out the fall (2021) as the most productive in terms of total biomass (18.49 t FM ha^{-1}), significantly higher than the spring biomass of both 2020 and 2021 statistically not dissimilar ($p \leq 0.001$).

Table 6. Results of nested ANOVA (mean values) for total epigeal biomass (t FM ha^{-1}), stems biomass (t FM ha^{-1}), leaves biomass (t FM ha^{-1}) and cones yield (t DM ha^{-1}) obtained from a 2-year cultivation trial at the UF/IFAS Gulf Coast Research and Education Center, Wimauma, FL.

	Total epigeal (t FM ha^{-1})	Stem biomass (t FM ha^{-1})	Leaf biomass (t FM ha^{-1})	Cone yield (t DM ha^{-1})
Year (Y)				
2020	11.82 b	4.82 b	5.00 b	0.51
2021	16.46 a	7.50 a	6.71 a	0.56
Significance of P value	***	***	**	†NS
Nested factor: Harvest season (year)				
Spring (2020)	14.86 b	5.89 b	6.34 b	0.69 a
Fall (2020)	8.79 c	3.75 c	3.67 c	0.33 b
Spring (2021)	14.43 b	7.27 ab	4.95 bc	0.54 ab
Fall (2021)	18.49 a	7.73 a	8.48 a	0.58 a
Significance of P value	***	*	***	**

Note: Means that do not share a letter are significantly different according the Tukey's method and 95% Confidence. *Significant at the .05 probability level. ** Significant at the .051 probability level. ***Significant at the .001 probability level. †NS, nonsignificant.

Appreciable differences were also observed for the biomass in stems produced between 2020 (4.82 t FM ha^{-1}) and 2021 (7.50 t FM ha^{-1}). Season-wise, in the fall (2021) the largest biomass yield was recorded (7.73 t FM ha^{-1}), while similar levels were located for spring (2021) (7.27 t FM ha^{-1}), both belonging to the same grouping a ($p \leq 0.001$). Much lower was the biomass in stems recorded for fall (2020), the only treatment belonging to the c grouping. The canopy produced by the crop was different depending on the year ($p \leq 0.005$) and the growing season ($p \leq 0.001$), showing the same patterns observed earlier for the stems. In 2021, the crop produced more leaves, showing an average increase of about 25% compared to 2020; the differences observed in harvest seasons instead allowed to discriminate fall (2021) as the most productive in terms of canopy produced (8,48 t FM ha^{-1}), followed by spring (2020) and spring (2021), statistically not dissimilar, with values of 6,34 and 4,95 t FM ha^{-1} respectively; and finally fall (2020), belonging to grouping c, which showed a value much lower than the average for the parameter under consideration ($p \leq 0.001$; table 6).

The cone yield was stable during the 2-year trial, not revealing significant differences between the average yields of 2020 (0.51 t DM ha^{-1}) and 2021 (0.56 t DM ha^{-1}). The leveling of production performance is attributable to the average yield obtained in spring (2020), statistically not dissimilar to the yields obtained in fall (2021) and spring (2021) of 0.69, 0.58, and 0.54 t DM ha^{-1} respectively ($p \leq 0.005$). Fall (2020), although belonging to the same group b, along with spring (2021), was the least productive season, with a reduction in yield of about 45% compared to the average value obtained in the other seasons (table 6).

The distribution of biomass (% FM) during the experiment was stable over the years, with no appreciable statistical difference for leaves and stems, while significant variation for cones, showing an average decrease of about 3% between 2020 and 2021 (figure 4). If the growing seasons were taken into account, there were no significant variations in the biomass distribution for cones and stems, but on the canopy only. Specifically, in fall (2021) the incidence of leaves was 46%, much higher than the spring season of the same year, although not statistically distinguishable from the average percentage values obtained in 2020, for both spring and autumn harvests ($p \leq 0.05$) (figure 4).

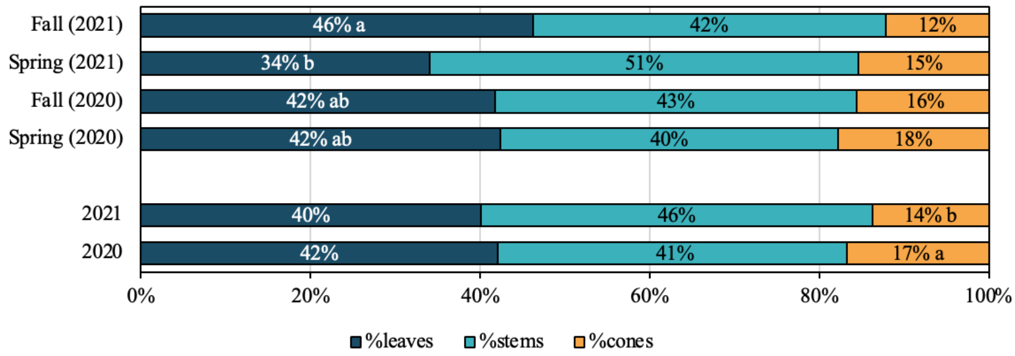

Figure 4. Plant repartition (leaves, stems and cones) during the 2-year trial at the UF/IFAS Gulf Coast Research and Education Center, Wimauma, FL. Means that do not share a letter are significantly different according to the Tukey's method and 95% Confidence.

As regards the quality parameters of hop cones, a nested ANOVA for three seasons out of four was carried out (figure 5 and 6).

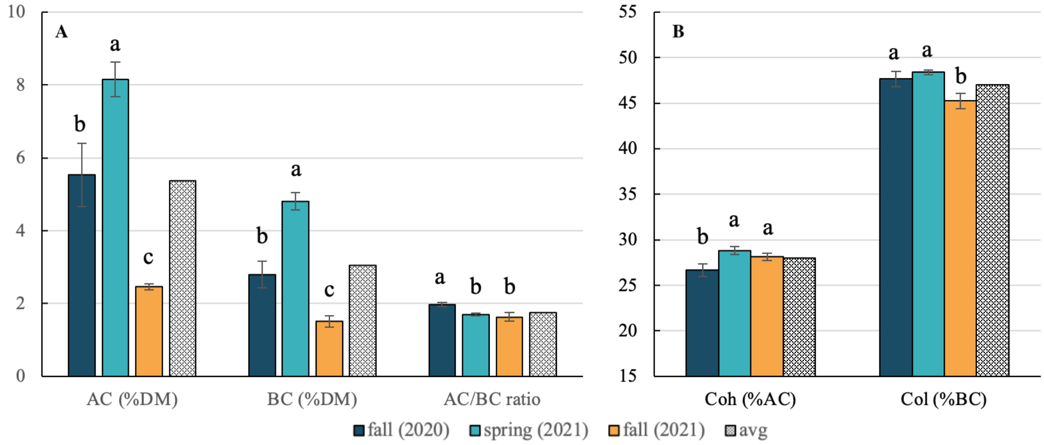

Figure 5 (A) α–acids, β–acids and α/β–acids ratio, (B) cohumulone and colupulone obtained in a 2-year trial at the UF/IFAS Gulf Coast Research and Education Center, Wimauma, FL. For each group of means, bars marked by the same letter are not significantly different at $p < 0.05$ (Tukey's test). The abbreviations used for the parameters are hereby listed: AC = α–acids; BC = β–acids; AC/BC = α/β-acids ratio; Coh = cohumulone; Col = colupulone.

AC (%) and BC (%) seemed to follow the same trend in the three growing seasons considered, with spring (2021) superior, followed by fall (2020), and finally by fall (2021), belonging as sole to "c" grouping for both parameters ($p \leq 0.001$) (figure 5); whereas, the AC/BC ratio was significantly higher in fall (2020) ($p \leq 0,001$) than the other two seasons, with average values of 1,69 and 1,63 respectively for spring (2021) and fall (2021) (figure 5a). The content in cohumulone (%AC) was increasing during the trial, with fall (2020) recording the lowest value (26.7%), while the following two seasons were significantly higher, with average values statistically not dissimilar, i.e., 28,8 and 28.1% for spring (2021) and fall (2021) respectively (figure 5b). Otherwise, it can be said for the content of colupulone (%BC) (table 5b), where in fall (2020) and spring (2021) they recorded values significantly advanced compared to fall (2021), pairs respective to 47.7%, 48.4% and 45.2% on the total BC.

EO content (ml 100 g^{-1}) appeared markedly higher in spring (2021), with a value of 0.72 ml 100 g^{-1}, about 52% higher than the average of the growth seasons taken into account ($p \leq 0.001$) (figure 6a).

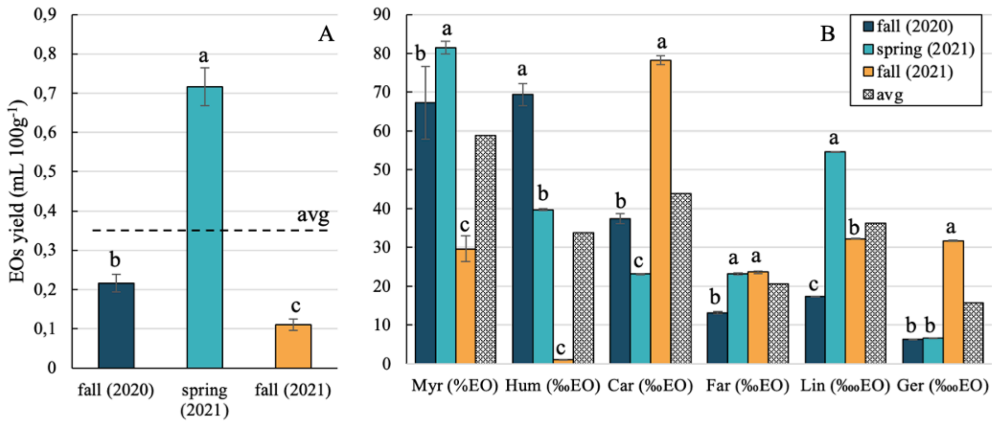

Figure 6. (A) Essential oils yield, (B) myrcene, humulene, caryophyllene, farnesene, linalool and geraniol, obtained in a 2-year trial at the UF/IFAS Gulf Coast Research and Education Center, Wimauma, FL. For each group of means, bars marked by the same letter are not significantly different at P < 0.05 (Tukey's test). The abbreviations used for the parameters are hereby listed: EOs = essential oils; Myr = myrcene; Hum = humulene; Car = caryophyllene; Far = farnesene; Lin = linalool; Ger = geraniol.

The content in myrcene (%EO) has varied depending on the season ($p \leq 0.001$), showing a first increasing trend, with an average initial value for fall (2020) of 63.3% and up to 81.5% in spring (2021), to then record a value much lower than the previous two seasons in fall (2021) (29.7%), as shown in figure 6b. A decreasing trend has been observed for the content in humulene (%EO) depending on the growing season ($p \leq 0.001$); the parameter showed a great variability, starting from average values of 69.4% for fall (2020) and up to 1.15% for fall (2021) (figure 6b). Trends complementary to the reduction in humulene instead have been observed for the average contents in caryophyllene and farnesene (%EO) in the three growing seasons. Specifically, caryophyllene content showed statistically not dissimilar average values for fall (2020) and spring (2021) (37.4 and 23.2% respectively), then reaching values of 78.2% in fall (2021). Instead, the rise for farnesene was milder, with fall (2020) that recorded the lowest values (13.1%), statistically different from spring (2021) and fall (2021), both belonging to the same "a" grouping ($p \leq 0.001$; figure 6b). The content in linalool (%) has varied depending on the season ($p \leq 0.001$), showing a trend before increasing, with an average initial value for fall (2020) equal to 0.17% and rising to 0.55% in spring (2021), to then record an intermediate value between the previous two seasons in fall (2021) (0.32%), as shown in Figure 6b. The geraniol content (%) was increasing, with fall (2020) and spring (2021) being on the same average levels (respectively 0.06 and 0.07%), statistically not differentiable, both belonging to the same grouping b; while, in fall (2021) there was a much higher value of 0.32% ($p \leq 0.001$; figure 6b).

4. DISCUSSION

The analysis of the variability between the different methods of calculation of GDDs, appreciated through the comparison of CV% and CQV%, showed the greater stability of Method I both for the vegetative and reproductive phases. However, Method II showed in general a better fit on the observed data during all the phenological stages. Differently from what was shown by Marceddu et al. (2020) in hop cultivation trials in a Mediterranean region, a little advantage was assessed by the use of correction for $T_{max} > 30$ °C in the sub-tropical environment, whose adoption led to the lowest variability and the highest R^2 values, even though each of these last showed a very low absolute value. Therefore, in our trial, none of the methods appeared to perform better than the others; a certain advantage, as expressed by the lower general variability, might however be attributed to the adoption of correction for $T_{max} > 30$ °C, although R^2 values appeared to be still too low to explain the observed variability according to the model's input.

In addition, the analysis of the variance performed between the different calculation methods of the GDDs, allowed us to appreciate, about the vegetative (0.9-2.9 BBCH scales) stages, a general increase in GDDs requirements during these stages, effect likely to be more influenced by the increase in the thermal averages in the 2-year trial. And this is made very clear by the difference between the average heat requirements recorded within the different growing seasons, i.e., the same statistical grouping for spring (2020) and fall (2020) in the method I and II, while the effect is more attenuated in method III for the correction effect for $T_{max} > 30$ °C. Similarly, it can be said for the reproductive phases (5.1-8.9 BBCH scales), with the sole exception of the method I, which recorded a reversal of the trend between years, determined by the high average value of GDDs recorded for spring (2020).

Eventually, the correlation analysis carried out among GDDs and biometric data showed, for both vegetative and reproductive stages, a positive correlation between these last and GDDs requirements (with all of the three GDDs' calculation methods tested); this is however in contrast with the results obtained in recent studies carried out in the Mediterranean area (Marceddu et al., 2020; Rossini et al., 2016), potentially proving that in a new environment such as sub-tropics different growing and productive responses could be spotted within hop cultivation. The α–acids content appeared negatively correlated for both vegetative and reproductive GDDs stages' requirements, even though without any statistical significance of the findings; whereas EO appeared poorly correlated and highly variable among all the methods tested and depending on the phenological stages taken into account. This seemed to suggest that the answers in terms of quality of hop production, especially in sub-tropical environments, are either little or not at all dictated by specific thermal requirements identified through the estimation of GDDs, regardless of the calculation method used. Biomass-wise, the increase in terms of total biomass produced between 2020 and 2021 is certainly associated with the greater capacity of the hypogeal root systems, although there have been no substantial increases in production (i.e., the cone yield has been stable between the years). The comparison between the growing seasons shows the higher productivity of the spring cycle compared to autumn. However, considering the cone's yield alone, the possibility of a double annual harvest could represent an excellent management strategy to integrate the medium-low production responses of hops in cultivation in sub-climate environments. These findings complement what has already been highlighted by Acosta-Rangel et al. (2024) in a recent study conducted in the same cultivation environments. Eventually, considering the quality parameters, we cannot fail to report how hop production in Florida has shown high variability in the composition of both bittering (α− and β−acids) and aromatic compounds (essential oil) within each growing season. From the results obtained so far, however, the spring season of 2021 was higher in terms of overall quality of production, showing on average high α−acid values, albeit with values in EO not optimal for the varietal standard (Forteschi et al., 2019). In fact, in fall (2021) only, an EO content of more than 0.2 ml 100 g^{-1} was observed, however, noting variations in the oil constituents (i.e., increase in caryophyllene and farnesene content compared to myrcene and humulene content). This would seem to underline a marked sensitivity of the species and/or the Cascade variety used in the test to the soil-climatic characteristics of the cultivation site, which can generate a single terroir for hop production on a local scale.

SUPPLEMENTAL MATERIAL

Table S4. PCA results for the 2-year trial data set: association of variables for the biomass and quality yield parameters during both vegetative and reproductive stages.

Vegetative stages				
Component	**Eigenvalue**	**Variables**	**Loadings**	**Variance**
PC1	5.532	Vegetative stages lenght	-0.004	46.1%
		Total biomass (t FM ha^{-1})	0.391	
		Stems and leaves (t FM ha^{-1})	0.401	
		Leaves (t FM ha^{-1})	0.406	
		Cones (t DM ha^{-1})	0.297	
		Leaves FM part%	0.25	
		Cones FM part%	0.174	
		AC10%	-0.187	

		BC10%	-0.319	
		AC/BC10	-0.277	
		EO	-0.289	
PC2	4.507	Vegetative stages lenght	-0.434	83.7%
		Total biomass (t FM ha^{-1})	0.176	
		Stems and leaves (t FM ha^{-1})	0.139	
		Leaves (t FM ha^{-1})	-0.047	
		Cones (t DM ha^{-1})	0.326	
		Leaves FM part%	0.316	
		Cones FM part%	-0.339	
		AC10%	0.263	
		BC10%	0.261	
		AC/BC10	0.314	
		EO	-0.254	
Reproductive stages				
Component	**Eigenvalue**	**Variables**	**Loadings**	**Variance**
PC1	6.467	Reproductive stages lenght	0.382	53.9%
		Total biomass (t FM ha^{-1})	0.355	
		Stems and leaves (t FM ha^{-1})	0.365	
		Leaves (t FM ha^{-1})	0.374	
		Cones (t DM ha^{-1})	0.265	
		Leaves FM part%	0.223	
		Cones FM part%	0.167	
		AC10%	-0.177	
		BC10%	-0.304	
		AC/BC10	-0.267	
		EO	-0.262	
PC2	3.715	Reproductive stages lenght	-0.028	84.9%
		Total biomass (t FM ha^{-1})	0.206	
		Stems and leaves (t FM ha^{-1})	0.16	
		Leaves (t FM ha^{-1})	-0.054	
		Cones (t DM ha^{-1})	0.375	
		Leaves FM part%	0.384	
		Cones FM part%	-0.397	
		AC10%	0.33	
		BC10%	0.249	
		AC/BC10	0.308	
		EO	-0.281	

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4.2. Phenological Assessment of Hops (*Humulus lupulus* L.) Grown in Semi-Arid and Subtropical Climates Through BBCH Scale and a Thermal-based Growth Model

Submitted to Agronomy

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SUMMARY

Plant growth, the increase of organ dimensions over time, and development, the change in plant structure, are often studied as two separate processes. However, there is structural and functional evidence that these two processes are strongly related. Today the BBCH scale is one of the main classification systems for growth and development stages for many plant species. Specifically, on the BBCH scale for hops (*Humulus lupulus* L.), the growth and development during the vegetative stage are reported separately. This research work, thus, aims to develop an integrated approach deepen the interaction between vertical growth rates and vegetative development in hops. Growth rates and development patterns of Cascade cultivar were determined in two growing environments: semi-arid environment (Sicily, Italy) and subtropical environment (Florida, US). Eventually, consistent likelihoods were obtained between growth and developmental stages, suggesting the possibility to determine growth dynamics from developmental observations at any time in hop plant vegetative phases. From an applied point of view, the knowledge of the growth characteristics associated with the growth developmental stages is definitely a key strategy to better understand, and possibly act upon, the interactions between plant development and pests and diseases in new growing areas.

1. Introduction

Crops' economic yield is often considered as a function of growth rates expressed over time (Egli, 2011). Time affects the potential yields of crops according to the duration of their growth cycle (planting to maturity), representing a measure of the resource used by crops in any specific growing environment (i.e., temperature or water availability) (Bruns, 2009).

Therefore, knowledge of growth and development patterns through phenological assessment plays a key role in developing crop management strategies, particularly when crops are grown in new production areas (Aitken 1974; Ong, 1986).

Hop (*Humulus lupulus* L.) is a perennial herbaceous plant cultivated for its female flowers, which are used as an essential ingredient of beer. Traditionally, hop production has been concentrated between latitudes 40° and 50° North or South (Haunold, 1981). However, driven by rapid growth in the global craft beer market, hop cultivation has recently expanded to new production regions worldwide, including Italy (Carrubba et al., 2022; Marceddu et al., 2024; Gresta et al., 2023; Rossini et al., 2021; Ruggeri et al., 2018), Brazil (Jastrombek et al., 2022; Leles et al., 2023), and Florida, United States (Acosta-Rangel et al., 2021, 2024). Although high phenological plasticity of hops to various climatic conditions has been indicated, there has been little research regarding the phenological classification for this crop in new production areas.

Growth patterns are characterized by cumulative increments of dimensions over time (Dambreville et al., 2015; Goudriaan & Van Laar, 2012), whereas development consists of a series of events resulting in a qualitative or quantitative change in plant structure (Bonhomme, 2000; Gatsuk, 1980). Today, the BBCH scale is a phenological classification system widely used for many plant species (Meier et al., 2009). Specifically, considering the BBCH scale for hops (Rossbauer et al., 1995), the growth and development during the vegetative stage are reported separately (i.e., stage 1: leaf development; and stage 3: elongation of bines), even though there is structural and functional evidence that these two processes are strongly related (De Wit et al., 2016; Lastdrager et al., 2014). Moreover, this scale characterizes a growth stage without considering potential complementary relationships with the previous or subsequent phenological phases.

Therefore, the main research question is: can we identify integrative “developmental growth stages” that account for both growth and developmental characteristics? To answer this question, this study aims to develop a new approach to integrate growth rate and vegetative development in hops. For experimentation purposes, growth rates and development patterns of Cascade cultivar were determined in two growing environments: a semi-arid climate in Sicily, Italy, and a subtropical climate in Florida, United States. We used the thermal time to consider the effects of temperature on growth and development, building a multi-stage model based on the height growth rate (HGR) sequences and relating it to the vegetative development of the species (Rossbauer et al., 1995). From an applied point of view, the knowledge of the association between growth characteristics and development events is also crucial to better understand, and possibly act upon, the interactions between plant development and biotic or abiotic stressors.

2. Materials and methods

2.1. Experimental sites and crop management

This study was performed in two different climates: (i) semi-arid Mediterranean (Sicily, Italy) and (ii) sub-tropic (Florida, United States). By employing the standard agronomic practices of these respective regions, the study aimed to understand the impact of these diverse climates on hop cultivation.

In the semi-arid environment, the field experiment was carried out at the "Sparacia" experimental farm (Cammarata, AG, Italy, 37° 38' 07'' N; 13° 45' 47'' E; 450 m a.s.l.), belonging to the Department of Agricultural, Food and Forest Sciences (D/SAAF) of the University of Palermo. The hopyard was built with a 3.60-meter-high V-trellis in 2021. Three replications on the row, each consisting of 6 hills, were set up. Distances of 0.7 m between plants in the row and 3 m between rows were adopted to allow mechanical weeding. Plastic mulch (2 mm polyethylene film) was also adopted to reduce the presence of weeds within the rows. During the growing season, two shallow mechanical interventions (about 10 cm in depth) were performed through a tractor-driven cultivator. Fertilization was carried out by supplying 30 t ha^{-1} of mature bovine manure at planting; a single foliar fertilization was performed, by distributing 2 hl ha^{-1} with doses of 150 ml hl^{-1} Upper Grow® and 150 ml hl^{-1} Tyson® at the 2.9 BBCH stage. A localized irrigation system was set up with a single dripline per row. The system was equipped with a closing valve and a pressure gauge to control the working pressure. Irrigation was supplied according to crop requirements.

In the sub-tropical environment, the field experiment was carried out at the Gulf Coast Research and Education Center (Wimauma, Florida, US, 27° 45' 36" N; 82° 13' 48 "W, 40 m a.s.l.), belonging to the University of Florida. The hopyard was built with a 4.57-meter-high V-trellis in 2019. The layout and configuration of the hopyard are as described by Agehara et al. (2020). Four replications on the row, each consisting of 10 hills, were set up. Distances of 0.9 m between plants in the row and 4.6 m between rows were adopted. Landscape fabric was also adopted as mulch for weed control within the rows, and bahiagrass (*Paspalum notatum*) was sown as a cover crop between the rows to prevent erosion and suppress weed growth.

Weeds were chemically controlled only along the edges of landscape fabric. Insecticides and fungicides were sprayed to prevent or limit the spread of spider mites, aphids' infestations, and fungal diseases. Fertigation was performed daily with a custom blend liquid fertilizer containing 5% of N, 0.87% of P, 6.64% of K, 1.4% of calcium, and 0.4% of magnesium, as well some micronutrients at <0.4%. Total inputs of N, P, K and Ca were 306, 51, 386 and 81 kg ha^{-1}, respectively. Additional micronutrients were applied through fertigation of a micronutrient fertilizer (Microplex; Miller Chemical and Fertilizer, LLC, Hanover, PA, USA). Each row had two drip tapes with emitters spaced 30 cm apart with a flow rate per emitter of 0.91 l h^{-1} (Netafim USA, Fresco, CA, USA). The irrigation system was equipped with a closing valve and a pressure gauge to maintain constant working pressure. Irrigation was supplied according to crop requirements. To prevent immature flowering induced by insufficient photoperiod in subtropical environments (Acosta-Rangel et al., 2021), photoperiod extension was performed with supplemental lighting until adequate vegetative growth was achieved as described by Agehara (2020).

Daily values of rainfall and maximum and minimum temperatures at the study sites during the 2022 growing season (Figure 1) were obtained from the Agrometeorology Service Network of the Sicilian Region (SIAS, 2023) and from the Florida Automated Weather Network (FAWN, 2023).

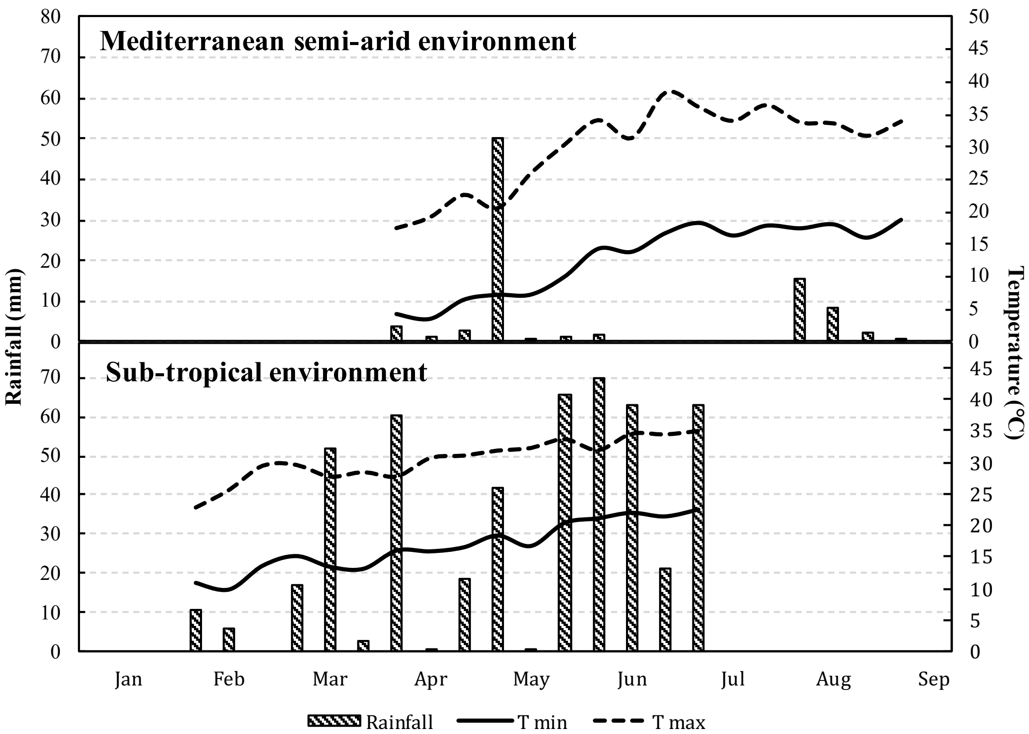

Figure 1 Rainfall and temperatures recorded at the "Sparacia" farm (Cammarata, AG, Sicily, Italy) (Mediterranean semi-arid environment) and at the Gulf Coast Research and Education Center (Wimauma, Florida, US) (sub-tropical environment) during the 2022 growing season.

2.2. Data collection

Hop plants were checked on weekly basis to monitor their phytosanitary and developmental conditions. Developmental stages were determined in an expert way by using the BBCH ("Biologische Bundesanstalt, Bundessortenamt and CHemical industry") scale (Rossbauer et al., 1995). Specifically, the study of the development of hop plants covered the phenological stage 1 of the BBCH, which corresponds to the development of the leaves along the bine, starting from phase 1.3 BBCH (3rd pair of unfolded leaves), also corresponding to the beginning of twinning.

Height growth rates (HGR) were calculated during the vegetative growing phase from objective measurements, according to the following formula, as shown in a recent study by Carrubba et al. (2022):

$$\mathrm{HGR} = H_t / \sum_{i=1}^{k} (T_{avg} - T_{base})_i \qquad \text{Eqn 1}$$

where H_t is the height value at a measurement time, t, and the $\sum_{i=1}^{k} (T_{avg} - T_{base})_i$ represents the cumulative growing degree-days (GDDs) within surveys, with i and k indicating the first and last days of measurement, respectively, T_{avg} is daily average temperature, and T_{base} is base temperature (i.e., temperature value below which plant growth is assumed to be zero). T_{base} = 0° C was adopted in the vegetative phases according to what shown in recent works dealing with hop phenological studies (Carrubba et al., 2022; Marceddu et al., 2022).

2.3. Growth stages identification

To adopt a multi-stage model, we divided the vegetative phenological stage (1 BBCH stage) into four growth stages, based on the growth rate sequences observed during the experiment. For both environments, four growth stages identified are as follows: Stage I is the initial slow growth phase with HGR values below 3 mm $GDDs^{-1}$; Stage II is the rapid growth phase until reaching the peak; Stage III is when growth becomes gradual following the peak; Stage IV is when little to no growth occurred (Figure 2).

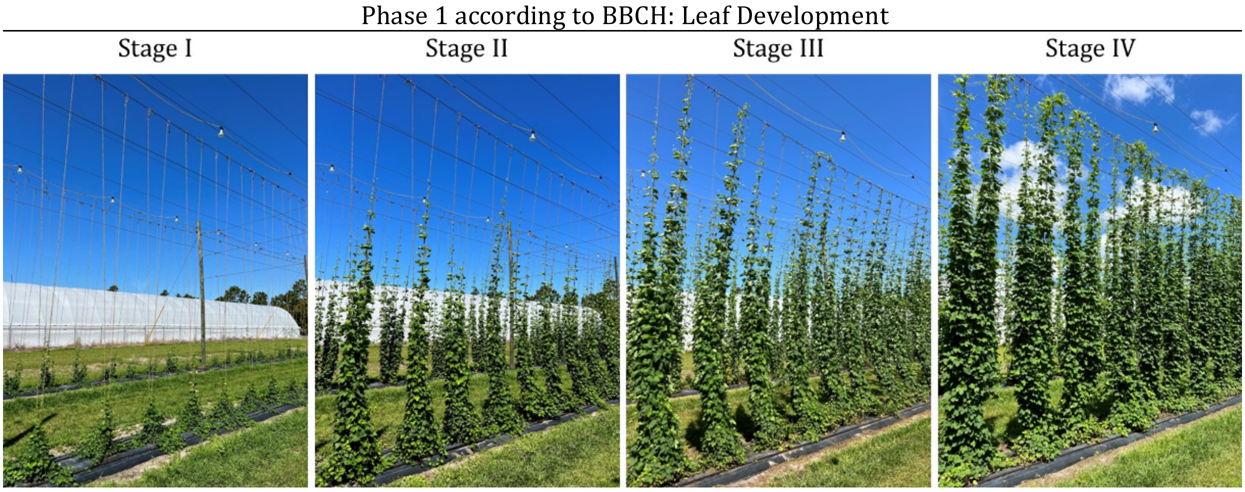

Figure 2 The four growth stages identified from the joint evaluation of developmental stages, determined from morphological observations in an expert way (BBCH), and HGRs during the vegetative growing phase. Stage I is the initial slow growth phase with HGR values below 3 mm GDDs^{-1}; Stage II is the rapid growth phase until reaching the peak; Stage III is the slow growth phase following the peak; Stage IV is the late growth phase when growth declines rapidly and finally ceases. Photographs were taken at the Gulf Coast Research and Education Center (Wimauma, Florida, US) during the 2022 growing season (photo credits: Shinsuke Agehara).

Table 1 Growth stages identification according to HGRs' ranges detected during BBCH stage 1 (Leaf development) in a Mediterranean and sub-tropical environment.

Location	**Growth stages**	**HGR values range (mm GDDs^{-1})**
Mediterranean environment	I	0.59→2.57
	II	3.09→5.96
	III	4.03→1.19
	IV	0.93→0.00
Sub-tropical environment	I	0.00→1.96
	II	2.99→11.2
	III	8.36→5.50
	IV	2.88→0.00

The number of growth stages was set arbitrarily to be fewer than the developmental stages. After defining the four growth stages, we evaluated their validity by comparing the derived growth stages (based on thermal time/HGR) with the developmental stages identified using the BBCH scale (Rossbauer et al., 1995).

2.4.Statistical Analysis

All the statistical analysis were performed using the statistical software Minitab (version 19.2.0.0). Plant height data and growth rates were related to the duration expressed in Julian calendar days. Considering the nonlinear relations between the introduced parameters, we compared different nonlinear regression models studied extensively in the literature (Archontoulis et al., 2015; Paine et al., 2012). The results of the comparative analysis (data not shown) have led to the adoption of the Gompertz model and the classic Gaussian type considering the high fitting between vertical heights and growth rates vs time, respectively. We provide details of all model forms adopted in Table 2.

Table 2 Nonlinear regression models tested in the study.

Variables	Model	Formula	Parameter definition
Eqn.	**Height vs Time**		
2.1	**Sigmoidal function**	$y = y_{asym}/\{1+\exp[-(t - t_0)/b]\}$	y is the response variable (e.g., height), t is the explanatory variable (e.g., time), y_{asym} is the asymptotic y value, t_0 is the inflection point of the curve, b controls the steepness and the shape of the curve
2.2	**Modified sigmoidal function**	$y = y_0 + y_{asym}/\{1+\exp[-(t - t_0)/b]\}$	
2.3	**Logistic function (Verhulst, 1838)**	$y = y_{asym}/[1+(t /t_0)^b]$	
2.4	**Modified logistic function**	$y = y_0 + y_{asym}/[1+(t /t_0)^b]$	
2.5	**Gompertz (1825)**	$y = y_{asym} \exp\{-\exp[-(t - t_0)/b]\}$	
2.6	**Modified Gompertz**	$y = y_0 + y_{asym} \exp\{-\exp[-(t - t_0)/b]\}$	
	HGR vs Time		
2.7	**Gaussian function**	$y = y_{asym} \exp\{-0,5[(t - t_0)/b]^2\}$	y is the response variable (e.g., HGR), t is the explanatory variable (e.g., time), y_{asym} is the asymptotic maximum y value, t_0 is the inflection point at which the growth rate is maximized a (default = 0.5 for the
2.8	**Modified Gaussian function**	$y = y_0 + y_{asym} \exp\{-0,5[(t - t_0)/b]^2\}$	

2.9	**Modified Gaussian function**	$y = y_{asym} \exp\{-k[(t - t_0)/b]^c\}$	Gaussian function), b, c and k are coefficients controlling the height and the width of the skew of the "bell"
2.10	**Modified Gaussian function**	$y = y_0 + y_{asym} \exp\{-k[(t - t_0)/b]^c\}$	
2.11	**Lorentzian peak function**	$y = y_{asym}/\{1+[(t - t_0)/b]^2\}$	
2.12	**Modiefied Lorentzian peak function**	$y = y_0 + y_{asym}/\{1+[(t - t_0)/b]^2\}$	

Also, a regression analysis was performed to study any possible asynchronism between axis growth and leaves emission, by calculating the coefficient of determination between relative axis length and vegetative development completion (i.e., 1 BBCH stage) for each of the two growing environments.

Finally, to identify any significant relation between the different measured growing rates and the observed developmental traits, two separated confusion matrices were built up depending on the growing environment. In fact, one of the aims of this study was to explore the relationships between growth stages and developmental stages. We hypothesized that there were correspondences between growth stages and developmental stages, combining succession constraints (i.e., preserving stage order) and detecting higher counts for the matching between a given developmental stage and a specific growth stage.

3. Results

Height growth of hop plants was described by the Gompertz model (Eq. 2.5; Table 2), which showed the highest goodness-of-fit among all tested models based on AICC values (data not shown). For both cultivation environments (Figure 3), after a first phase of initial stasis, or slow growth, a substantial increase in height between the 30th and 60th day of cultivation was recorded, then followed by a slow growth phase until reaching the top of the trellises.

HGRs trends were described by a modified Gaussian function (Eq. 2.8; Table 2), because of the lowest AICc value obtained among all tested functions and considering the high goodness-of-fit with the empirical data (data not shown). Crop growth rate dynamics were similar between the two environments, although the values recorded in the sub-tropical environment were on average greater than those in the Mediterranean environment (Figure 3). Nevertheless, in both environments, HGRs increased to a maximum value approximately 3 to 4 weeks following the initiation of growth and subsequently declined throughout the remainder of the growing season. The maximum HGR value achieved by plants in the subtropical environment was consistently greater than that achieved in the Mediterranean environment (i.e., 13.8 and 8.8 mm GDD^{-1} respectively) (Figure 3).

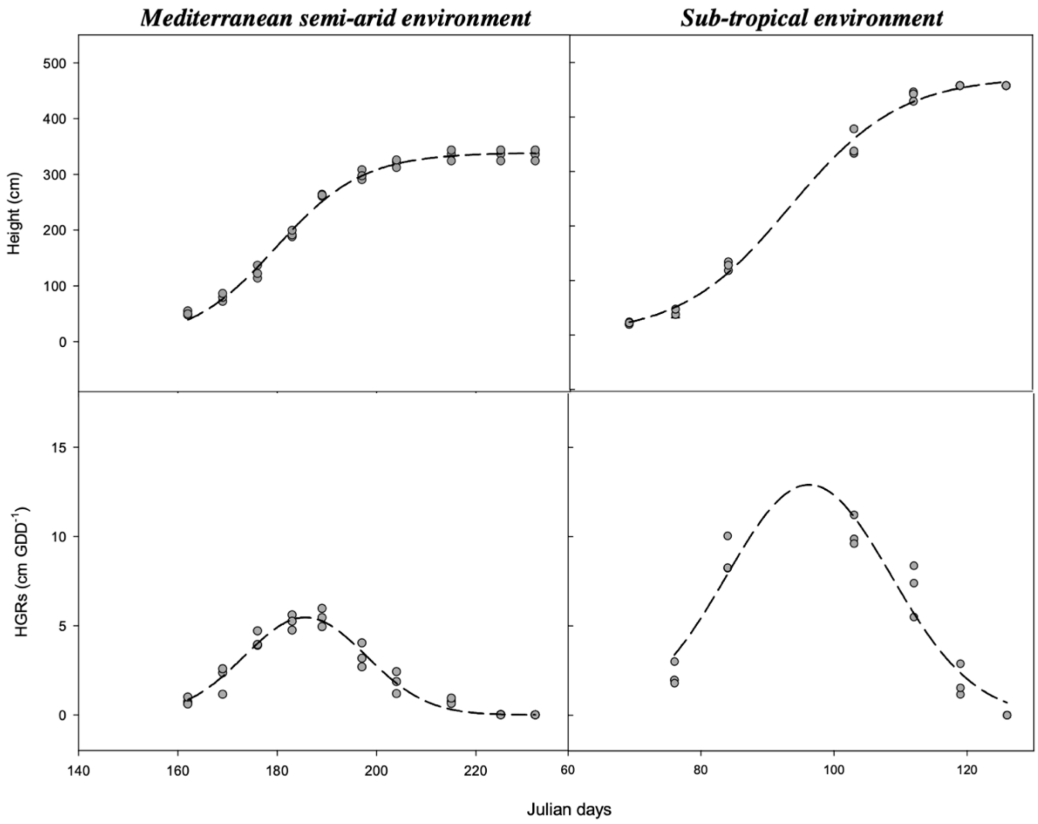

Figure 3 Height vs Time (Julian calendar days) and HGR vs Time (Julian calendar days) nonlinear regressions during the 2022 growing season for Mediterranean semi-arid and sub-tropical environment. Regression models for semi-arid Mediterranean environment: Height vs Time (n = 170), y = 349.07 exp(–exp(–(x – 174.4)/13.6)); HGR vs Time (n = 170) y = 0.12 + 5.3 exp(–0.5((x – 185.6)/11.9)^2). Regression models for sub-tropical environment: Height vs Time (n = 210) y = 499.9*exp(–exp(–(x – 87.8)/13.7)); HGR vs Time (n = 180) y = –3.6 + 15.6 exp(–0,5((x – 96.5)/16.5)^2).

To deepen the association between the leaf emission process (1 BBCH stage) and vertical elongation of bines, a regression analysis was carried out. The results indicated that in both environments, the length of the axis increased gradually following certain advancements in the leaf development process (Figure 4).

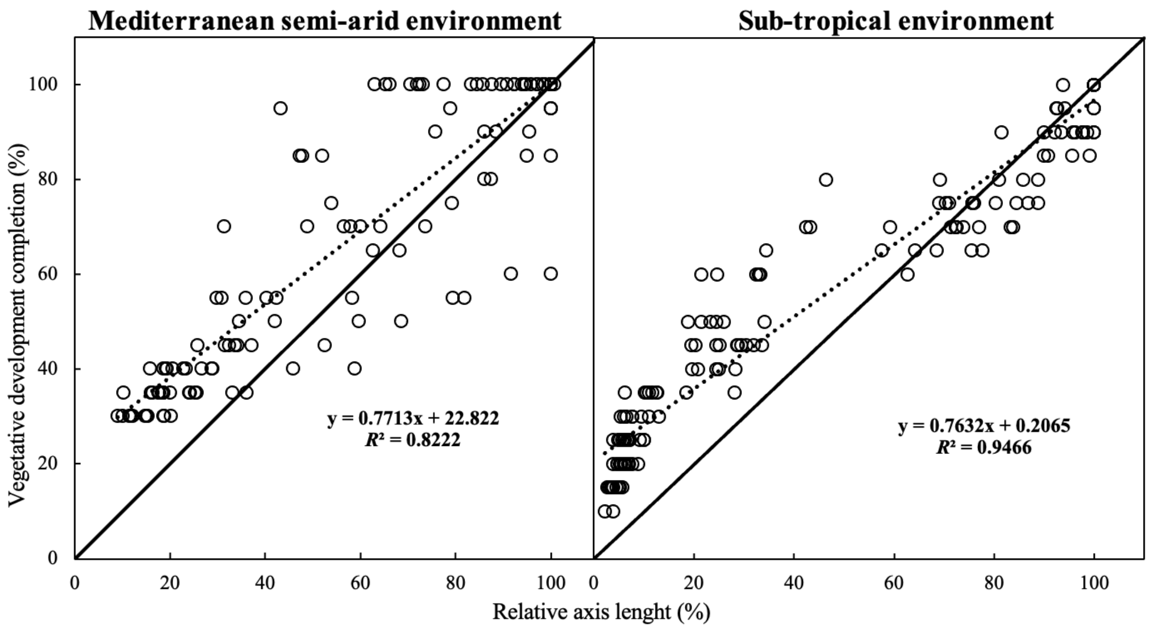

Figure 4 Relative vegetative development completion in relation to relative axis length during cultivation in two growing environments, Mediterranean semi-arid and sub-tropical. Percentages are calculated as the length of the axis divided by the final length and the completion of the 1 stage according to the BBCH scale, respectively. The solid line on each graph represents $x = y$.

Specifically, a more marked asynchronism between axis length and vegetative development completion for the Mediterranean environment was recorded. On contrary, in the subtropical environment, the use of a supplemental lighting system prevented premature flowering, which otherwise is a common phenological phenomenon under insufficient day length conditions in subtropical and tropical climates (Acosta-Rangel et al., 2021, 2024; Agehara et al., 2020), allowing adequate vegetative growth before transitioning into the reproductive phase.

The last step in our research was to explore the relationship among growth and developmental stages. The four growth stages identified for the vegetative phase, according to the HGR distributions, were related to the developmental stages, determined based on morphological observations (1 BBCH phase) (Table 3 and 4). The match rates obtained by the confusion matrices between growth and developmental stages were equal to 71% for both Mediterranean and subtropical environments. More in detail, developmental stages 1.5–1.8

corresponded to growth stage I (no to little growth occurred) in the Mediterranean environment (Table 3), whereas in the subtropical environment the growth stage I corresponded with earlier development stages 1.3–1.6 (Table 4). Growth phases II and III appeared to match with slightly shifted development phases according to the growing environment, with slight deviations, however, as shown in Tables 3 and 4. Growth stage IV (growth arrest), marked by reduced HGRs values (equal or close to zero), was synchronous for both environments, corresponding in fact to developmental stages 1.17–1.20.

Table 3 Confusion matrices between growth stages and developmental stages determined from morphological observations in an expert way during the vegetative growing phase in a Mediterranean semi-arid environment.

Developmental stages	**Mediterranean semi-arid environment**				
	Growth stages				
	I	**II**	**III**	**IV**	**Total**
1.3	0	0	0	0	0
1.4	1	0	0	0	1
1.5	**1**	0	0	0	1
1.6	**11**	0	0	0	11
1.7	**13**	2	0	0	15
1.8	**8**	4	0	0	12
1.9	1	**6**	0	0	7
1.10	0	**4**	0	0	4
1.11	1	**4**	3	0	8
1.12	0	**0**	1	1	2
1.13	0	0	**2**	0	2
1.14	0	6	**1**	0	7
1.15	0	1	**1**	0	2
1.16	0	0	**2**	0	2
1.17	0	3	1	**1**	5
1.18	0	0	4	**0**	4
1.19	0	1	1	**2**	4
1.20	0	1	15	**52**	68
Total	34	32	31	56	153
				Match:	*71%*
				Mismatch:	*29%*

Table 4 Confusion matrices between growth stages and developmental stages determined from morphological observations in an expert way during the vegetative growing phase in a sub-tropical environment.

Developmental stages	Sub-tropical environment				
	Growth stages				
	I	II	III	IV	Total
1.3	**12**	1	0	0	13
1.4	**13**	1	0	0	14
1.5	**10**	3	0	0	13
1.6	**4**	4	0	0	8
1.7	5	**4**	0	0	9
1.8	1	**3**	1	0	5
1.9	2	**5**	3	0	10
1.10	0	**5**	1	0	6
1.11	0	0	0	0	0
1.12	0	4	**3**	0	7
1.13	0	3	**2**	1	6
1.14	0	1	**10**	0	11
1.15	0	0	**9**	1	10
1.16	0	0	5	0	5
1.17	0	0	4	**0**	4
1.18	0	1	6	**5**	12
1.19	0	0	2	**3**	5
1.20	0	0	1	**39**	40
Total	49	35	47	49	180
				Match:	*71%*
				Mismatch:	*29%*

4. Discussion

As far as we are aware, there is still little knowledge about the vegetative growth rate dynamics of hop plants. Our results suggest that the height growth function of hops is similar to that recorded for the genus Cannabis (Yoshimatsu et al., 2004; Morello et al., 2022), which may not be surprising as both species belong to the same botanical family of the Cannabinaceae.

The Gompertz model appeared to be the best fitting model with the height growth data of hop plants in the Mediterranean environemnt. In fact, the study found that the height growth of hop plants was best described by the Gompertz model, showing distinct phases: an initial slow growth, a substantial increase in height between days 30 and 60, and a subsequent slow growth until reaching the trellis tops. Indeed, the model has also been used widely by previous studies to describe growth of other plant species (Gachoki et al., 2022; Tjørve & Tjørve, 2017; Wardhani & Kusumastuti, 2014). Height growth rates (HGRs), modeled by a modified Gaussian function, exhibited similar dynamics in both semi-arid Mediterranean and sub-tropic environments, with the sub-tropic environment displaying higher overall HGR values. Considering the HGRs trends outcomes, it seems that the vegetative growth rate of hops is greatly influenced by external environmental factors. Indeed, according to our results, it becomes evident that there are comparable growth trends between these regions; however, it is worth noting that under sub-tropical conditions, there is a propensity for accelerated development because of the beneficial environmental factors in effect.

Regression analysis indicated a close correlation between leaf emission and vertical elongation in both environments, although a more pronounced asynchronism was observed in the Mediterranean setting. Specifically, there was a slight mismatch between axis length and vegetative development completion in the Mediterranean environment, likely due to the premature cessation of axis growth before reaching the trellis top, causing an early transition to the reproductive stage. The relationship between growth and developmental stages was initially synchronous in both environments but diverged as growth progressed. The strong correlation between growth and developmental stages across different environments suggests that morphological discontinuities are closely related to changes in growth dynamics. While further research is needed to fully understand hop growth dynamics in varying cultivation conditions, understanding the growth characteristics associated with developmental stages is crucial for addressing interactions between plant development, pests, and diseases.

5. Conclusions

The strong correlation across different environments underscores the importance of understanding growth characteristics associated with developmental stages. Such understanding is crucial for managing interactions between plant development and environmental factors, including pests and diseases.

From a practical standpoint, these findings highlight the potential for targeted agronomic practices to optimize hop growth in diverse climates. Further research is needed to refine our understanding of hop growth dynamics and to develop tailored strategies for different cultivation conditions. This knowledge will be essential for improving hop production, particularly in new and challenging growing areas.

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4.3. Conclusions

The two studies presented in this chapter highlight the critical importance of understanding the interplay between environmental factors and hop growth dynamics across different developmental stages. The analysis of various Growing Degree Days (GDD) calculation methods revealed that while no single method outperformed the others in explaining variability, the inclusion of corrections for extreme temperatures improved model stability. Notably, the findings indicate that hop cultivation in sub-tropical environments may yield different growth and quality responses compared to Mediterranean settings, as evidenced by biomass production and the variability in essential oil composition.

Furthermore, the strong correlation between GDD requirements and biometric data emphasizes the need for tailored agronomic practices to optimize hop growth in diverse climates. This is particularly crucial for managing potential interactions with pests and diseases. Moving forward, targeted research will be essential to refine our understanding of these dynamics and to develop specific strategies that enhance hop production in both established and emerging cultivation regions. By integrating these insights, growers can better navigate the challenges posed by varying climatic conditions, ultimately improving both yield and quality in hop cultivation.

5. CONCLUSIONS AND PERSPECTIVES

The field trials and experimental studies in this PhD thesis showcase the adaptability and potential of hop cultivation in non-traditional and environmentally challenging regions, such as the semi-arid Mediterranean and subtropical climates.

Impact of Environment, Agronomy, and Genetics

Environmental factors such as temperature extremes and water availability play crucial role in hop growth and secondary metabolite production. Agronomic practices like sub-surface drip irrigation (SSDI) have been highly effective in improving resilience and yield under high-temperature, low-water conditions, providing a sustainable solution for drought-prone areas. The genetic variability of hop cultivars also influences growth and quality, emphasizing the importance of aligning farming techniques with both the environment and the specific genotype.

Performance of Hop Cultivars

Significant differences exist between hop varieties, particularly Cascade and Chinook. Cascade consistently outperformed Chinook in terms of biomass production and essential oil yields, critical to brewing quality. This suggests the need for cultivation practices that are tailored to the genetic traits of each variety. Additionally, the local terroir—soil composition, microclimate, and environmental interactions—shapes the unique characteristics of hops grown in different regions, further highlighting the importance of genotype-environment matching.

Expanding Hop Cultivation to New Regions

Hop cultivation is viable in non-traditional growing regions, including Mediterranean and subtropical climates, when paired with appropriate agronomic strategies such as low-trellis systems, multiple harvests, and refined water management. Importantly, the distinct terroir of these new regions could introduce unique flavor profiles and characteristics to hops, adding diversity to brewing ingredients. By selecting resilient genotypes and optimizing farming techniques, these areas can contribute to a broader range of hop flavors influenced by their unique environmental conditions.

Potential for Sustainable Hop Expansion

There is potential for sustainable hop production across diverse climates, meeting the increasing demand from brewers while maintaining high-quality standards. Expanding hop farming into these regions could introduce new terroir-driven hop varieties, which may offer distinct flavors and aromas that appeal to brewers seeking diverse ingredients. The ability to cultivate hops in varied environmental conditions ensures the industry's resilience and long-term growth.

Future Research Directions

Further research should focus on maximizing genotype-environment interactions to refine farming practices and explore the terroir effect in non-traditional hop-growing areas. Understanding how local soil, climate, and environmental factors influence the flavor profile of hops is critical for expanding cultivation. Breeding more resilient hop varieties with improved tolerance to drought, heat, and disease will also be essential. Additionally, exploring the long-term sustainability of SSDI and low-trellis systems in regions like semi-arid and subtropical climates can improve resource efficiency. Expanding hop production into tropical and high-altitude regions will require continued investigation into terroir-specific strategies to enhance both sustainability and the diversity of hop characteristics.

6. OTHER ACTIVITIES DEVELOPED DURING THE PhD COURSE

This chapter presents three research articles resulting from additional studies conducted with the Department of Agricultural, Food, and Forest Sciences at Palermo University during my doctoral program. These works build upon a previous research contract associated with the "C.O.S.A. – Conservazione e caratterizzazione di Accessione Siciliane di Specie Agrarie" project, part of the integrated Measure 10 "Agro-climatic-environmental Payments Sub-measure 10.2 'Support for the Conservation, Use, and Sustainable Development of Genetic Resources in Agriculture' Operation 10.2," under the scientific coordination of Prof. Giuseppe Di Miceli.

The first article investigates the potential of pea (*Pisum sativum* L.) intercropped with cereals and annual grasses in the semi-arid Mediterranean region, revealing that intercropping can significantly improve biomass production and silage quality (DOI: 10.1002/agj2.21300). The study highlights the advantages of specific mixtures, such as pea–wheat and pea–ryegrass, and demonstrates the efficacy of sub-surface drip irrigation in enhancing crop resilience and productivity.

The second article (DOI 10.17660/ActaHortic.2023.1376.8) explores the productive stability of asparagus (*Asparagus officinalis* L.) cultivars in southern Italy's Mediterranean climate. The research provides valuable insights into the yield performance of various cultivars, emphasizing that while asparagus can achieve high yields, the suitability of different genotypes varies significantly. This information aids local farmers in selecting appropriate cultivars for optimal production. This paper was previously orally presented at the 15th International Asparagus Symposium, organized by the Asparagus Working Group of the International Society for Horticultural Science (ISHS) and the University of Córdoba (UCO), on June 12th-15th 2022

These contributions collectively enhance our understanding of crop adaptation and optimization in diverse and challenging environments, supporting sustainable agricultural practices and advancing the cultivation of underutilized species.

6.1. Forage Mixture Productivity and Silage Quality from a Grass/Legume Intercrop in a Semiarid Mediterranean Environment

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Agronomy Journal

ORIGINAL ARTICLE

Agronomy, Soils, and Environmental Quality

Forage mixture productivity and silage quality from a grass/legume intercrop in a semiarid Mediterranean environment

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Abstract

In semiarid environments of the Mediterranean region, intercropping is a sustainable agricultural system of long standing. In this area, the pea (*Pisum sativum* L.) is one of the most commonly grown legume crops. Little information is available on the quality of silages to be obtained from forage mixtures of pea intercropped with cereals or annual grasses. In this study, two experiments were conducted over the course of two growing seasons in Sicily (Italy) with the aim to determine the biomass production of forage crop mixtures and assess, only in the second experiment, the silage quality of grass and legumes. Four cereals and one annual grass species were grown in pure stand and in mixture with pea, and their main agronomic traits were determined. The land equivalent ratio (LER), competitive ratio, and aggressivity index were also calculated. A number of parameters were considered to assess the quality of silage obtained from fermented biomasses derived from pea–ryegrass (*Lolium multiflorum* Lam. var. Westerwoldicum) intercropping. In the first experiment, the best performance between the intercrops was recorded for the pea–wheat mixture. The total LER calculated for fodder yields was always greater than 1, indicating crop yield advantages ranging from 2.0% to 47.0%. In the second experiment, the pea–ryegrass mixture appeared to respond well, depending on plant arrangement and seeding ratio factors: the ratios 50:50 and 100:50 showed the greatest crop yield advantages, of 12.0% and 11.0%, respectively. All silages revealed a very good suitability of a pea–ryegrass intercropping system with high-quality silage production in the Southern Mediterranean region.

1 | INTRODUCTION

The intensification of dairy farming systems worldwide has led to increases in silage consumption, usually from high-input grass species (e.g., maize [*Zea mays* L.] and sorghum [*Sorghum bicolor* L.]), with protein supplement charged to soybean meal (Lehuger et al., 2009). In fact, the most important crops for ensiling are cereals. Other common silage crops include legumes and industrial byproducts (e.g., pineapple peel, sweet corn husk and cob mixed with bagasse and vinasse) (Wilkinson et al., 2003). Particularly, small grain cereals provide high yields in terms of dry matter (DM) but produce forage and silage with low crude protein. On the

Abbreviations: ADF, acid detergent fiber; AR, alternate rows; CER, cereals; CP, crude protein; CR, competitive ratio; DM, dry matter; LER, land equivalent ratio; NDF, neutral detergent fiber; PR, pea and ryegrass; SR, same row; WSC, water-soluble carbohydrates.

contrary, silage of legumes is rich in protein and gives high nutritional values in comparatively lower quantities (Primi et al., 2019; Ruggeri et al., 2017; Yucel et al., 2018).

According to a survey by Hutnik et al. (2012), greater silage of cereals and legumes is produced in countries with predominantly wet climates. In countries with good weather for haymaking, such as France and Italy, about half of the forage is ensiled (Wilkinson et al., 2003). When considering the semiarid Mediterranean environments, dairy farming systems tend to vary due to the physical and chemical characteristics of the land such as soil type, altitude, and landscape. This deeply affects how local livestock areas managed (Gibon et al., 1999; Peco, 2002). These physical characteristics go along with the distances from great inhabited centers and a land use model (Corbacho et al., 2003; Pflimlin et al., 2003). Hadjigeorgiou et al. (2005) report that this orientation contributes to plant biodiversity and may favor adoption of more sustainable livestock farming, such as routine crop rotation and intercropping.

Many studies on intercropping systems have been conducted in Mediterranean environments to evaluate forage quality suitable for ensiling (Annicchiarico et al., 2017; Iannucci et al., 2006; Monti et al., 2016). In intercrops, cereals provide structural support for legume growth, improve light interception, and facilitate mechanical harvest, while legumes generally increase the protein and mineral content of the forage (Yucel et al., 2018). Among cereal/grass species, ryegrass (*Lolium multiflorum* Lam. var. Westerwoldicum) represents a relevant asset for livestock farming (Giambalvo et al., 2011; Saia et al., 2016; Topcu et al., 2021; Tsiplakou et al., 2014). The species consistently shows excellent adaptability to the pedoclimates of the inland regions of the Southern Mediterranean (Bacchi et al., 2021; Bonanno et al., 2012; Saia et al., 2016). It is well known for remarkable production and qualitative performance of the forage, both in pure stand and when grown in mixtures (García de Arévalo et al., 1994; Kramberger et al., 2012). Among legume crops, the pea (*Pisum sativum* L.) shows higher yield potential than other grain legumes (Annicchiarico et al., 2008; Carrouée et al., 2003; Ruisi et al., 2012) and is frequently used in a mixture with barley (*Hordeum vulgare* L.) or wheat (*Triticum* spp.) (Monti et al., 2016). This species has great adaptability through intercropping with cereals as a whole-crop forage, as documented by Mustafa et al. (2003) and Bacchi et al. (2021). It is ensiled as a multifunctional crop, providing protein and starch sources for livestock (Danieli et al., 2011; Mustafa et al., 2004; Pursiainen et al., 2008). Mustafa et al. (2000) and Salawu et al. (2002) reported that ruminal digestibility and total nutrients from pea silage were similar to alfalfa (*Medicago sativa* L.). Borreani et al. (2009) found that pea and other legumes, such as faba bean (*Vicia faba* L.) and white lupin (*Lupinus albus* L.) could be successfully ensiled after a wilting period in good weather conditions.

Core ideas

- Intercrops represent a key strategy to increase the sustainability of forage production in Sicily.
- Legumes in forage mixtures provide potential benefits to soil fertility and fodder quality for livestock feeding.
- Plant arrangement and seeding ratio should be jointly assessed to maximize yield and pea–ryegrass silages' quality.
- Pea–ryegrass intercrop is very suitable for quality silage production in the Southern Mediterranean regions.

Few studies have been conducted on the quality of silage obtained from forage mixtures of pea and other crops, such as triticale (x *Triticosecale* Wittm. ex A. Camus), durum wheat (*Triticum durum* Desf.), barley, oats (*Avena sativa* L.), and other cereals or annual grasses. However, the yield potential of forage mixtures and the quality of silage largely depend on the crop's selection and their relative combination in the mixed seeded crops, as reported by Soufan et al. (2021). For this purpose, a trial was carried out across two growing seasons by testing several intercropping combinations to establish the most suitable approach for ensiling in semiarid environments. Bacchi et al. (2021) reported an excellent qualitative response of a mixture of pea and ryegrass for forage production in the Mediterranean environment. We sought to complete the framework those researchers introduced, integrating the excellent forage characteristics of the mixture with the benefits of the intercropping system for production of quality silage. Two experimental trials were performed for an initial evaluation of the forage mixture with reference to its suitability for silage cultivation in a semiarid climate.

2 | MATERIALS AND METHODS

2.1 | Test site

Trials were conducted in two consecutive growing seasons (the first was 2017–2018, the second was 2018–2019) at the experimental farm "Don Pietro Canicarao," located near Ragusa in southwest Sicily (36.964140, 14.629026; 220 m asl).

Before sowing, three soil sample points per plot were sampled at 0–30 cm, combined, and analyzed. The soil type in the area is mostly clay (40% clay, 35% silt, and 25% sand), classified as Pachic Calcixerolls (USDA Classification, 1975). The soil chemical characteristics (0–30 cm) were pH 8.0 (1:2

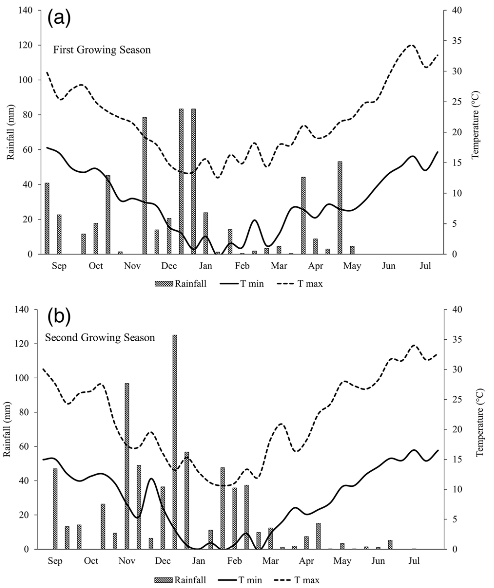

FIGURE 1 Rainfall and air temperature trends during the growing seasons 2017–2018 (a) and 2018–2019 (b) at "Don Pietro Canicarao" farm (Ragusa, Italy). In the charts, 10-day values are shown.

H_2O); total carbon, 1.73% (Walkley & Black, 1934); and total nitrogen, 1.75% (Kjeldhal method [Kjeldahl, 1883]).

The study location has a warm temperate climate according to the Köppen–Geiger classification (Kottek et al., 2006), with dry summers (Csa). The average annual rainfall is about 541 mm, mainly distributed in autumn (34%) and winter (43%). The annual average temperature is 16.5˚C, the average maximum temperature is 20.1˚C, and the average minimum temperature is 13.1˚C.

2.2 | Weather data

A weather station belonging to the Sicilian Government (SIAS, 2021) collected climate data. It measured air temperature, rainfall, relative humidity, solar radiation, and wind speed. A data logger (model WST1800) recorded daily minimum and maximum air temperatures and rainfall data for this study.

Figure 1 shows the trends of average maximum and minimum air temperature and total rainfall during the first and the second growing seasons.

During the first growing season, the total rainfall that occurred was 583 mm, higher than the long-term average (482 mm), mostly in November and December. Rainfall in November and December (32% of annual total) enabled optimal seedbed preparation. Small rains (10.6 mm total) were recorded from early February until mid-March. This led to reduced crop growth causing shifts in species composition in mixtures. Temperatures were close to the long-term average.

In the second growing season, total rainfall was 840 mm, much higher than the long-term average. Temperatures were close to the long-term average.

2.3 | Experimental design and crop management

2.3.1 | Experiment 1

Experiment 1, begun in December 2017, focused on pea agronomic performance in a mixture with different cereals and grass species, typically used for silage production in Mediterranean semiarid environments (Figure 2).

The treatments consisted of four cereals (see below) and one annual grass species each grown singly in a pure stand or in mixture with pea, adopting a randomized complete block design with four replications. The seedbed preparation used ploughing (25 cm deep) in August. Surface harrowing was conducted in November to control emerged weeds before sowing. The crops were sown in late December. No fertilization was performed according to experimental protocol adopted. A sufficient nutrients availability was, however, guaranteed by the previous fertilization carried out in the same field cultivated with carrot crop, as confirmed by other studies (D'Haene et al., 2018; Nendel et al., 2013). For all species in pure stands, 350 pure live seeds m^{-2} were used for barley (variety Diomede), durum wheat (variety Simeto), triticale (variety Catria), and oats (variety Argentina); 450 pure live seeds m^{-2} for ryegrass (variety Elunaria) and 80 pure live seeds m^{-2} for pea (variety Baccarà, semi-leafless).

The seeding ratio between the species was based on number of pure live seeds m^{-2} per species and it was equal to 50:50. The mixed species were sown 3–5 cm deep in alternate rows (AR) with a modified drill. Each plot was 3 × 25 m (25 rows, 0.12 m apart). Seeds were not inoculated with *Rhizobium* spp. During the winter–spring growing season, plant height, soil cover rate, and plants per m^2 were assessed. All crops, both in pure stands and mixtures, were cut ~3 cm from ground level at soft dough stage (stage 85) for grain cereals (Zadoks et al., 1974), medium milk (stage 75) for ryegrass (Lancashire et al., 1991; Witzenberger et al., 1989), and pod filling stage (stage 79) for pea (Meier, 1997). Mowing began in early May, starting with pea–triticale and triticale in pure stands, and then the intercrops were harvested. At harvest, total fresh biomass was measured, including biomass of legume, grass, and weeds. A representative sample of

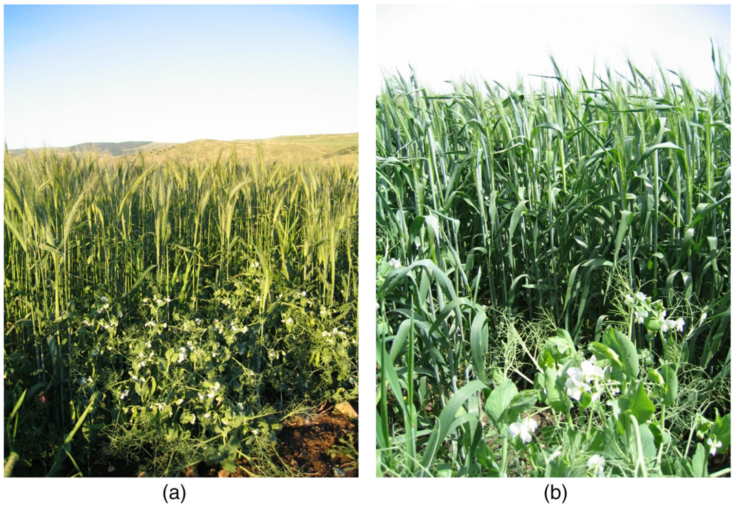

FIGURE 2 A view of some intercrops planted in experiment 1 at "Don Pietro Canicarao" farm (Ragusa, Italy) during the growing season 2017–2018. (a) Pea–wheat intercrop. (b) Pea–triticale intercrop.

FIGURE 3 Pea–ryegrass intercrop.

plant material was obtained from each treatment plot, divided by hand in botanical components, dried at 60˚C for 36–48 h to constant weight, and then weighed to obtain DM content.

2.3.2 | Experiment 2

The trial conducted in the second growing season focused on the agronomic performance of pea–ryegrass intercropping (Figure 3) and on the quality of silages obtained. The pea–ryegrass intercropping was mainly chosen taking the silage quality into consideration, regardless of yields of this system.

The experiment deepened on the "intercropping ratio" between pea and ryegrass based on number of pure live seeds m^{-2} per species (100:50, 75:25, 50:50, and 25:75) and by applying different sowing methods (in AR or in the same row [SR]). Moreover, the pea (100:0) and ryegrass (0:100) were also grown in pure stands as control treatments.

A randomized block design was adopted with three replications per treatment. The plot was 3 × 25 m (25 rows, 0.12 m apart).

Crops were sown in late December in tilled soil with a ploughing depth of about 25 cm, followed by complementary operations (surface harrowing) for seedbed preparation. The ryegrass grown as pure stands was sown at 450 pure live seeds m^{-2}. For pea in pure stands, a density of 80 pure live seeds m^{-2} was adopted. The mixed species were sown through a modified seed drill. Crops were sown and harvested in the first 10-day period of May, adopting the same techniques carried out in experiment 1.

2.4 | Ensilage procedure and analysis

At the end of the second growing season (experiment 2), using 10 L airtight micro-silos, silages obtained from fermented biomasses derived from pea–ryegrass intercrops were tested.

Fresh biomasses were used to conduct silage tests. Biomass was chopped to 10–20 mm with a shredder (OMAS Molino Elettrico M12) and put in the micro-silos and tightly compacted to expel air. Micro-silos were made of transparent plastic and evenly lined with dark polyvinyl chloride. They were placed in a shaded environment at an average temperature of approximately 21˚C to avoid the UV light damage to the plastic film and limit the effect of direct light on temperature measured on the micro-silos (Borreani et al., 2018). After around 150 days, in the first 10-day period of October, they were opened for sampling. A 5-month period was chosen to evaluate not only the chemical and fermentative characteristics, but also the shelf-life of the silage. This experimental setting best reflects the realistic conditions of silage harvesting on farms (personal consideration).

The following analyses were carried out for each sample: pH and DM (Association of Official Analytical Chemists International 930.15); ethanol content (Fussell et al., 1987); lactic acid, acetic acid, propionic acid, and butyric acid (Fussell et al., 1987); ammoniacal nitrogen ($N\text{-}NH_3$) (Weatherburn, 1967); crude protein (Association of Official Analytical Chemists International 984.13); ash content (Association of Official Analytical Chemists International 942.05); neutral detergent fiber (NDF), and acid detergent fiber (ADF) content (Association of Official Analytical Chemists International 973.18); and water-soluble carbohydrates (WSCs) (AOAC, 2006; Thomas, 1977).

2.5 | Calculations

In each year-test, intercrops were assessed through a comparison of the DM yield and the use of indices such as the land equivalent ratio (LER), competitive ratio (CR), and aggressivity index (A). The LER values were estimated using the following equation (De Wit et al., 1965; Willey, 1979), similar to those adopted by Giambalvo et al. (2011) and Saia et al. (2016). This evaluates the indices of intercrop efficiency in a similar cultivation environment:

$$\mathrm{LER} = \mathrm{LER}_{\mathrm{pea}} + \mathrm{LER}_{\mathrm{cer}} = \frac{Y_{\mathrm{pea(Mix)}}}{Y_{\mathrm{pea}}} + \frac{Y_{\mathrm{cer(Mix)}}}{Y_{\mathrm{cer}}}$$

where Y_{cer} and Y_{pea} are the biomass yields of cereal/grass species and pea in pure stand, respectively, and $Y_{\mathrm{cer(Mix)}}$ and $Y_{\mathrm{pea(Mix)}}$ represent the biomass yield component of cereal/grass species and pea, respectively, in mixture. The CR index was obtained using the equation proposed by Willey (1979):

$$\mathrm{CR}_{\mathrm{pea}} = \frac{\mathrm{LER}_{\mathrm{pea}}}{\mathrm{LER}_{\mathrm{cer}}} \text{ and } \mathrm{CR}_{\mathrm{cer}} = \frac{\mathrm{LER}_{\mathrm{cer}}}{\mathrm{LER}_{\mathrm{pea}}}$$

where the maximum value of the parameter refers to the larger competitive capacity of the species in the mixture. The intensity of competition between the intercropped species was calculated by the aggressiveness index, A_{pea}, (McGilchrist et al., 1971) and the competitiveness ratio:

$$A_{\mathrm{pea}} = \frac{Y_{\mathrm{pea(Mix)}}}{Y_{\mathrm{pea}}} - \frac{Y_{\mathrm{cer(Mix)}}}{Y_{\mathrm{cer}}}$$

Positive values A_{pea} indicate pea dominates the ryegrass; negative values indicate the reverse.

2.6 | Statistical analysis

Statistical analysis was performed using the software MINITAB 19 for Windows (version 19.2.0.0). The data obtained for each growing season were separately analyzed and standardized to facilitate the comparisons. In experiment 1, a general linear model determined the influence of the intercropping system on the recorded agronomic performances. For experiment 2, the general linear model procedure was used to analyze the variance. When this produced significant results, the differences between means were established using Tukey's test (Gomez et al., 1984).

3 | RESULTS

3.1 | Experiment 1

3.1.1 | Agronomic performance

Among all Gramineae in pure stand, triticale, wheat, and barley had high yields, although no significant differences were detected (Table 1).

The pea in pure stand recorded a biomass yield of 4.38 t ha^{-1}, including within this value stems, leaves, and grain. The best performance between the intercrops was recorded for the pea–wheat mixture ($p \leq 0.05$), with percentage increases of +65% compared to the corresponding wheat in pure stand. Among all intercrops in the study, the pea–ryegrass mixture recorded the lowest biomass yield. For all combinations tested, the incidence of pea among mixtures was low (18.0% average DM).

3.1.2 | Land equivalent ratio, competitive ratio, and aggressivity index (experiment 1)

Regarding total LER, no significant differences were found between the treatments. The competitive ratios of cereals in terms of the biomass yield were all higher than 1 (Table 2).

The competitive ratio for pea varied from 0.11 to 0.22; this was a sign of its reduced competitive ability. In the case of aggressivity index for pea, the various treatments did not show significant differences.

TABLE 3 Forage dry matter yield for pure stands and mixtures during growing season 2018–2019 at "Don Pietro Canicarao" farm (Ragusa, Italy), experiment 2 (stage 87 for cereals and stage 79 for legumes)

	Pea biomass yield (t DM ha^{-1})	Cereal/grass biomass yield (t DM ha^{-1})	Total biomass yield (t DM ha^{-1})
P (100:0)			5.87 a
R (0:100)			3.37 b
p-Value			*
Mean effect of plant arrangement			
AR	4.24	1.02	5.26
SR	4.38	0.97	5.35
p-Value	ns†	ns	ns
Mean effect of seeding ratio			
PR (100:50)	5.15 a	0.71 c	5.85 a
PR (75:25)	5.11 a	0.69 c	5.81 a
PR (50:50)	4.64 a	1.09 b	5.74 a
PR (25:75)	2.34 b	1.48 a	3.82 b
p-Value	***	***	***
Mean effect of the interaction plant arrangement × seeding ratio			
AR × PR (100:50)	4.88 ab	0.86	5.73 ab
SR × PR (100:50)	4.85 ab	0.59	5.49 abc
AR × PR (75:25)	4.85 ab	0.72	5.57 ab
SR × PR (75:25)	5.38 a	0.68	6.06 ab
AR × PR (50:50)	4.44 ab	1.18	5.61 ab
SR × PR (50:50)	5.25 a	0.88	6.14 a
AR × PR (25:75)	2.79 bc	1.33	4.13 bc
SR × PR (25:75)	1.88 c	1.64	3.52 c
p-Value	***	ns	**

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.
Abbreviations: AR, alternate rows; P, pea; PR, pea and ryegrass; SR, same row.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
†ns, not significant at the 0.05 probability level.

factors plant arrangement and seeding ratio, showing de facto the reduced productivity of the interaction SR × PR (25:75) ($p \leq 0.01$).

3.2.2 | Land equivalent ratio, competitive ratio, and aggressivity index (experiment 2)

The total LER was assessed by the seeding ratio, which was always greater than 1 on average, except for the PR (25:75) ($p \leq 0.05$) (Table 4).

The plant arrangement factor did not show any significant influence on the recorded means. LER_{pea} appeared to be adequately assessed by the seeding ratio with better values recorded for mixtures in PR (100:50) (0.89), PR (75:25) (0.87), and PR (50:50) (0.79) ($p \leq 0.001$). LER_{pea} was much lower for PR (25:75) (0.39). In addition, in this case, no influence was due to the plant arrangement; however, the interaction between the two factors revealed significant differences among the treatments ($p \leq 0.001$). The interaction SR × PR (75:25) showed a higher absolute value for LER_{pea} (6.14), although it was not statistically different from other treatments, except SR × PR (25:75), solely belonging to the grouping c. Again, the lowest absolute values were recorded for the interactions SR × PR (25:75) and AR × PR (25:75), but the latter showed a slight superiority, perhaps induced by the reduced interspecies competition in the row due to the plant arrangement. When analyzing the mean effect of the seeding ratio for the recorded LER_{cer} values, the PR (25:75) had the highest value (0.44) ($p \leq 0.05$). No significant differences were recorded when comparing the means of different plant arrangement treatments with those of the two fixed factors' interaction. Taking into consideration the mean effect of the seeding ratio, CR_{pea} showed the greater competitiveness of the pea in the mixture 100:50 (5.43) and 75:25 (4.48), followed by

TABLE 1 Dry matter biomass yield for pure stands and 50:50 mixtures during growing season 2017–2018 at "Don Pietro Canicarao" farm (Ragusa, Italy), experiment 1 (harvest stage 87 for cereals and harvest stage 79 for legumes).

Crops	Pea biomass yield (t DM ha^{-1})	Cereal/grass biomass yield (t DM ha^{-1})	Total biomass yield (t DM ha^{-1})
Pea (P)	4.38 a		4.38 ab
Triticale (T)		4.60	4.60 ab
Wheat (W)		3.48	3.48 ab
Barley (B)		3.41	3.41 ab
Oat (O)		3.07	3.07 ab
Ryegrass (R)		2.53	2.53 b
P-T	0.59 b	4.06	4.65 ab
P-W	1.07 b	4.27	5.34 a
P-B	0.91 b	3.54	4.45 ab
P-O	0.58 b	3.62	4.20 ab
P-R	1.11 b	2.85	3.96 ab
p-Value	***	ns†	*

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's Test.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
†ns, not significant at the 0.05 probability level.

TABLE 2 Mean values of indices of intercropping efficiency: Land equivalent ratio (LER), competitive ratio (CR), and aggressivity index (A) of intercrops in terms of dry matter during growing season 2017–2018 at "Don Pietro Canicarao" farm (Ragusa, Italy), experiment 1.

Intercrops	LER_{pea}	LER_{cer}	LER_{tot}	CR_{pea}	CR_{cer}	A_{pea}
P-T	0.13	0.88	1.01	0.15	6.77	−0.75
P-W	0.24	1.23	1.47	0.19	5.13	−0.99
P-B	0.21	1.04	1.25	0.20	4.95	−0.83
P-O	0.13	1.18	1.31	0.11	9.10	−1.05
P-R	0.25	1.12	1.37	0.22	4.48	−0.87

Abbreviations: B, barley; O, oat; P, pea; R, ryegrass; T, triticale; W, wheat.

3.2 | Experiment 2

3.2.1 | Agronomic performance

The biomass yield for pea was assessed by the seeding ratio factor and the interaction between the latter and plant arrangement factor ($p \leq 0.001$) (Table 3).

The mean effect of the seeding ratio factor demonstrated the higher production of the mixtures at 100:50, 75:25, and 50:50 compared to 25:75. Taking the interaction effect of the two factors into consideration, SR × PR (75:25) recorded the highest level of production in absolute value (5.38 t DM ha^{-1}), although statistically this was not differentiable from other intercropping combinations, with the exception of PR (25:75), both AR and SR, which had values of 2.79 and 1.88 t DM ha^{-1}, respectively. No significant influence was found that was due to the plant arrangement factor. Instead, the opposite trend was observed for ryegrass biomass yield, which was influenced only by the seeding ratio ($p \leq 0.001$). As the percentage of the grass within the mix grew, its average biomass contribution increased. In fact, in the PR (25:75) treatment the greater contribution of ryegrass in the total biomass yield was recorded (1.48 t DM ha^{-1}), even though the latter was the lesser productive combination with respect to the other treatments. With reference to the total biomass yields, a significant difference was observed between the production levels of the two species in pure stands: the pea produced about 57.0% more biomass than ryegrass ($p \leq 0.05$). No significant influence was found due to the plant arrangement factor, while the influence of the seeding ratio on total biomass yield was significant. As the pea percentage increased in the mixture, significantly higher production levels were found, increasing from 3.82 for PR (25:75) to 5.85 t DM ha^{-1} for PR (100:50), on average. Indeed, PR (100:50), (75:25), and (50:50), all belonging to the same grouping, were certainly more productive than PR (25:75) ($p \leq 0.001$). In addition, a significant difference was observed among the mean values of DM yields obtained from the combination of the two fixed

TABLE 4 Land equivalent ratio (LER), competitive ratio (CR), and aggressivity index (A) of intercrops in terms of dry matter during growing season 2018–2019 at "Don Pietro Canicarao" farm (Ragusa, Italy) (experiment 2).

	LER_{pea}	LER_{cer}	LER_{tot}	CR_{pea}	CR_{cer}	A
Mean effect of plant arrangement						
AR	0.72	0.30	1.02	2.91	0.50	0.46
SR	0.75	0.29	1.04	4.01	0.63	0.42
p-Value	ns[†]	ns	ns	ns	ns	ns
Mean effect of seeding ratio						
PR (100:50)	0.86 a	0.25 b	1.09 a	5.43 a	0.24 b	0.66 a
PR (75:25)	0.87 a	0.21 b	1.08 a	4.48 a	0.25 b	0.66 a
PR (50:50)	0.79 a	0.32 ab	1.12 a	2.80 ab	0.43 b	0.47 a
PR (25:75)	0.40 b	0.44 a	0.84 b	1.12 b	1.33 a	−0.04 b
p-Value	***	*	*	*	**	***
Mean effect of the interaction plant arrangement × seeding ratio						
AR × PR (100:50)	0.83 ab	0.25	1.08	3.45 b	0.30 b	0.57 a
SR × PR (100:50)	0.83 ab	0.18	1.01	7.39 a	0.21 b	0.66 a
AR × PR (75:25)	0.82 ab	0.39	1.04	4.25 ab	0.28 b	0.61 a
SR × PR (75:25)	0.92 a	0.20	1.12	4.72 ab	0.22 b	0.71 a
AR × PR (50:50)	0.75 ab	0.35	1.10	2.47 abc	0.48 b	0.41 a
SR × PR (50:50)	0.89 a	0.26	1.16	4.21 ab	0.31 b	0.63 a
AR × PR (25:75)	0.48 bc	0.39	0.87	1.48 bc	0.91 ab	0.08 ab
SR × PR (25:75)	0.32 c	0.49	0.81	0.76 c	1.74 a	−0.17 b
p-Value	***	ns	ns	*	*	**

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.
Abbreviations: AR, alternate rows; PR, pea and ryegrass; SR, same row.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
[†]ns, not significant at the 0.05 probability level.

the 50:50 (2.80) ($p \leq 0.001$). The PR (25:75) showed a slight increase for the cereal species over the legume. This aspect was confirmed by the CR_{cer} (1.33) that was subsequently determined ($p \leq 0.01$). The results of analysis of variance also revealed significant differences between the means of the different treatments, confirming the trend previously observed by the comparison of the mean effect of the seeding ratio: the CR_{pea} values recorded for the interactions AR × PR (25:75) and SR × PR (25:75) were significantly lower ($p \leq 0.05$). In contrast to CR_{cer}, the SR × PR (25:75) had the highest value, about 52% higher than the average value obtained for the AR × PR (25:75) ($p \leq 0.05$). A_{pea} was assessed according to seeding ratio ($p \leq 0.001$) and varied from 0.66 (100:50) to −0.04 (25:75) ($p \leq 0.001$). The interaction between the two fixed factors also revealed significant differences among treatments ($p \leq 0.01$). SR × PR (25:75) had the lowest value (−0.17), significantly lower than all other treatments, perhaps a sign of the major interspecies competitions between pea and cereal in the same row. Finally, no influence was due to plant arrangement.

3.2.3 | Chemical composition and fermentation parameters of silages

The chemical composition of silages obtained from pea–ryegrass intercrops and pure stands is shown in Table 5.

The raw protein content of sampled silage varied significantly between crops in pure stands ($p \leq 0.001$) and between mixtures, in both AR and SR ($p \leq 0.001$). For the latter, the interactions AR × PR (75:25) and SR × PR (75:25), together with AR × PR (50:50), had average higher absolute values of crude protein than the other treatments: 18.69%, 17.97%, and 17.76% DM, respectively. Contrary to expectations, the application of an additive seeding ratio (100:50), both in AR and SR, did not lead to appreciable increases in crude protein content, while showing final mean values not significantly different to the results obtained in the above treatments. A significant difference was found as a function of plant arrangement ($p \leq 0.05$). In particular, the AR treatments showed an average crude protein content of 17.28% DM, significantly higher than SR intercrops (16.41% DM). Finally, taking into consideration the mean effect of the seeding ratio,

TABLE 5 Chemical composition of silages obtained from pea–ryegrass intercrops and pure stands harvested during growing season 2018–2019 at "Don Pietro Canicarao" farm (Ragusa, Italy), experiment 2.

	CP (% DM)	Ash (% DM)	NDF (% DM)	ADF (% DM)	WSC (% DM)
P (100:0)	19.04 a	7.16	25.40 b	20.56 b	2.31
R (0:100)	11.55 b	10.37	53.95 a	34.60 a	1.40
p-Value	***	ns[†]	***	***	ns
Mean effect of plant arrangement					
AR	17.28 a	7.49 b	32.08	22.67 b	1.66
SR	16.41 b	9.28 a	32.85	24.15 a	1.90
p-Value	*	*	ns	*	ns
Mean effect of seeding ratio					
PR (100:50)	17.16 b	9.40	29.22 b	22.98 b	1.99
PR (75:25)	18.33 a	7.50	29.18 b	21.41 b	1.15
PR (50:50)	17.33 ab	8.24	31.91 b	23.25 b	2.26
PR (25:75)	14.55 c	8.41	39.54 a	26.01 a	1.72
p-Value	***	ns	***	***	ns
Mean effect of the interaction plant arrangement × seeding ratio					
AR × PR (100:50)	17.17 ab	9.64 ab	29.89 c	23.12 abc	1.38
SR × PR (100:50)	17.01 ab	10.53 a	27.67 c	22.77 abc	2.24
AR × PR (75:25)	18.69 a	6.89 ab	30.23 c	20.08 c	1.01
SR × PR (75:25)	17.97 a	8.11 ab	28.13 c	22.74 abc	1.29
AR × PR (50:50)	17.76 a	6.34 b	29.32 c	22.29 bc	2.46
SR × PR (50:50)	17.04 ab	9.21 ab	33.45 abc	23.90 abc	2.38
AR × PR (25:75)	15.50 b	7.11 ab	38.87 ab	25.21 ab	1.79
SR × PR (25:75)	13.60 c	9.70 ab	40.21 a	26.82 a	1.64
p-Value	***	*	***	**	ns

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.
Abbreviations: ADF, acid detergent fiber; AR, altnerate rows; CP, crude protein; NDF, neutral detergent fiber; P, pea; PR, pea and ryegrass; R, ryegrass; SR, same row; WSC, water-soluble carbohydrates.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
[†]ns, not significant at the 0.05 probability level.

a very significant difference ($p \leq 0.001$) between means was found: a higher crude protein content was recorded for PR (75:25), an average of 12.00% higher than the other treatments in absolute value, while the mean value obtained by the PR (25:75) (14.55% DM) was much lower. No significant differences were found for the recorded ash contents among crops in pure stands or due to the seeding ratio factor, although an influence of plant arrangement emerged, with SR crops having a significantly higher ash value (9.28% DM) ($p \leq 0.05$). The interaction between the two fixed factors was also significant, which led to discrimination between the treatments, showing the superiority of SR × PR (100:50) for the higher recorded ash content. The fiber content (both NDF and ADF) was, as expected, significantly affected by the seeding ratio in the mixtures ($p \leq 0.001$), and the mean fiber content of silage from crops in pure stands was found to be heterogeneous ($p \leq 0.001$). The interaction between the two fixed factors was also significant, both for NDF ($p \leq 0.001$) and ADF ($p \leq 0.01$). For both parameters, the superiority of SR × PR (25:75) and AR × PR (25:75) emerged, belonging to the same grouping, and were therefore much richer in fiber than the others in absolute value. No significant differences were found for WSC content among the treatments.

The fermentation characteristics of the silages obtained from pea–ryegrass intercrops and pure stands are shown in Table 6.

The seeding ratio factor significantly influenced the pH values, with lower absolute values within the PR (25:75) and PR (50:50), probably in response to the greater percentage of grass species in the mixtures ($p \leq 0.01$). A comparison of the mixtures showed that the additive intercropping system PR (100:50) significantly reduced this pH-lowering effect (approximately 9.00% higher) due to the increased buffering power of the leguminous silage biomass. The

TABLE 6 Fermentation characteristics of silage obtained from pea–ryegrass intercrops and pure stands harvested during growing season 2018–2019 at "Don Pietro Canicarao" farm (Ragusa, Italy), experiment 2.

	pH	LA (% DM)	AA (% DM)	PA (% DM)	BA (% DM)	Ethanol (% DM)	N-NH_3 (% N_{to})
P (100:0)	4.58 ab	10.87	1.79	0.26	0.28	4.43	10.74
R (0:100)	4.34 b	11.53	0.94	0.19	0.18	6.11	9.21
p-Value	ns[†]	ns	ns	ns	ns	ns	ns
Mean effect of plant arrangement							
AR	4.44	1.38	0.14	0.20	0.22	3.02	10.60
SR	4.58	1.33	0.14	0.15	0.18	4.21	8.33
p-Value	ns	ns	ns	ns	ns	ns	ns
Mean effect of seeding ratio							
PR (100:50)	4.82 a	1.31	0.16	0.23	0.22	2.81 b	8.39
PR (75:25)	4.50 ab	1.44	0.14	0.20	0.22	4.82 a	9.34
PR (50:50)	4.39 b	1.38	0.15	0.14	0.23	2.64 b	9.27
PR (25:75)	4.33 b	1.28	0.12	0.12	0.14	4.24 a	10.87
p-Value	**	ns	ns	ns	ns	*	ns
Mean effect of the interaction plant arrangement × seeding ratio							
AR × PR (100:50)	4.88 a	13.20	1.98	0.32	0.29	2.10 b	8.49 ab
SR × PR (100:50)	4.92 a	12.45	1.36	0.18	0.16	4.53 ab	7.87 ab
AR × PR (75:25)	4.39 ab	15.26	1.34	0.23	0.29	2.91 ab	11.66 ab
SR × PR (75:25)	4.61 ab	13.51	1.41	0.16	0.16	6.72 a	7.01 b
AR × PR (50:50)	4.29 b	14.28	1.15	0.12	0.18	2.50 ab	8.87 ab
SR × PR (50:50)	4.49 ab	13.50	1.53	0.15	0.25	2.53 b	9.53 ab
AR × PR (25:75)	4.22 b	12.40	1.22	0.11	0.14	4.65 ab	13.39 a
SR × PR (25:75)	4.44 ab	13.20	1.10	0.14	0.14	3.93 ab	8.35 ab
p-Value	**	ns	ns	ns	ns	*	*

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.

Abbreviations: AA, acetic acid; AR, alternate rows; BA, butyric acid; LA, lactic acid; P, pea; PA, propionic acid; PR, pea and ryegrass; SR, same row.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]ns, not significant at the 0.05 probability level.

interaction between the plant arrangement and the seeding ratio was significant ($p \leq 0.01$), resulting in appreciable differences among the means of the treatments in the study. In addition, in this case, higher pH levels were recorded for the additive combination (100:50), for both AR and SR seeding. It was interesting to observe how PR (25:75) and PR (50:50), both seeded AR, had the lowest pH values (in absolute value terms), all belonging to the same grouping (b). No significant differences were observed among the means for the acetic acid, butyric acid, lactic acid, and propionic acid parameters. However, assessment of the organic acid profile indicated a preponderance of lactic acid. There were small amounts of acetic, propionic, and butyric acid. The seeding ratio factor significantly influenced the ethanol content, with significantly lower values within the PR (100:50) and PR (50:50) ($p \leq 0.05$). The interaction between the plant arrangement and the seeding ratio was also significant ($p \leq 0.05$), resulting in appreciable differences between the averages of the treatments analyzed. Minor levels of ethanol were recorded on average for AR seeding with additive combinations (100:50). For PR (50:50) in both AR and SR seeding, mean values were not significantly different. No significant differences were found for the ethanol content recorded among crops in pure stands and because of the plant arrangement factor. The NH_3-N content was also evaluated. Although there was no significant influence with regards to the mean effect of seeding ratio, the values were slightly below the threshold identified by Haigh (1996 and 1998) for PR (100:50), PR (50:50) and PR (75:25), with average values of 8.39%, 9.27%, and 9.34% N, respectively. Ultimately, the interaction between plant arrangement and seeding ratio was significant ($p \leq 0.05$), allowing for clear discrimination between the different treatments. Higher NH_3-N levels were recorded for AR × PR (25:75) (13.39% N), while SR × PR (75:25) was the best combination, with a much lower NH_3-N value than the other treatments ($p \leq 0.05$).

4 | DISCUSSION

The knowledge of the cultivation environments is an aspect of great importance in order to better target the most sustainable productive addresses for a given area. Surely semiarid climate environments represent a challenge for farmers, but the joint knowledge of natural inputs (distribution of rainy events, soil types, etc.) can only be a clear reference for the farmer in the choice of crop systems most suitable for local production contexts. Crop-wise, the morphological traits of pea have been extensively studied, bringing out the greater adaptability of the semi-leafless and leafless varieties to environments with reduced water availability (Harvey, 1980; Silim et al., 1992). Thus, with the right agronomic management (cropping technique and choice of the most suitable genotype) pea might represent an important asset for livestock farming. Moreover, its use for forage purpose, namely for silage production, has shown promising results for its wider spread in the agricultural areas of southern Mediterranean regions.

4.1 | Experiment 1

In accordance with previous studies (Bacchi et al., 2021; Baxevanos et al., 2017, 2019; Dordas et al., 2012; Monti et al., 2016), we found that pea in pure stands and in mixtures with cereal and annual grass species is a valid crop for production of high-quality fodder in a semiarid environment. Focusing on biomass yield values obtained in the first trial, a greater productivity of the pea–wheat (5.34 t ha^{-1}) and pea–triticale (4.86 t ha^{-1}) intercrops was found. Furthermore, taking the indices of intercrop efficiency into consideration, the total LER values of all intercrop combinations were good, highlighting the more efficient use of growth resources relative to sole crops. This is consistent with previous results (Bacchi et al., 2021; Giambalvo et al., 2011; Monti et al., 2016, 2019; Saia et al., 2016; Yilmaz et al., 2008). The amount of DM produced by pea within various intercropping systems has proven to be low, contributing on average 18.0% of total DM yields. Taking into consideration CR and *A*, our findings highlight that increasing the seeding ratio in favor of pulse species is a necessary strategy to improve the balance of DM yields and protein content in fodder crop mixtures.

4.2 | Experiment 2

In the second trial, significant differences were also found in biomass yields as the seeding ratio varied. Particularly, greater grass concentrations in the mixture significantly depressed the productive performance of the intercrops so designed. The same trend was confirmed by observing the indices of intercrop efficiency, where total LER values were greater than 1 for all intercrops except for the PR (25:75). Contrary to what was found in experiment 1, in the second trial, the pea was better able to realize its productive potential, which resulted in a more balanced contribution in biomass between the legume and cereal fractions within the mixtures. We believe that the productive differences in and contribution to biomass yield within the mixtures between the two years were due to the different climatic conditions that prevailed during the germination period. This is consistent with the weather data, where the rainfall distribution after sowing in the first growing season led to a reduced emergence of pea seeds. Moreover, as the emergence phase was completed, this lower seeding ratio of pea in the mixtures exposed the crop to strong interspecific competition originating in cereal and annual grass species, which explained the small amounts of DM produced by the pea within the several intercropping combinations that were tested in the first year of experimentation. The plant arrangement factor also influenced the intercrop performance. It was evident that AR seeding led to, on average, more protein-rich silage; thus, this plant arrangement strategy might be advisable to increase the crude protein content of pea–ryegrass intercrops. As a result of our second trial, we argue that AR seeding leads to a clear spatial separation of crops by reducing the competitive advantage of the most competitive crop in the intercropping system (e.g., cereals and annual grass species). Moreover, it also allocated a given amount of space with corresponding resources to each species, thereby delaying interactions (positive or negative) until one species was able to reach the resource pool of the other, which is consistent with previous studies (Chapagain et al., 2014; Cheriere et al., 2020; Kermah et al., 2017; Martin et al., 1982). Taking into consideration the qualitative characteristics of the silages, the protein content also varied considerably according to the seeding ratio that was applied. It was found that both plant arrangement and seeding ratio should be jointly evaluated to maximize the protein concentration of silage obtained from pea–ryegrass intercrops. As reported previously (R. T. Ward, personal communication, 2009; Kung et al., 2001), the highest pH values were recorded within treatments with the most favorable seeding ratios for legumes, and this results from the increased buffering power of the leguminous ensiled biomass. The combination of the two fixed factors made it possible to appreciate the differences between the means, depending on the factors applied. Alternate seed mixes always showed the highest pH values due to the increased contribution of legumes in the biomass yield obtained. Conversely, for SR mixtures, the interspecific competition affected in fact the development of the pulse, reducing the buffer effect during silage's fermentation processes. The chemical compositions of silages obtained from the mixtures were optimal; ash and fiber (NDF and ADF) values were similar to those reported in previous studies in other regions (Copani et al., 2016, 2009; Dewhurst et al., 2003; Kaiser et al., 2007; Yucel et al., 2018).

The WSC of silages appeared to be acceptable, always above the threshold of 3.70% DM, identified by Haigh (1996) for successful ensilage without the use of additive. The fermentation processes during ensiling appeared to be similar to the ensiling processes of other common forage intercrops (Bergen et al., 1991; McDonald et al., 1991). Silage fermentation was dominated by the production of lactic rather than acetic acid. The profile of the acids showed a preponderance of lactic acid, which was always above the target threshold of 6.50% DM identified by Haigh (1998). Although no significant differences were found among treatments during the test, the DM of silage had, on average, less than 0.30% propionic and butyric acids. The NH_3-N content in silage was another indicator of fermentation. Silage is considered excellent when the NH_3-N content is less than 7.0% of total N, and it is considered good when it is between 7.0% and 10.0% N (Haigh, 1996, 1998). High ammonium concentrations (>12.00%–15.0% N) are therefore the result of excessive degradation of the proteins in the silo, which is caused by a slow decrease in pH or clostridial action (McDonald et al., 1991; Woods, 1961). In this study, the level of NH_3-N was lower than 10.0% N, except for the sole pea and PR (25:75) silage samples. Adding additives to the green forage could have further reduced the level of NH_3-N, according to Umana et al. (1991) and Ojeda et al. (2001).

5 | CONCLUSIONS

This study identified potential benefits from planting pea within forage mixtures in a Southern Italian environment. In addition to the well-known beneficial effects of increasing the fertility of the soil in which the crop is grown, the reintroduction of legumes within the forage systems of a Mediterranean environment would enrich agrobiodiversity, increase the sustainability of agricultural systems, and at the same time provide protein-rich forage biomass for livestock. According to our findings, LER_{tot} was always greater than 1 and corresponded to crop yield advantages ranging from 2.0% to 47.0%. However, the results highlighted the poor competitive ability of pea in intercrops, contributing on average 18.0% of total DM yields from the mixtures tested in the first test. Our findings highlighted that increasing the crop combination ratio in favor of pulse specie is a necessary strategy to achieve a better balance within DM yields and protein content in fodder crop mixtures. For CP content, the pea–ryegrass mixture appeared to respond adequately depending on the plant arrangement and seeding ratio factors adopted. Thus, AR seeding might be advisable to increase the CP content of pea–ryegrass intercrops; the crop combination rates 50:50 and 100:50 showed the greatest crop yield, with advantages of 12.0% and 11.0%, respectively. All silages obtained, except samples derived from the mixture PR (25:75) and pea in pure stands, showed fermentation characteristics and chemical compositions comparable to those reported in the literature.

AUTHOR CONTRIBUTIONS

Giuseppe Di Miceli: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; writing–original draft; writing–review and editing. **Mario Licata**: Formal analysis; investigation; validation; visualization; supervision; writing–original draft; writing–review and editing. **Roberto Marceddu**: Data curation; formal analysis; investigation; methodology; software; validation; writing–original draft; writing–review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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6.2. *Asparagus officinalis* (L.): Yield and Field Performance of 10 Genotypes Cultivated in a Semi-arid Environment

Asparagus officinalis (L.): yield and field performance of 10 genotypes cultivated in a semi-arid environment

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Abstract

***Asparagus officinalis* (L.) (*Asparagaceae*) is widely cultivated throughout the world, mostly as a traditional food item but, in recent times, also due to its acknowledged many benefits for human health. In Mediterranean environments, this crop proved able to achieve high spear yields, which in many cases may be marketed earlier than competing products, with a significant advantage for local growers. Although the cropping technique of *A. officinalis* has already been deepened, there is still scarce knowledge about the year-to-year productive stability of the main commercial cultivars in the pedoclimatic conditions of the southern regions of Italy, potentially suitable to the cultivation of this species. This research aimed to provide local farmers with useful information to facilitate the introduction of this species in their farming systems, also giving information about the major factors affecting yield outcomes in these environments. A 3-year cultivation trial was therefore carried out, comparing the productive performances of 10 genotypes of *A. officinalis* ('UC157', 'Purple', 'Italo', 'Darbella', 'Giove', 'Placoseps', 'Grande', 'Jaleo', 'NJ953' and 'Atlas'). For experimental purposes, a randomized complete block design was adopted. The results showed that the farming suitability of *A. officinalis* in the Mediterranean environment is highly variable among cultivars.**

Keywords: agronomy, cultivation trial, crop yields, green asparagus, semi-arid Mediterranean environment

INTRODUCTION

The growth and biochemical properties of asparagus are significantly influenced by the cultivar and the surrounding environment (Zhang et al., 2016; Fukuda et al., 2018). Choosing the most suitable cultivar for cultivation in a given environment is a very important factor in yielding (Liao et al., 1999; Rolbiecki et al., 2021). Cultivation systems, temperature, soil water content, and light intensity can drastically affect plant growth characteristics, as well as bioactive compounds and bioactive properties of asparagus (Motoki et al., 2012; Zhang et al., 2016; Ku et al., 2018a, b; Pegiou et al., 2019). Thus, it is recommended to create optimal growth and development conditions during the growing season to obtain maximum marketable yields of a given asparagus cultivar, i.e., by limiting interspecific competition with weeds, applying organic and mineral fertilization tailored to nutritional needs and ensuring water supply levels appropriate to the growing conditions.

The profitability of the asparagus crop is also partly attributable to the health and nutritional aspects brought about by the consumption of green spears (Ji et al., 2012; Lee et al., 2015; Jaramillo et al., 2016; Iqbal et al., 2017; Ku et al., 2018a; Lee et al., 2014; Solana et al., 2015). Green asparagus is one of the vegetables with the greatest anti-oxidant capacity among the most commonly consumed vegetables in Italy (Pellegrini et al., 2003) and USA (Vinson et al., 1998) due to the high content of phenols (Mitchell and Waring, 2014; Hamdi et al., 2018; Ku et al., 2018a, b; Chitrakar et al., 2019; Pellegrini et al., 2003; Rodríguez et al., 2005).

In Italy, asparagus production markedly increased from 2000 to 2020, both as concerns cultivated area (+34%) and yield (+15%) (FAOSTAT, 2022). However, the spread of the

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cultivation in the Mediterranean area must consider the effects of climate change. It appears quite necessary, nowadays, to implement innovative adaptations in order to mitigate the adverse effects of climate change, while profiting from higher annual yields of crops. They include a wider use of drip irrigation systems, soil mulching, aquifer management and of course choosing the most suitable cultivars to the given agricultural context.

Much research around the world has been carried out about the performance of this perennial crop, but relatively few studies have compared the productive performances of different genotypes in a new growing environment. Therefore, a 3-year experiment was carried out to assess the outcome of cultivation and the productive performances of ten asparagus cultivars in a typical area of the semi-arid Mediterranean environment. The results will be useful for understanding the interactions between cultivars and the growing environment, given also the management system adopted and seasonal climatic conditions.

MATERIAL AND METHODS

Experimental sites

The field experiment was carried out in the years 2008-2010 at the experimental farm "Don Pietro", located in the district of Canicarao, about 10 km northwest of Ragusa, Sicily, Italy (36°58'51.0"N, 14°38'36.5"E, 220 m a.s.l.). The soil was classified as a Typic Calcirellos (United States Science, & Education Administration, 1975), and its main characteristics (0-40 cm) were as follows: clay, 36%; silt, 39.6%; sand, 24.4%; pH 8.0 (1:2 H_2O); total C, 1.74% (Walkley Black method); total N, 1.78‰ (Kijeldhal method).

The climate of the experimental site is semi-arid Mediterranean, with an average annual precipitation of 482 mm, mainly distributed in the autumn (34%), winter (43%) and spring (19%) seasons, respectively (SIAS, 2021). In 2008, 2009 and 2010, average air temperatures were 8.8, 9.5, 10.8°C in winter, 16.0, 17.1, 16.8°C in spring, 23.1, 24.7, 23.8°C in summer, and 16.4, 15.8, 14.4°C in autumn, respectively. The total annual precipitation during the trial reached 479, 760, and 809 mm for the 2008, 2009, and 2010 growing seasons.

Experimental plantings

For experimental purposes, a randomized complete block design (RCBD) with 4 replications was adopted (15×6 m plot with about 200 plants each). Ten *A. officinalis* cultivars were tested: 'UC157', 'Purple', 'Italo', 'Darbella', 'Giove', 'Placoseps', 'Grande', 'Jaleo', 'NJ953' and 'Atlas'. In the year before planting, a starter organic fertilization of mature manure (160 t ha^{-1}) and a plowing (45 cm deep) were carried out in winter. These operations were followed by additional weeding operations. At the beginning of spring, a starter P fertilization with 150 kg ha^{-1} of P_2O_5 was carried out and grooves were mechanically excavated 35 cm deep and 150 cm apart. The asparagus crowns were planted in May 2007, adopting a plant density of 2.23 crowns m^{-2} by placing the propagation material at 30 cm on the previously obtained row. Considering the hot and dry weather during the following summer season, the irrigation practice appeared necessary; a surface-drip-irrigation system was therefore set up, adopting a PE pipe D16 mm, with drippers placed every 30 cm.

Terms of each watering were determined based on crop needs, with single interventions carried out based on estimated evapotranspiration, according to IRRISIAS alerts (SIAS, 2008-2010). The irrigation system was also equipped with a fertigation system and volumetric meters for the measurement of irrigation volumes.

In the first year of planting, from June to October, 180 kg ha^{-1} of N, P_2O_5 and K_2O kg ha^{-1} were distributed by fertigation. Moreover, through a complex fertilizer (MgO 16%, SO_3 32%, and microelements), 45 kg ha^{-1} of magnesium oxide were distributed. In the second and third years, macronutrient fertilization reached 200 kg ha^{-1} of N, P_2O_5 and K_2O.

Field measurements

At harvest time (i.e., as soon as green spears reached a height of 17 to 27 cm) total spear yield (kg ha^{-1}) was measured, further classifying the harvested product, according to the measured diameters, into marketable spears (Ø >10 mm) and thin spears (Ø <10 mm), otherwise termed "asparagine". In all experiment years, the harvest took place simultaneously for all cultivars tested in the trial.

Statistical analysis

Statistical evaluations were performed by means of the software Minitab® version 17.1.0 (Minitab Inc., State College, PA, USA, 2013). All data were preliminary tested for normality applying the Ryan-Joiner test (α=0.05), and a pooled variance analysis (ANOVA) including both experimental factors "year" and "cultivar" was possible only for the variable "total spear production". In the data sets including the two yield components, namely "marketable yield" and "thin spears yield", two separate one-way ANOVA procedures were run for each experiment year, only including the factor "cultivar". When the ANOVA offered statistically significant results, the differences between mean values were appreciated through Tukey's test (p≤0.05) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Productive performances (total spear production, marketable yield and asparagine yield)

The results of the pooled ANOVA of "total spear production" are shown in Table 1. Since the Y×C interaction turned significant, it was necessary to evaluate its size relative to the average effects of the cultivar.

Table 1. Pooled ANOVA of "total spear production" data over the 3-year trial at the experimental farm "Don Pietro" (Comiso, RG, Italy).

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F
Year (Y)	2	260.842	130.421	20.03**
Reps. within year	9	58.608	6.512	
Cultivar (C)	9	345.010	38.334	5.34*
Y×C	18	711.569	39.532	3.92*
Pooled error	81	302.297	3.732	
Total	**119**	**1678.325**		

* significant at 5% level; ** significant at 1% level.

Upon examination of the mean values across years for each of the ten cultivars (Figure 1), a generally constant trend of yields shows up, and in almost all cultivars 2009 was the most productive year, whereas the first and third trial years have generally lower yields. The only exception to this general trend was 'Darbella', which, in contrast to the other cultivars, exhibited a decreasing yield trend among years. Based on the observation of mean values, two groups of cultivars can be identified. The first group consists of 'Placoseps', 'Giove', and 'NJ953', which in all three trial years reached higher yields than other cultivars. The cultivars of the second group, including 'UC157', 'Jaleo', 'Atlas', 'Grande', 'Darbella', 'Italo', and 'Purple', showed a higher variability of yield values around the respective year averages. Even though it is clear that a consistently superior cultivar could not be identified in this trial, emphasis in future trials should probably be given to the cultivars 'Placoseps' and 'Giove', which both exhibited a high degree of consistency, giving the highest mean yield (11.55 and 10.80 t ha^{-1}, respectively) and performing rather well during the whole 3-year-trial.

Data about marketable spear yield (Ø>10 mm) and thin spear yield (Ø<10 mm) were analysed separately by year (Table 2). In 2008, yields in marketable spears were significantly higher for cultivars 'Giove' (7.70 t ha^{-1}), 'Grande' (7.11 t ha^{-1}) and 'Placoseps' (6.96 t ha^{-1}). In 2008, except for 'Purple', all the cultivars tested reached average production levels above 4 t ha^{-1}. 'NJ953' had the highest thin spear yield, approximately 45% higher than the average for 2008.

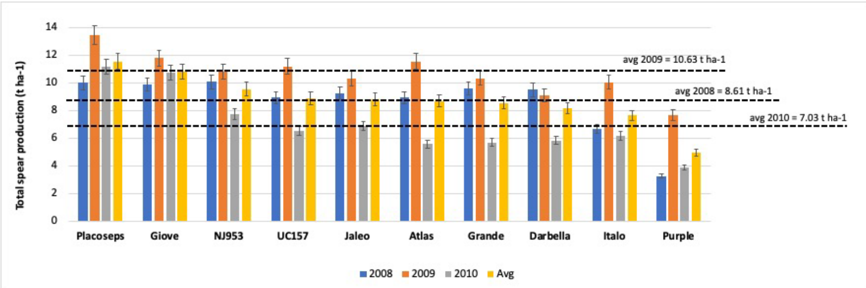

Figure 1. Total spear production (t ha^{-1}) of the 10 chosen cultivars in 2008-2010 at the experimental farm "Don Pietro" (Comiso, RG, Italy). Dashed horizontal lines indicate the average yield values of all cultivars in each year.

Table 2. Results of ANOVA (mean values) for marketable yield of green spears (t ha^{-1}) and asparagine yield (t ha^{-1}) obtained from the 3-year cultivation trial at the experimental farm "Don Pietro" (Comiso, RG, Italy).

Cultivars	2008		2009		2010		avg	
	Marketable spear yield (t ha^{-1})	Thin spear yield (t ha^{-1})	Marketable spear yield (t ha^{-1})	Thin spear yield (t ha^{-1})	Marketable spear yield (t ha^{-1})	Thin spear yield (t ha^{-1})	Marketable spear yield (t ha^{-1})	Thin spear yield (t ha^{-1})
Placoseps	6.96 ab	0.91 b	10.72 a	0.88 ac	8.28 ab	1.39 a	9.47	1.06
Giove	7.70 a	0.79 b	10.45 ab	0.32 d	9.25 a	0.35 c	9.53	0.49
NJ953	6.88 ab	1.72 a	8.93 ab	1.03 ab	5.65 ab	1.33 a	8.25	1.36
UC157	6.13 ac	0.72 bc	9.23 ab	1.08 a	4.42 ab	1.27 a	8.20	1.02
Jaleo	5.20 bc	0.19 d	8.42 ab	0.39 cd	4.95 ab	0.65 bc	7.35	0.41
Atlas	6.33 ac	0.57 bd	9.89 ab	0.75 ad	4.37 ab	0.60 bc	8.70	0.64
Grande	7.11 ab	0.77 b	9.21 ab	0.50 bd	4.19 ab	0.58 bc	8.51	0.62
Darbella	6.62 ab	0.81 b	8.09 ab	0.41 cd	4.60 ab	0.49 c	7.60	0.57
Italo	4.41 cd	0.97 b	8.68 ab	0.67 ad	4.49 ab	1.02 ab	7.26	0.89
Purple	2.53 d	0.22 cd	6.64 b	0.28 d	3.26 b	0.19 c	5.27	0.23
Significance of P-value	***	***	***	***	**	***	-	-

, *: significant differences at $P<0.01$, $P<0.001$, respectively. Means that do not share a letter, including intermediate not reported, are significantly different according to the Tukey method and 95% confidence.

The 2009 commercial production figures partially confirmed those of 2008. In this case also, the most productive cultivars were 'Placoseps' (10.72 t ha^{-1}) and 'Giove' (10.45 t ha^{-1}), although no appreciable differences with the remaining cultivars were detected by statistical analysis. The incidence of thin spears in total production has decreased for all cultivars, with 'UC157' that recorded the highest thin spear yield in 2009 (Figure 2).

In 2010, the productive performances suffered a slight decrease, on average 35% less than in 2009. 'Giove' (9.25 t ha^{-1}) proved to be the most productive, recording on average the highest yield in commercial spears, also being statistically different from all the other genotypes. 'Purple', instead, exhibited the lowest yields, although statistically not different from many other cultivars, also belonging to the same statistical group.

CONCLUSIONS

In conclusion, it is worth to be noted that the most productive cultivars, in terms of marketable spears, have shown a tendency to substantially reduce the incidence of the non-commercial component on total production (thin spears). This result must be of course considered based on the previous responses of the genotypes in terms of total spear productions, but might be eventually interpreted as a clue of the higher suitability of these cultivars to the management techniques applied.

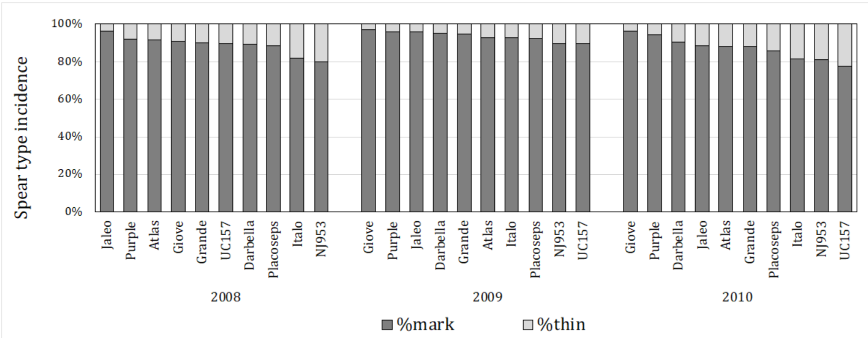

Figure 2. Spear type (mark = Ø>10 mm and thin = Ø<10 mm) incidence during the 3-year trial at the experimental farm "Don Pietro" (Comiso, RG, Italy).

On the other hand, the increase in the incidence of non-commercial spears for the cultivars 'NJ953' and 'UC157', although it was balanced in the first years by average high marketable spear yields, showed the strong dependence of the productive responses of those cultivars on the interaction between the environment and the applied cropping technique. Therefore, emphasis in future trials should probably be given to varieties that have performed best, also in terms of yield stability along testing.

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