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Adaptation of tropical horticultural species

to a changing Mediterranean climate

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Dedicated to the memory of Dr. Salvatore Scianò

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1   Introduction

The Mediterranean Sea has always represented a busy crossroad at which people and cultures from all over the world have met and exchanged their knowledges and goods. Its own name derives by its position, as stated firstly by Isidore of Seville: the sea "is called Mediterranean because it crosses the *media terra*, which is the central region of the World, and its waters extend from West to East, dividing Europe, Africa and Asia" (Barbera, 2021). We can rephrase the theologian's last sentence, objecting that rather than dividing three continents, the Mediterranean Sea unites them, it puts them in communication and represents the common meeting point between human beings from very distant origins. A common way of life takes place on the shores of this sea, which have been the fertile soil where a significant part of the human history has taken place.

Obviously, plant species and crops follow humans closely, and they have been carried by traders and travellers everywhere around the globe. Nowadays, the landscape of the territories bordering the Mediterranean Sea, governed by a climate which unifies many different landscapes, is dotted by citrus and loquat trees coming from China, prickly pear shrubs coming from Mexico, Bougainville from the Caribbean, *Robinia* trees from North America, *Eucalyptus* and *Ailanthus* from Australia, *Carpobrotus* from South Africa and many more, and now all these species form part of the collective imagination of the Mediterranean Sea.

During the last decades, a significant increase in the agricultural surfaces dedicated to the cultivation of tropical horticultural species has been observed in the Mediterranean basin. Crops such as avocado, mango, passionfruit, papaya, litchi, just to cite the most important, are now grown extensively in all the countries bordering the Mediterranean sea, from the Levantine countries such as Egypt, Lebanon and Palestine, to the Gibraltar strait (Ahmed *et al.*, 2010; Aiello *et al.*, 2021; Cano *et al.*, 1996, 1996; Continella *et al.*, 1992; Farina *et al.*, 2017b, 2020c, 2020b; Kourgialas and Dokou, 2021; Lionakis and Loxou, 1996; Medany *et al.*, 2009; Merheb

et al., 2018; Nave *et al.*, 2010; Takeuchi *et al.*, 2017; Tzatzani *et al.*, 2022; Younis *et al.*, 2022). The diffusion of these crops is undoubtedly driven by the preference that European consumers express towards this kind of fruit (CBI, 2021a, 2021b; Migliore *et al.*, 2017), by the increasing globalization of the European food market following the migratory events (Mintz, 2008; Möhring, 2008; Nützenadel and Trentmann, 2008; van Andel and Fundiko, 2016) but is only made possible by the ongoing climate change that is happening in the Mediterranean region (Giorgi and Lionello, 2008). However, the historical unpredictability of the Mediterranean weather is attested even in a major pillar of the Italian literature of the XX century such as *Il Gattopardo*, where Giuseppe Tomasi di Lampedusa reflects on the effects of the climate on the psychology of the Sicilian people, referring to an alternation between "a torrid 40-degrees hell for six months" and "rains, always in the form of storms, which flood the dry riverbeds, drowning humans and beasts, which were dying of thirst just one week before" and soils which can change from scorched clay hills to breeze-kissed rocky bays in a distance of few kilometres.

Such diversity and variability of climates and soils determine and abundance of ecological niches which allow many plant and animal species to coexist and made the Mediterranean basin one of the most important hot spots of biodiversity in the world (Barbera, 2021).

1.1 *Climate change in the Mediterranean basin and implications for agriculture*

Low total annual rainfall and high variability between the years characterize the climate of the Mediterranean basin. Unfortunately, the climate change observed since the second half of the XX century contributed to an even greater reduction of the annual rainfall in the area, at a rate of -3.2 mm/year in the period 1951-1995 (Piervitali and Colacino, 2003). This translates into a state of almost constant water unavailability in most of Northern African and Middle eastern countries (Tuel and Eltahir, 2020), with some serious economic and social consequences (Kelley *et al.*, 2015). The territories which face the northern side of the Mediterranean Sea,

however, are not exempt from these issues. Several areas of the Iberian Peninsula and of the northern Aegean Sea, in facts, already show a shift from a Temperate to an Arid type of climate (Cui *et al.*, 2021). Along with this, an increase in the frequency of heavy precipitation events is forecasted (Gao *et al.*, 2006; Ozturk *et al.*, 2015), meaning that the already decreasing rainfall will be more poorly distributed throughout the year, which will significantly affect water accumulation in the reservoirs.

The Mediterranean climate is officially classified as Csa in the Köppen classification (Köppen, 1936), which is the most widely used climate classification scale in the world: this type of climate is identified by $T_{hot} > 10°C$ and $0°C < T_{cold} < 18°C$; $P_{sdry} < 40$ and $P_{sdry} < P_{wwet}/3$; $T_{hot} > 22°C$ (M. C. Peel *et al.*, 2007). Each of these variables represent:

- · T_{hot}: temperature of the hottest month;
- · T_{cold}: temperature of the coldest month;
- · P_{sdry}: precipitation of the driest month in summer;
- · P_{wwet}: precipitation of the wettest month in winter.

It is expected that temperatures will increase in the Mediterranean area at an even stronger rate than they will do in the Earth as a whole, with an enhanced amplitude in the temperatures recorded between day and night, and between winters and summers (Lionello and Scarascia, 2018), probably due to the peculiar presence of the large body of water at the centre of many continents. Temperature in the Mediterranean region is expected to increase by a worrying 3.5°C by the end of the current century by a median of 21 different models (Calbó, 2010), with maximum summer temperatures likely to increase more than the average in southern and central Europe. However, the Mediterranean region is characterized by the occurrence of both cold and hot temperature extreme events, often even in the same year or fraction of year. In particular, the Mediterranean basin is the only area of the world, along with SE USA, where an increase in the average daily thermal amplitude is forecasted (Tebaldi *et al.*,

2006), this reflecting an expected increased frequency of "too cold" and "too hot" days in the same year. Still, also a decrease in the number of frost days, where minimum temperature falls below 0°C, is expected (Hertig *et al.*, 2010).

All these premises require the human activities, which in no case are unaffected by the natural events, to change in response to the changes in the climate, in order to adapt to the mutating conditions and to still allow for social, economic and cultural viability of the people. It will be fundamental, thus, a shift in the mentality of stakeholders at all levels of the economic system, because it will no longer be possible to do things "the way we used to" but it will be necessary to work in the most efficient way, enhancing the available resources at the maximum with new, smart, and careful choices.

Agriculture – which is one of the main causes of pollution and climate change – is the first of the human activities which must deal with the effects of changing environmental conditions: the exchange of information between different people in different parts of the world has always allowed to experiment the cultivation of different vegetal species in many areas, sometimes with success and sometimes with failure. One of the solutions that proved most successful in the last years for the growers in the Mediterranean basin has been the shift from traditional cultures such as olive or citrus to ones that provide a richer yield per hectare such as avocado or mango. Despite the previously described weather conditions that have become the norm in the region and do not correspond to the ideal habitat for these species, they seem to have adapted well to the new cultivation environment, especially in some areas which turned out to be more suitable. This is confirmed by the ever increasing availability of regionally-grown produce, as evidenced by market trends (CBI, 2021b, 2021a), mainstream media reportages (The Guardian, 2021) and scientific literature (Sáez *et al.*, 2014). The duty of the research institutions is to study the feasibility of the cultivation of these new species and the application of the most efficient growing techniques, in one sense and the other, meaning that

the highest returns for the growers must be obtained while at the same time preserving the future productivity of the territories.

1.2   Tropical crops in the Mediterranean climate: research state of the art

The climatic conditions found in the Mediterranean areas are not the ones at which tropical crops have evolved throughout their history: in facts, the main centres of diversification for these species are located in areas with tropical ($T_{cold} > 18°C$) or temperate climates without an actual dry season (Hu *et al.*, 2022; Scora and Bergh, 1990; Silva and Souza, 2020; Singh, 2016). Research on the cultivation of tropical species in the Mediterranean climate is unfortunately still limited to some experiences carried out by a limited number of research teams belonging to few institutions, even more so when restricting the focus to the Mediterranean Sea basin.

A systematic review conducted on the number of scientific articles investigating tropical crops cultivation in the countries where areas with a Mediterranean climate are found, using "*species_name*" + "*Country_name*" as keywords on the Scopus database, allowed to find only 130 papers as of July 2023, the oldest one dating back to 1960. The most studied species are mango, avocado and cherimoya, and the most active countries in terms of published studies are Italy, Spain, and Egypt. Few research groups are responsible for much of the scientific production, therefore only some aspects of the species' adaptation are covered, corresponding to each group's field of expertise: the most investigated aspects are pathology (Ahmed *et al.*, 2014; Aiello *et al.*, 2022, 2021, 2015; Fiorenza *et al.*, 2023; Guarnaccia *et al.*, 2016; Gutiérrez-Barranquero *et al.*, 2019a, 2019b, 2013, 2012; Ismail *et al.*, 2012; Torta *et al.*, 2003; Youssef *et al.*, 2014), irrigation (Durán Zuazo *et al.*, 2019, 2011, 2006; Durán Zuazo and Tarifa, 2001; Rodríguez Pleguezuelo *et al.*, 2018, 2011b, 2011a) and post-harvest fruit behaviour (Farina *et al.*, 2017a, 2017b, 2018, 2020b, 2020c; Gianguzzi *et al.*, 2021; Passafiume *et al.*, 2020, 2022, 2023; Sortino *et al.*, 2017; Tinebra *et al.*, 2021, 2022a, 2022b). A thorough study on the

behaviour and productivity of any of these species in the Mediterranean climate is yet to be conducted. Most importantly, it is fundamental to monitor the tolerance of such species to the most extreme environmental conditions to which they are exposed in the new cultivation areas; to assess the productive potential of these territories, in relation to the existing conditions, both natural and infrastructural; to formulate a guide to the cultivation of each of the most important species, rather than letting each grower test his own agronomic techniques, with a high risk of economic loss and improper land use, resulting in degradation.

1.2.1   Plant pathology

Insect pests do not constitute, to the present day, a major concern for the growers of tropical fruits in the Mediterranean basin. A comprehensive description of the arthropods found in mango trees in the Levantine coast was only carried out at the beginning of the 1990's (Wysoki *et al.*, 1992) and never repeated for other countries. Most of the mango trees in Sicily, Italy, are host to the scale insect *Aulacaspis tubercularis* (Pellizzari and Porcelli, 2014), which however does not constitute an economically relevant problem. Still, the cultivation of these exotic species can act as a reservoir of potential new infestations by alien insects: in facts, in the recent years the Seychelles scale *Icerya seychellarum* (Westwood) was observed for the first time in Europe, in mango trees growing in the open air in Sicily (Lo Verde *et al.*, 2020).

The most significant threat to the health of the mango trees in the Mediterranean basin, instead, comes from bacterial populations. In facts, the cosmopolite bacterium *Pseudomonas syringae* pv. *syringae* proved to be the causative agent of the disease called Bacterial Apical Necrosis (BAN) of mango (Cazorla *et al.*, 1998). This bacterium is widely diffused on plants which are typical of the Mediterranean agriculture, such as olive and tomatoes, causing little to no alteration to the plant's health or productivity. Following its spread caused by wind and rain events, the bacterium usually ends up in the concave parts of the plant's canopy, such as the leaves pits or, especially, the closed buds. Here, it has the ability to perform Ice Nucleation

Activity (INA) when temperatures fall below 4-6°C: the bacterial colony can lower the temperature of the water droplets around it, causing it to turn into ice crystals and thus increasing their volume (Cazorla *et al.*, 1995). This causes the plant tissues to crack and offer an entrance site to the bacterium, which then installs itself into the plant's vases, causing embolisms and obstructing the lymphatic flow. This results in rotting of the plant's tissues, which start turning black and rotting, proceeding top-down from the terminal shoot's apex down to the whole branch.

The most important abiotic stress factor for tropical crops in the Mediterranean basin is represented by the excessive solar radiation to which they are exposed, especially because it happens in the hot season, paired with extreme high temperatures that are occurring more and more frequently in the last years. Reports of sunburn of the fruits of avocado and mango are common in the Mediterranean producing countries (Alon *et al.*, 2022; Shaban *et al.*, 2021; Singh *et al.*, 2019) while the issue is known also in litchi (Huang, 2019; Kumar, 2020; Mitra *et al.*, 2010; Sanyal *et al.*, 1990). Sunburn fruits are immediately depreciated and lose marketability as fresh produce. They can still be recycled to obtain transformed product, but this means a lower price with a higher amount of work required for the producers.

1.2.2   Irrigation strategies

The Mediterranean summers offer very good temperature conditions for the development of tropical fruit. But, contrary to the tropics, precipitation in this period is absent, and it is necessary to meet the plants' demands with irrigation. At the same time, the Mediterranean tropical fruit production prides itself of its sustainability, motivated by the proximity to the destination markets and lower environmental costs. Therefore, it proves necessary to find solutions for optimizing water consumption of the orchards and making irrigation schedules as efficient as possible.

Several experiments have been carried out in Mediterranean countries on the possibility of providing water with different deficit regimes to tropical tree crops. Sustained deficit irrigation to 75% of the mango tree's evapo-transpirative demand allowed to achieve non-significantly different fruit yields, average fruit weight and subsequent water use efficiency over a three year period in Spain (Durán Zuazo *et al.*, 2011). The same percentage of supplied water was enough to obtain mango fruits of the highest quality, with regards to both total soluble solids and titratable acidity, but reducing fruit weight (Lipan *et al.*, 2021). This might actually be a desirable result for the growers, considering that smaller fruits are in many cases easier to place on the market (Ahmad *et al.*, 2001). Deficit irrigation up to 50% of the mango tree's calculated demand, finally, did not cause a significant decrease in the plant's trunk cross sectional area, and even allowed to reach the highest values of canopy diameter and tree height in the work of Rodríguez Pleguezuelo *et al.* (2018).

Various studies showed, instead, that the avocado fruit is sensible to deficit irrigation treatments: reduced supplies of water altered the fruit's diurnal diameter variation and even halved the tree's yield (Silber *et al.*, 2013, 2012). Interesting results, in the optics of increasing the sustainability of the avocado cultivation, were obtained by enlarging the wetted soil volume from 25% to 75%, for the same volume of water, which allowed to reduce leaf temperature and thus transpiration (Cantuarias and Tomer, 1995). The supply of 75% of the calculated water demand of the plant was optimal for reducing water consumption and achieving comparable yields and fruit quality in two independent studies carried out at opposite locations in the Mediterranean basin (Durán Zuazo et al., 2021; Levin et al., 2011).

1.2.3 *Post-harvest fruit behaviour and handling*

Due to the high investment costs for their cultivation, the high value and their scarcity, the need to valorise even the lower grades of tropical fruits cultivated in the Mediterranean countries emerged soon for growers and marketers alike. A significant amount of work in this

sense has been conducted especially in Italy: several post-harvest techniques, with the goal of extending the fruits' shelf-life and marketability, have been experimented and evaluated. Among them, the application of edible coatings to achieve a longer storage of fruits was successful in mango and papaya (Farina *et al.*, 2020c; Passafiume *et al.*, 2022); Modified Atmosphere Packaging (MAP) proved to extend the shelf life of litchi and cherimoya fruits (Passafiume *et al.*, 2023; Tinebra *et al.*, 2022b); the transformation of the pulp of fresh fruits that were unmarketable due to mere aesthetic flaws into dried products was a success in giving these food wastes a second life (Farina *et al.*, 2020a; Fratianni *et al.*, 2020; Tinebra *et al.*, 2022a).

All these transformed products found appreciation by the market, also in light of the new dietary habits of European consumers (Tarancón *et al.*, 2021; Testa *et al.*, 2023). This implies that while researchers, agronomists and growers must do all to ensure sustainable production of tropical fruits in the Mediterranean is achieved, the know-how for how to deal with the inevitable part of unmarketable product is already present and available to all actors of the supply chain.

1.3 Objective of the study

The objective of the Ph.D., and *trait d'union* of the work conducted over the three years, was to better understand the effect of the environmental conditions of the Mediterranean area on the phenological and physiological behaviour of tropical crops and fill the knowledge gap in this regard.

This Ph.D. thesis illustrates the advances made in understanding the possible cultivation of two of the most important horticultural species of tropical origin, mango and coffee, in Mediterranean climate. We show the comprehensive and detailed findings of different experiments, that have been carried out on these crops in the various environmental and cultivation conditions that the island of Sicily offers.

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2 Mango

Mango (*Mangifera indica* L.) is the most important species of the family of the Anacardiaceae, which also includes other widely diffused dry fruits crops such as pistachio (*Pistacia vera* L.) and cashew (*Anacardium occidentale* L.). Mango is now the third most cultivated fruit species in the world with regards to the surfaces invested and the sixth for tonnes harvested every year (FAO, 2022), with his cultivation area that spans on more than 100 countries, from 33° S in South Africa to 37° N in Italy (Mitra, 2016).

Mango is a species native to the highlands located between the Indian subcontinent and South-East Asia (Singh, 2016; Vavilov, 1926). The word "mango", in facts, comes from the Portuguese "manga", originated from the Malay word "mangga" and the Tamil one "mankay" (Hoad, 2003). From its original territories, the species spread all over the tropical belt of the Earth thanks to the commercial exchanges between people, first via land, reaching the Malaysian peninsula and East Asia around the 7th century A.D., or East Africa in the 10th century A.D. thanks to the Persians, and then also by marine transport, especially after the colonial era of the 16th century A.D. with the Portuguese colonizers carrying the species to their newly subjected territories such as West Africa and Brazil (Litz, 2009; Purseglove, 1969). Mango was later brought to the Caribbean islands by British botanists at the end of the 18th century A.D. (Powell, 1977; Sheridan, 1989). After the plant reached Oceania at the end of the 19th century A.D. (Morton, 1987), Europe was the last continent to be colonized by the mango tree (Calabrese, 1978; Mukherjee, 1953). Nowadays, then, mango can be considered a real cosmopolitan species, diffused and consumed all over the world.

Mango is an evergreen tree which, in the wild, can reach heights up to 40 meters and live for several hundreds of years (**Figure 2.1**). Leaves are simple, petiolate, and alternate, can span from 12 to 38 cm at maturity and their shape and colour are variety-related: they can be lanceolate, oblong, ovate, completely plain or curled, the colour of the lamina can go from light

to dark green and the veins are in general yellowish, but can assume pink / reddish nuances. They are copper coloured at emergence, and at each flush they appear following a spiral arrangement (**Figure 2.2 a, b**).

Figure 2.1. Mango tree in Réunion island. Photo by the author.

When left to grow undisturbed, the canopy has a heliotropic growth, but in cultivation the tree is generally pruned so that it does not exceed 6 meters in height. In this case, it will assume a globous or dome shaped aspect.

The root system is formed by a long, consistent taproot and a wide network of surface feeder roots. In facts, most of the living roots of the tree are concentrated in the shallow soil, so the ground around the trees is almost never subject to tillage operations and the plant does not do well in clay-rich soils.

The inflorescence of mango is a terminal pyramidal panicle (**Figure 2.2 c, d**), which can be as long as 50 cm and bears numerous branches. Each flower is small, with four or five white to red petals, and can be monoecious or hermaphrodite, both appearing on any panicle. The pollination of mango flowers can be both anemophilous and entomophilous (Kumar *et al.*, 2016; Popenoe, 1917; Ramírez and Davenport, 2016). The fruit of the mango tree is a climacteric drupe, with a pale to golden yellow resinous mesocarp of varying texture and fibrousness on the basis of the variety. Each fruit can measure from 5 to > 30 cm, and weigh

from 100 to 2000 g. It extends in the three dimensions, length, width and thickness, and its shape goes from a flattened globe, like in the "José" variety, to a sword-like shape in the "Nam Dok Mai" variety (**Figure 2.2 e, f**). Also the external colour varies greatly among the fruits of all the mango varieties, which in general start forming with a green or purple exocarp, until they turn to a golden, orange, red or blushed colour at maturity (Mukherjee and Litz, 2009).

The single seed contained inside the mango drupe can be mono-embryonic or contain nucellar embryos besides the zygotic ones. This is a character that reflects the origin of the varieties, traditionally divided in Indian (monoembryonic) and Indo-chinese

Figure 2.2. Young (a) and mature (b) leaves of mango; Flowers of the Irwin (c) and Caro (d) varieties; Fruits of the José (e) and Nam Dok Mai (f) varieties. All photos by the author.

(polyembryonic). The latter character is particularly sought after in the breeding programs, especially for obtaining new varieties to use as rootstocks to be propagated easily.

To the palate, the ripe mango fruit has a pleasant, consistent texture with different degrees of firmness and a richly aromatic, sweet taste, generally not sour. The main harvesting indexes used by the growers and traders are the fruit's skin colour and the total soluble solids content (°Brix) which has to reach a certain threshold before the fruit can be harvested and

marketed, specific for each variety (Ketsa *et al.*, 1991). Being climacteric, the fruits that have to travel for long distances in containers are usually harvested at the "green ripe" stage (Vincenot and Normand, 2009) and shipped at a controlled temperature, the optimum being 13°C (BMT Netherlands B.V., n.d.; The German Insurance Association, n.d.). In many tropical countries, however, mango is also consumed when unripe, when the flesh still has a pale straw yellow colour and is shredded to be eaten in salads with several condiments. Some varieties such as "Caro" are consumed mostly in this form. The ripe fruit, instead, is consumed mostly as a dessert or as juice in the Western countries while it takes part in many recipes in the cuisines of countries where its cultivation dates back to many centuries (**Figure 2.3**).

Figure 2.3. Ripe and unripe mangoes sold at a street market (left) and mango with sticky rice (right) in Bangkok, Thailand. Photos by the author.

Mango buds are not predetermined, but while still closed, they hold in themselves the primordia of both vegetative and reproductive organs. If a mango bud is initiated during vegetative inductive conditions, bracts develop as small leaves and the leaf primordia will develop as the full-sized leaves of vegetative shoots. On the other hand, under floral inductive conditions, the leaf bracts and primordia fail to develop, and the meristems inside the bud begin to elongate and branch, with each branch point differentiating into an axis of the flower panicle.

Mixed shoots, bearing both leaves and inflorescences at each node, result from development of both the primary leaf primordia and the lateral meristems, which form the inflorescences in the same nodes as leaves (Chaikiattiyos *et al.*, 1994; Davenport, 2009; Naik and Rao, 1943).

But which are, respectively, the vegetative and floral inductive conditions that we mentioned? We have seen how the vegetative or reproductive fate of mango buds remains undetermined until shoot growth starts to take place. Mango trees develop vegetative shoots when shoot initiation occurs in warm temperatures (30°C day/25°C night), whereas inflorescences develop when shoots initiate growth in cool temperature conditions (18°C day/10°C night; or 15°C day/10°C night) (Davenport, 2009; Núñez-Elisea and Davenport, 1994; Whiley *et al.*, 1989). It appears, then, that the conditions that the mango bud encounters at the moment of its opening are what determines which type of development it will undergo.

The mango fruit, for its part, is also sensitive to the climatic conditions that occur during its development (Carella et al., 2021). The process of acclimating to high temperatures and light exposure involves morphological changes and up- or down-regulation of physiological and biochemical processes. The position of the fruit on the tree, for example, influences the fruit cuticle's water vapour permeance and its irrigation requirements. Excessive irradiation or high temperature can be the causes of oxidative stress, thus affecting the accumulation of metabolites such as sugars and ascorbate in the fruit pulp and peel and affect the fruit's post-harvest behaviour (Léchaudel et al., 2013).

If environmental conditions play such an important role in the biology of the mango plant and fruit, we can rightfully assume that their behaviour and quality will be deeply affected by the cultivation in a new climate. Therefore, it is important to put strong effort in understanding the relations between the environment and the mango plant in the Mediterranean basin, where the crop is spreading significantly.

2.1   What climate is suitable for mango cultivation?

According to the literature, mango trees grow and produce more successfully in frost-free subtropical latitudes with a marked dry season (Schaffer *et al.*, 2009), reportedly surviving without rain or irrigation for up to 8 months (Gandhi, 1955), though with obvious detrimental effects on productivity (Pongsomboon, 1991). Shoot growth ceases below 15°C (Issarakraisila *et al.*, 1991; Whiley *et al.*, 1989), and cool temperatures are known to be an inductive factor for flowering (Davenport 2009; Luo *et al.* 2019). However, too cold or too high temperatures during flower development have been shown to hinder pollen germination and viability (Issarakraisila and Considine, 1994; Pérez *et al.*, 2019).

We conducted an analysis of short-term historical data in three islands, with significantly different climates, at which mango is currently successfully cultivated at commercial level. The simultaneous comparison of the environmental conditions that the mango trees encounter in the three islands will allow to reconsider the actual constraints for the species and understand its response to new growing conditions, thus offering researchers and growers new, challenging insights.

2.1.1   Material and methods

Daily values of minimum, average and maximum temperature (°C), total rainfall and potential evapotranspiration (mm), and hourly values of solar radiation (W/m^2) for the period 2018-2022 were collected from weather stations located in a radius of < 5 km from experimental mango orchards, namely the station of Caronia Buzza (38°03'N 14°48'E, 50 m a.s.l., climate according to the Köppen classification: Csa) (Fratianni and Acquaotta, 2017), managed by the Servizio Informativo Agrometeorologico (SIAS) of Regione Siciliana for Sicily; the station of Guía de Isora (28°13'N 16°50W, 48 m a.s.l., climate according to the Köppen classification: BWh) (Chazarra *et al.*, 2011), managed by the Dirección General de Agricultura y Desarrollo Rural, for Tenerife; and the station of Ligne Paradis (21°32'S 55°48'E, 154 m a.s.l., climate

according to the Köppen classification: Aw) (www.meteofrance.re, 2015), managed by Cirad – Centre de coopération Internationale en Recherche Agronomique pour le Développement – for Réunion.

2.1.2   Results and Discussion

2.1.2.1   Temperatures

Figure 2.4 reports the absolute minimum, mean average and absolute maximum daily temperatures recorded in the three islands at each date over the 5-year observation period.
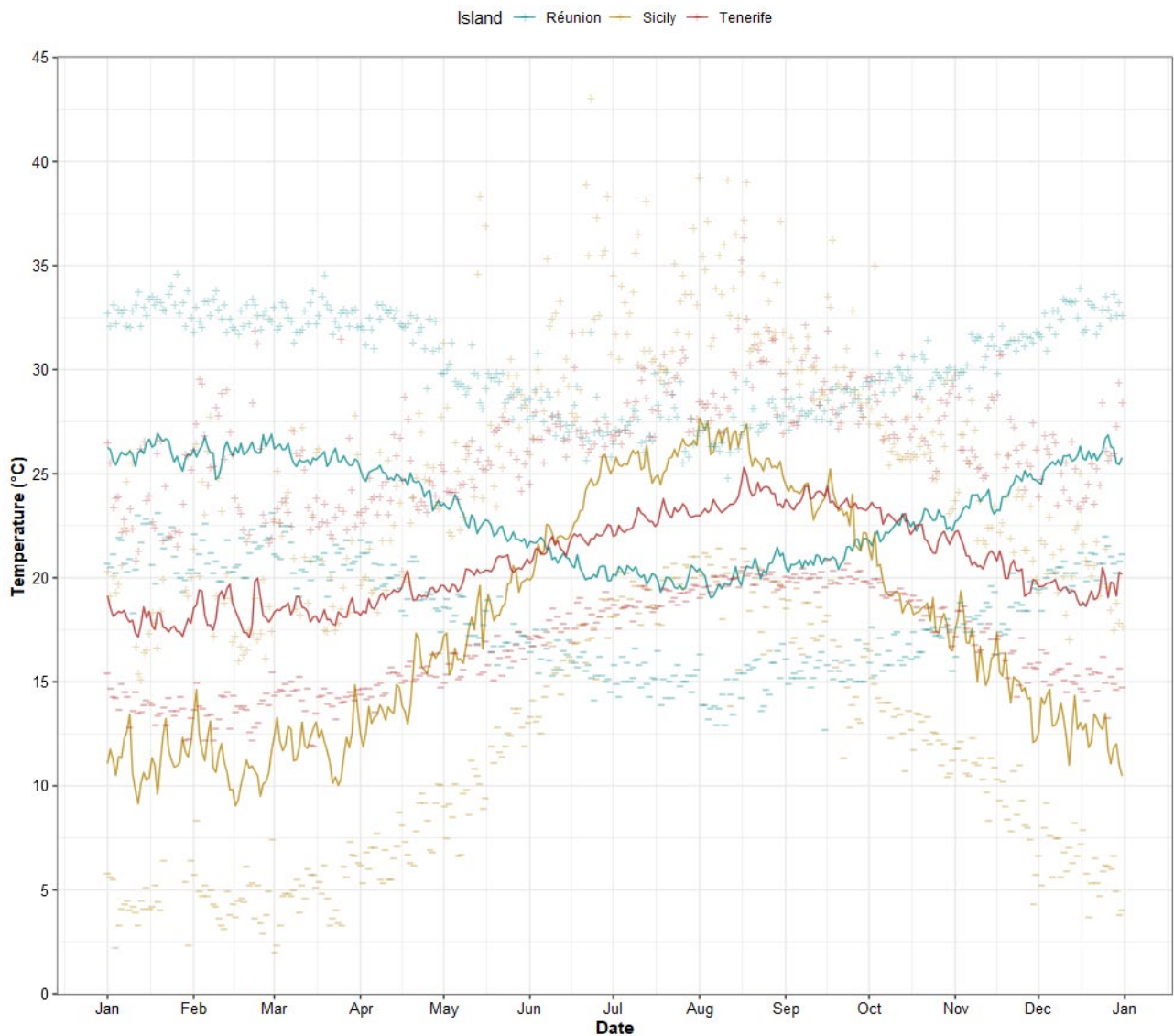

Figure 2.4. Average (lines), minimum ("-" symbols) and maximum ("+" symbols) temperatures recorded each date over the observation period in the three islands.

Being located in opposite hemispheres, the temperatures in Réunion follow an opposite trend compared to the ones in Sicily and Tenerife, with the hottest months in the southern hemisphere corresponding to the coldest ones in the northern one. However, it can be observed that the temperatures in the two subtropical climate islands fluctuate in a range comprised between 12.5 and 35 °C at all dates. Temperatures in the Mediterranean climate of Sicily, instead, reach in many dates values as low as 2 °C and as high as 45 °C. Moreover, for large parts of the year the average daily temperature in Sicily is lower than the minimum temperature observed in the other islands.

This reflects in different daily thermal amplitude ranges among the islands at each month of the year (**Figure 2.5**).
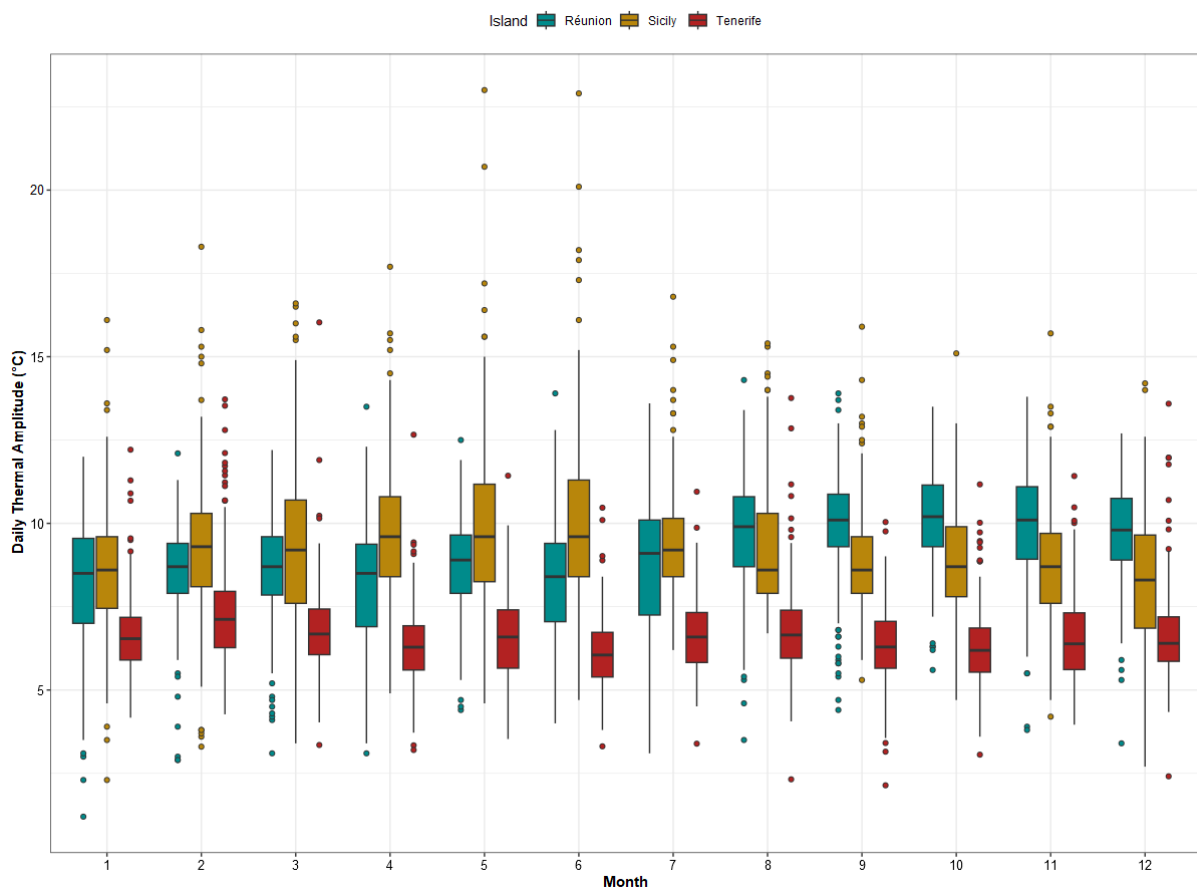

Figure 2.5. Daily thermal amplitude (°C) ranges observed in the three islands at each months of the year during the observation period.

Statistical analysis conducted via a Student's t-test for any pair of locations at each month (data not shown) confirms that differences in the thermal amplitude values between any

two islands are non-significant only in 4 cases, precisely in the months of January, February, March and August between Sicily and Réunion islands. These months correspond to the coldest and hottest ones in each of the two locations, respectively. Thermal amplitude values in Tenerife, instead, are significantly lower than in any of the other islands, remaining in a range between 5 and 7.5°C at any month of the year, indicating a much smaller daily variability of the temperatures in the Spanish island.

2.1.2.2   Solar radiation

The range of hourly solar radiation values at which mango is grown in the three islands object of the study varies greatly along the year (**Figure 2.6**). The lowest daily maximum solar radiation values (< 500 W/m2/h) are found in Sicily during the winter and autumn seasons, while the highest values, with peaks of 800 W/m2/h have been recorded in Tenerife in the spring and summer periods. Mango is a sun-adapted plant and leaves are only light-saturated at high radiation values, but strong solar radiation can cause sunburn damage on the leaves and fruits, especially if paired with high temperatures (Weng *et al.*, 2006). Net photosynthesis, in facts, is positively correlated with leaf temperature in a normal range. Leaf temperature is not a driving variable of photosynthesis, but it is the single most important rate-determining factor after the photosynthetically active photon flux density (Schaffer *et al.*, 2009).
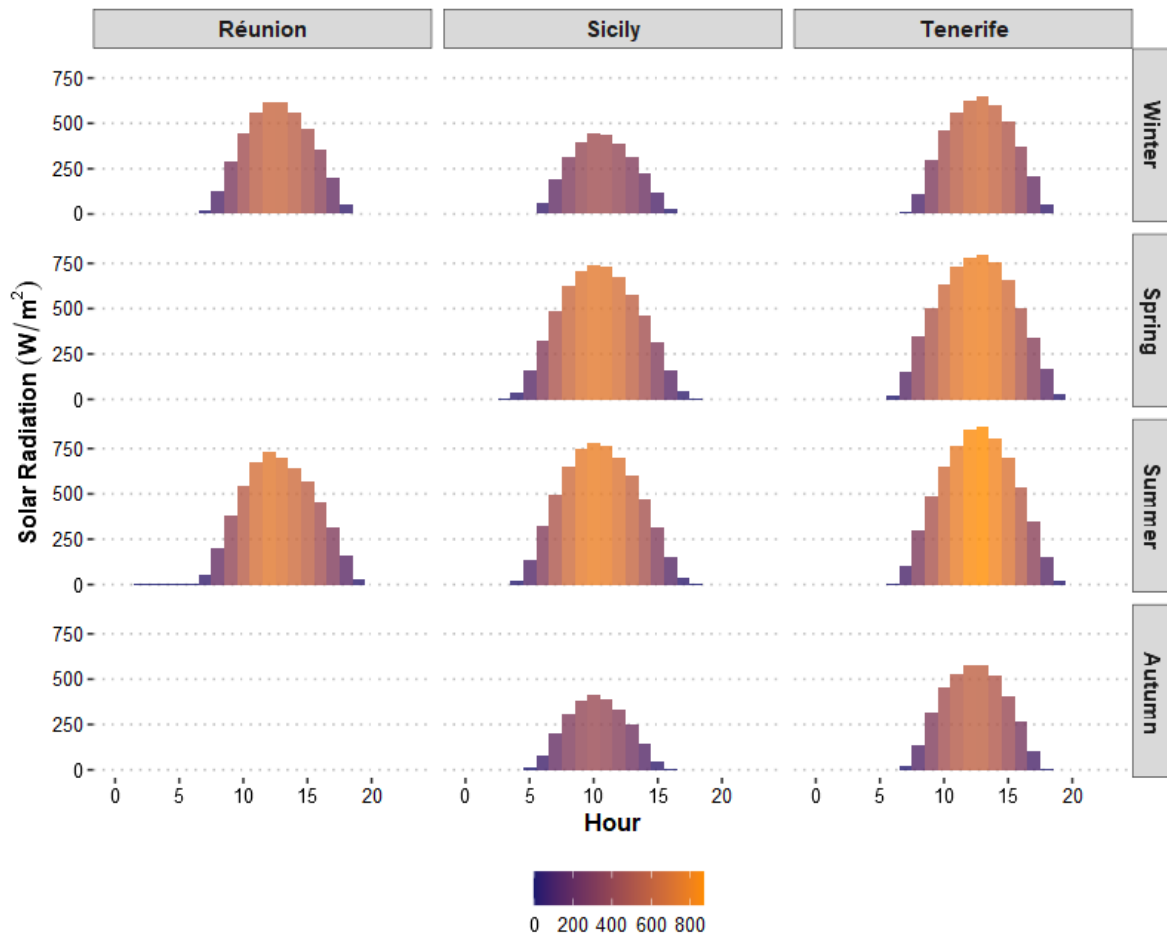
Figure 2.6. Average hourly solar radiation values (GHI, W/m2) recorded at each period of the year in the three islands object of the study. Only two periods, corresponding to the hot and cold seasons of the year, have been considered for the subtropical climate of Réunion island.

2.1.2.3 Rainfall and evapotranspiration

Average daily total rainfall and potential evapotranspiration at all dates of the year in the three islands are reported in **Figure 2.7**. The trendlines reveal that the periods with the highest evapotranspiration (November to April) overlap with the wettest periods of the year in Réunion island. Here, then, rainfall occurs especially when the mango plants need it the most. In the island of Sicily, instead, the opposite happens, with most of the rainfall happening during the coldest months when the potential evapotranspiration is low. This means that precipitation water will not immediately be used by the plants and should hopefully go to refill the water reservoirs of the region. Finally, the trends for the island of Tenerife reveal the almost desertic climate of the Southern part of the island, where mango cultivation is carried out and the

weather station was located, and total yearly cumulated rainfall ranged between just 50 and 200 mm in the observed period.
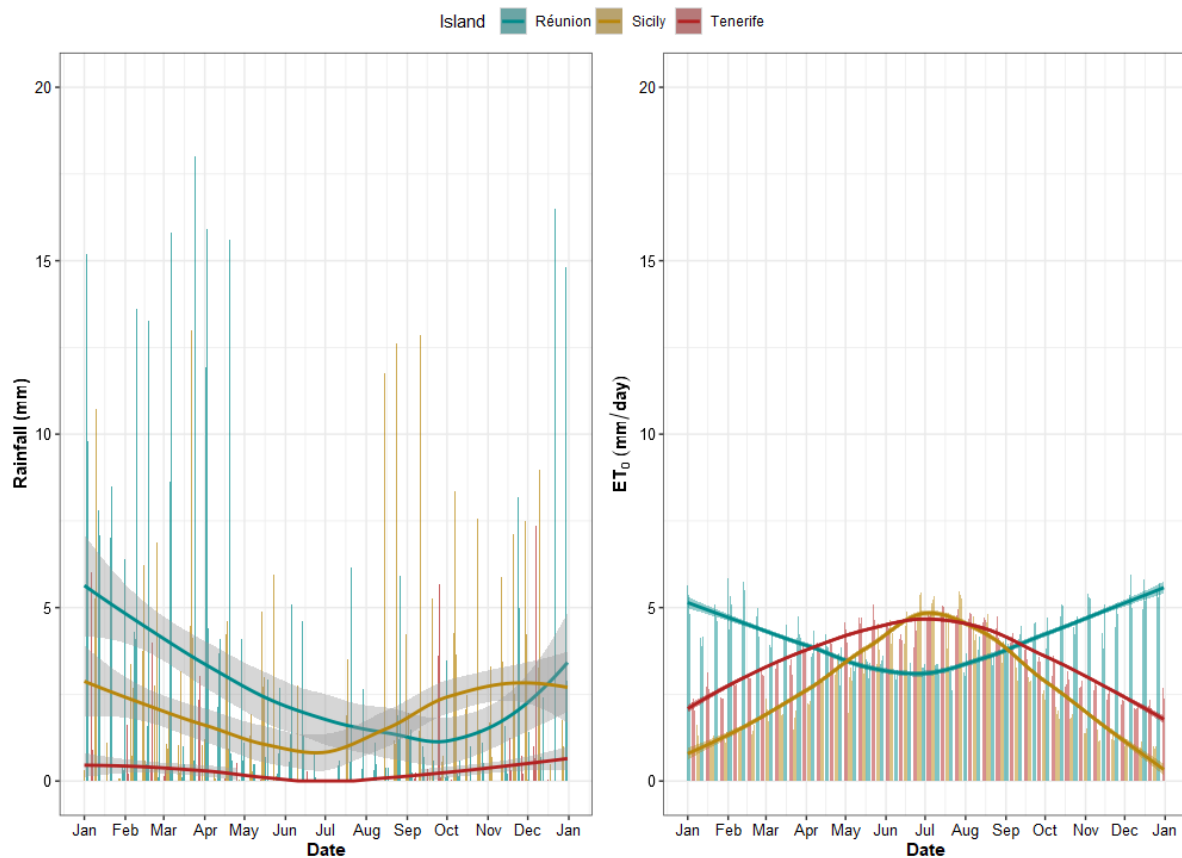

Figure 2.7. Average rainfall (left) and potential evapotranspiration (right) values at each date over the observation period in the three islands object of the study.

The cumulated combination of rainfall and evapotranspiration translates into the pluviometric deficit for each island (**Figure 2.8**). It is immediately noticeable that the lines depicting the rainfall deficiency have different patterns in the three islands: in Réunion, the cumulated difference between rainfall and potential evapotranspiration is positive only for half of the year. In Sicily, the same cumulated difference is positive for two thirds of the year, while in Tenerife it is positive throughout all the year. This means that mango plants (and their growers) in the three islands have to adapt to significantly different water availabilities over the course of the year, and this can affect greatly the plant's physiology and the relative crop management.
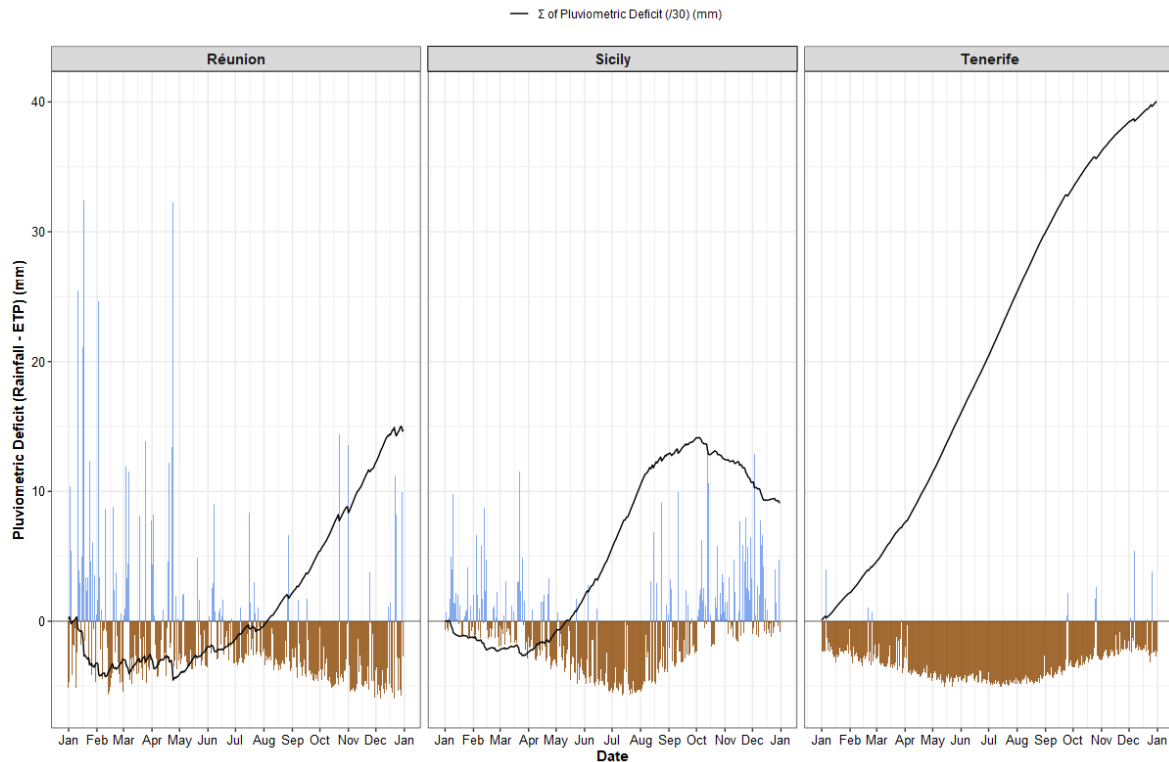
Figure 2.8. Average pluviometric deficit (*30) (black line) (mm) in each island object of the study over the course of the year, obtained as the cumulated difference between rainfall (blue columns) and potential evapotranspiration (brown columns) of each date of the year.

The main features of the climate of each island, along with their possible consequences, are summarized in **Table 2.1**. The extreme daily temperatures, both low and high, that are found in Sicily, can have a detrimental effect on the population of pests. In facts, in many cases in the Mediterranean island, mango can be cultivated in an organic cultivation regime. Tenerife has the highest solar radiation among the three islands, but at the same time precipitations are absent, implying the need for sourcing irrigation water elsewhere. The conditions in Réunion are the most favourable for a strong vegetative and architectural development of the plants, which, however, could be at the base of alternate bearing.

Table 2.1. Main features and relative consequence of the climate of each island, as emerged from the analysis of the 5-year observation period.

RÉUNION		TENERIFE		SICILY	
Feature	**Consequence**	**Feature**	**Consequence**	**Feature**	**Consequence**
High water availability during and after fruit harvest	Strong vegetative development / alternate bearing	Constant temperature range	Uninterrupted plant development / possible unsatisfactory flowering	Low temperatures down to 0°C and frost events	Bacterial diseases and tissue freezing / Interruption of the life cycle of pests
No chilling temperature	Constant presence of pests through the years	Strong solar radiation	Optimal photosynthetic conditions / possible fruit sunburn	Temperatures over 40°C during summer	Fruit sunburn and thermal stress for the plants / Interruption of the life cycle of pests
		Complete absence of rainfall	Necessity for irrigation with water sourced elsewhere	Harvest followed by cold season	Poor vegetative development / alternate bearing
				Persisting conditions for flowering	Poor fruit set / fungine infection on panicles

2.1.3 Conclusions

Our results confirm the plasticity of the mango tree to adapt to very different environmental conditions. Literature has shown that the environment significantly affects the plant's physiology and phenology (Núñez-Elisea and Davenport, 1994; Pongsomboon, 1991; Whiley, 1992), and even more does the current climate change (Makhmale *et al.*, 2016; Normand *et al.*, 2015; Rajan *et al.*, 2013). Acknowledging the conditions that mango is able to tolerate in its new cultivations areas is a starting point for better understanding of its physiology and relations with the environment.

This study was presented as an oral presentation at the ISHS XIII International Symposium on Mango which took place in Málaga, Spain, from September 29th to October 3rd, 2023.

2.2 *What is the phenological cycle of mango in the Mediterranean?*

The understanding of a species' adaptation to a cultivation environment requires the knowledge of the temporal distribution and intensity of the key events of the species' life cycle. The plant phenology is defined as the recurring sequence of plant developmental stages (Piao *et al.*, 2019). A thorough description of the phenological cycle of mango can represent a guide for growers needing to plan their orchard's management and helps to understand the response of the plant to the new environmental conditions that it encountered. To obtain it for mango in the Mediterranean climate, the phenological evolution of mango trees was observed over a three-years period in two different growing conditions, open air and greenhouse.

2.2.1 *Material and Methods*

Mango trees of the varieties Keitt, Osteen, and Tommy Atkins were selected for the study. They were growing in two cropping conditions, open air and greenhouse, in two plots located in the countryside of Sant'Agata di Militello (ME, Italy) (38.04 N – 14.39 E), respectively at 6 and 9 m a.s.l. The climate of the area is defined as Mediterranean – Csa, Temperate climate with Dry and Hot Summers – in the Köppen classification (Köppen, 1936), with a mean annual precipitation of 885 mm and an average annual temperature of 18.56 °C (M. C. Peel *et al.*, 2007).

The greenhouse roof was made of corrugated polymethacrylate sheets, while the side walls were realised in PVC film. The greenhouse has an automated, temperature-controlled system of roof ridges and walls opening. Temperatures in the greenhouse and in the open-air plots were measured at 30-minute intervals using two data-logger equipped sensors (RC-51, Elitech Europe Ltd, London, UK), sheltered from direct sun and rain, throughout the study period. The average daily and night temperatures were calculated as the mean of the temperature values measured between sunrise and sunset and between sunset and sunrise, respectively.

Three plants per variety were chosen, and on each plant, 12 terminal shoots were selected randomly around the tree canopy. For each recording occasion 36 readings per variety were carried out in each cropping condition. Since in some trees more than one vegetative flush was observed during the same year, precise phenological stages on the terminal entity of each shoot were identified on the basis of the extended BBCH scale for mango (Rajan *et al.*, 2011).

In order to simplify the phenological cycle description, the precise phenological stages of the BBCH scale for mango were grouped into 5 main phenological phases of the plants' growing cycle, summarised in Table 1:

- BBCH stages 630, 631, 010, 011, 119, 319 and their and their analogous in the subsequent vegetative flushes were considered to belong in the "Quiescent" phase;

- BBCH stage 013 was considered as the "Swollen Bud" phase, when it is still impossible to determine whether the new entity will undergo reproductive or vegetative development;

- BBCH stages 017; 110 to 117; 311 to 317 and their analogous in the subsequent vegetative flushes were considered to belong in the "Vegetation" phase;

- BBCH stages 510 to 619 were considered to belong in the "Flowering" phase, which includes the inflorescence emergence and flower opening;

- BBCH stages 701 to 809 were considered to belong to the "Fruiting" phase.

Table 2.2. Correspondence of the BBCH scale stages to the main phenological phases of the mango cycle. All photos were taken by the author in the two orchards of the study.

Phenological phase	Quiescent	Swollen Bud	Vegetation	Flowering	Fruiting
BBCH stages	010, 011, 119, 319, 3x9, 630, 631	013	017, 019 110-117, 311-317, 3x1-3x7	510-619	701-809
Photo					

Observations were carried out at fortnightly intervals over the course of three years: 2019, 2021 and 2022. In total, 5872 phenological stage readings were carried out and analysed.

For each recording occasion, the frequency of occurrence of each main phenological phase in each variety for each cropping condition was calculated and expressed as the percentage on the total observations.

Statistical analysis was conducted using R statistical software (R Core Team, 2022).

2.2.2 Results

2.2.2.1 Temperatures

Figure 2.9 reports the values of minimum, average and maximum temperatures recorded each day of the year in the two cropping conditions over the three-year observation period.
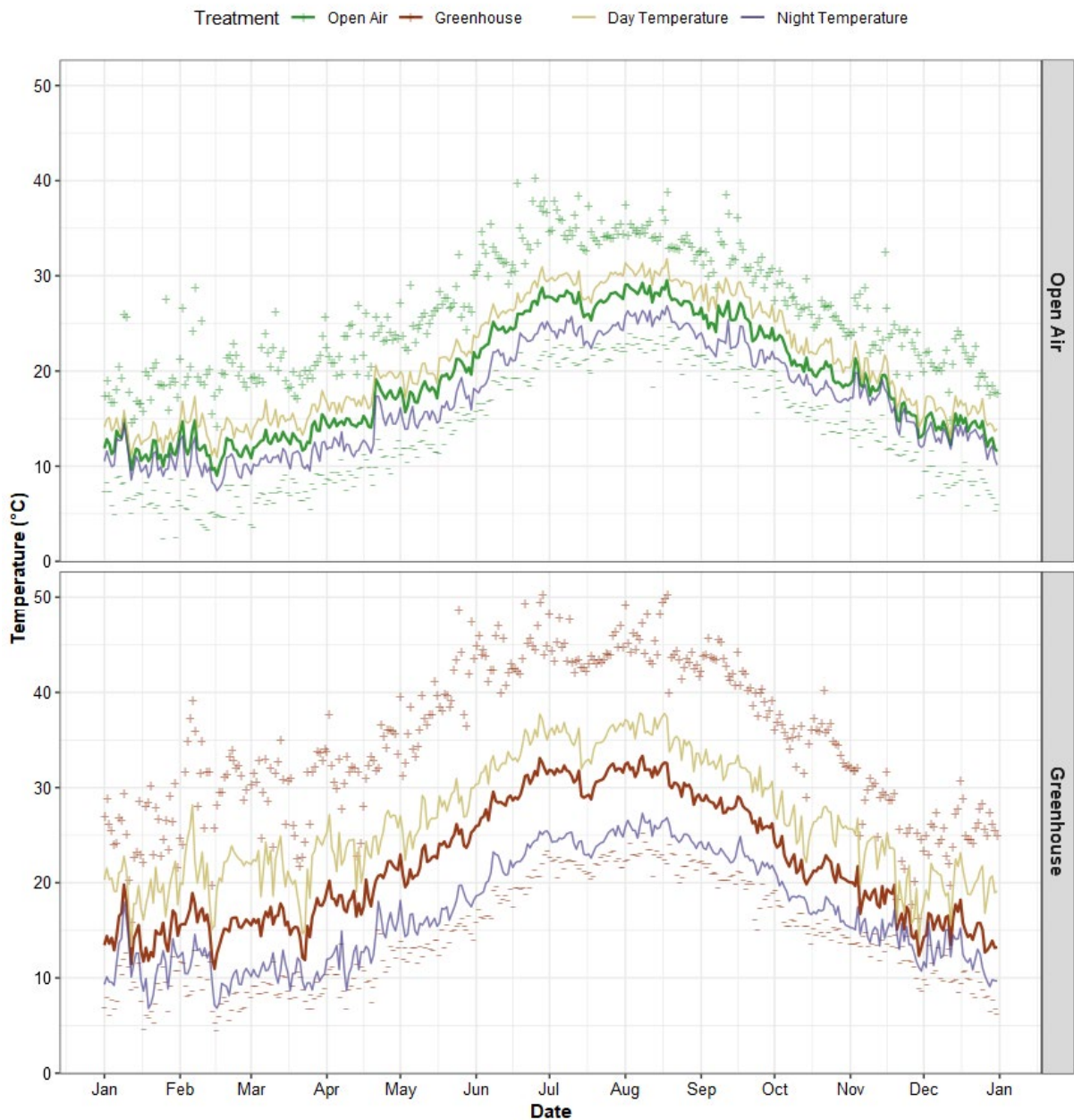

Figure 2.9. Average (solid lines), daily (yellow lines), night (blue lines), absolute minimum ("-" symbols) and absolute maximum ("+" symbols) temperatures measured each day of the year in the open air (green) and in the greenhouse (red) over the three-year recording period.

Average values of temperature span from 10°C to 30°C, with the highest maximum temperatures recorded between June and August. Over the whole 3-year observation period, only on 21 dates the minimum daily temperature was ≤ 5°C in the open air and only twice in the greenhouse. The coldest minimum temperatures were always recorded between mid-January and mid-February. A marked increase in the temperature values is observed starting from the second half of the month of April, until average daily temperatures stabilise around the value of 30°C during the summer months and then decrease constantly until the end of December.

The average daily temperature is almost always higher in the greenhouse than in the open air, except in the winter months (November and December in particular) where the values in the two conditions are almost coinciding. An even greater difference between the two cropping conditions is observed in the maximum daily temperatures, which in the greenhouse reach values close and above 50°C, two degrees higher than the previously considered tolerance limit for the species (Galán Saúco, 2009; Ramírez and Davenport, 2010), on several occasions, while they only get close to 40°C on some days in the open air. These values are always observed between June and August.

The data points relative to the minimum temperatures, interestingly, do not show marked differences between open air and greenhouse cultivation. Statistical analysis (**Table 2.3**) confirms that there was no significant effect of the presence of the greenhouse on the minimum daily temperatures recorded in both cropping conditions.

Table 2.3. *F* and *p* values and significance of the effect of the cropping condition on the minimum, average and maximum daily temperatures observed in each date over the course of the study period.

	Minimum Temperature	**Average Temperature**	**Maximum Temperature**
F	1.011	16.03	275.3
p	0.315	< 0.001	< 0.001
Significance	ns	***	***

2.2.2.2   Phenological phases observed on the mango tree

The percentages of main phenological phases observed in the mango trees during each month are reported in **Table 2.4** and **Figure 2.10**. Some differences between the open air and greenhouse cultivation are immediately noticeable: in the first case, mango trees show no activity (100% of the observed shoots in the Quiescent phase) for a period of at least two months between harvest and the following bud swelling, while in all the three observed varieties cultivated in the greenhouse, some activity is always observed at any moment of the year.

In the three varieties a significant anticipation of the period of flowering is observed in the greenhouse. Here, panicle development begins in late January or February and flowers are open until May. In the open air, on the other hand, no flowering is observed before March. This anticipation, however, is not reflected in the fruiting period of the three varieties in the two cropping conditions: in fact, fruits reach commercial maturity in the same period in varieties Osteen and Tommy Atkins in both cases, while in trees of the cv. Keitt fruits in the greenhouse reach commercial maturity later than those in the open air.

Besides the marked differences between the treatments observed in all the cultivars under study, some differences also emerged between the varieties. In particular, the trees of cv. Keitt start showing some vegetation activity earlier than the other varieties, in January in the open air and in December in the greenhouse. However, the percentage of shoots in the Vegetation phase decreases in the trees of Keitt in the open air between January and February: this is due to the apical necrosis – and consequent return to the Quiescent phase – of shoots that have started vegetative development very early but encountered unfavourable climate at a stage (BBCH stages 017 to 117) where leaves are very sensitive to cold temperatures. This is not seen in cvs. Osteen and Tommy Atkins because in the month of January, the shoots of these two varieties in the open air are mostly still in the Quiescent or Swollen Bud stages, which are less susceptible to cold damage.

The duration of each major phenological phase in each cropping condition is presented in **Figure 2.11**. The Vegetation phase extends for a longer period in the greenhouse in all three varieties compared with the open air due to the higher temperatures: in the greenhouse, in fact, vegetative flushes follow one another without periods of quiescence between them.

Significant differences have emerged for the duration of the fruiting phase in both Keitt and Osteen cvs., where fruits need more time to reach maturity in the greenhouse than in the open air. Only in cv. Tommy Atkins, instead, significant differences have emerged in the duration of the Flowering and Quiescent phases, which last longer in the open air than in the greenhouse.

Table 2.4. Percentages of shoots found in each Phenological phase during each month of the year in the two cropping conditions (OA: "Open Air"; GH: "Greenhouse").

Variety	Pheno. phase	Treatment	January	February	March	April	May	June	July	August	September	October	November	December
KEITT	Quiescent	**OA**	52.27	68.63	30.43	11.85	20.93	18.10	52.42	58.97	34.48	45.45	100.00	100.00
		GH	79.31	40.43	18.06	14.06	18.09	16.54	13.49	12.24	21.54	45.45	65.91	76.47
	Swollen Bud	**OA**	6.82	11.76	11.96	14.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		GH	3.45	6.38	6.94	1.56	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Vegetation	**OA**	40.91	19.61	28.26	12.59	6.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		GH	17.24	14.89	13.89	10.94	4.79	2.26	5.56	6.12	7.69	0.00	0.00	23.53
	Flowering	**OA**	0.00	0.00	29.35	61.48	72.09	43.97	0.00	0.00	0.00	0.00	0.00	0.00
		GH	0.00	38.30	61.11	73.44	63.30	3.76	0.00	0.00	0.00	0.00	0.00	0.00
	Fruiting	**OA**	0.00	0.00	0.00	0.00	0.00	37.93	47.58	41.03	65.52	54.55	0.00	0.00
		GH	0.00	0.00	0.00	0.00	13.30	77.44	80.95	81.63	70.77	54.55	34.09	0.00
OSTEEN	Quiescent	**OA**	90.59	90.00	50.52	7.75	16.54	15.13	32.76	43.33	43.75	100.00	100.00	100.00
		GH	59.09	33.33	19.48	8.95	17.93	12.50	16.10	12.94	33.33	89.47	91.11	83.33
	Swollen Bud	**OA**	9.41	6.67	17.53	23.24	7.87	0.84	0.00	0.00	0.00	0.00	0.00	0.00
		GH	9.09	8.33	9.09	4.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Vegetation	**OA**	0.00	3.33	20.62	38.03	10.24	0.84	0.86	0.00	0.00	0.00	0.00	0.00
		GH	25.00	10.42	10.39	10.53	2.17	2.34	1.69	5.88	5.00	5.26	8.89	16.67
	Flowering	**OA**	0.00	0.00	11.34	30.99	65.35	60.50	0.00	0.00	0.00	0.00	0.00	0.00
		GH	6.82	47.92	61.04	75.79	67.39	4.69	0.85	0.00	0.00	0.00	0.00	0.00
	Fruiting	**OA**	0.00	0.00	0.00	0.00	0.00	22.69	66.38	56.67	56.25	0.00	0.00	0.00
		GH	0.00	0.00	0.00	0.00	12.50	80.47	81.36	81.18	61.67	5.26	0.00	0.00
TOMMY ATKINS	Quiescent	**OA**	60.23	56.67	16.13	12.90	12.93	10.81	48.18	36.84	66.67	98.28	100.00	100.00
		GH	86.96	39.58	14.67	3.65	8.33	16.13	22.31	14.63	42.00	75.00	95.83	83.33
	Swollen Bud	**OA**	32.95	21.67	5.38	9.68	1.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		GH	6.52	12.50	10.67	2.60	0.52	0.81	0.00	0.00	0.00	0.00	0.00	0.00
	Vegetation	**OA**	6.82	21.67	24.73	4.30	2.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		GH	6.52	14.58	6.67	5.73	3.13	0.81	0.00	1.22	12.00	20.83	4.17	16.67
	Flowering	**OA**	0.00	0.00	53.76	73.12	82.76	52.25	0.00	0.00	0.00	0.00	0.00	0.00
		GH	0.00	33.33	68.00	88.02	75.00	1.61	0.00	0.00	0.00	0.00	0.00	0.00
	Fruiting	**OA**	0.00	0.00	0.00	0.00	0.00	36.94	51.82	63.16	33.33	1.72	0.00	0.00
		GH	0.00	0.00	0.00	0.00	13.02	80.65	77.69	84.15	46.00	4.17	0.00	0.00

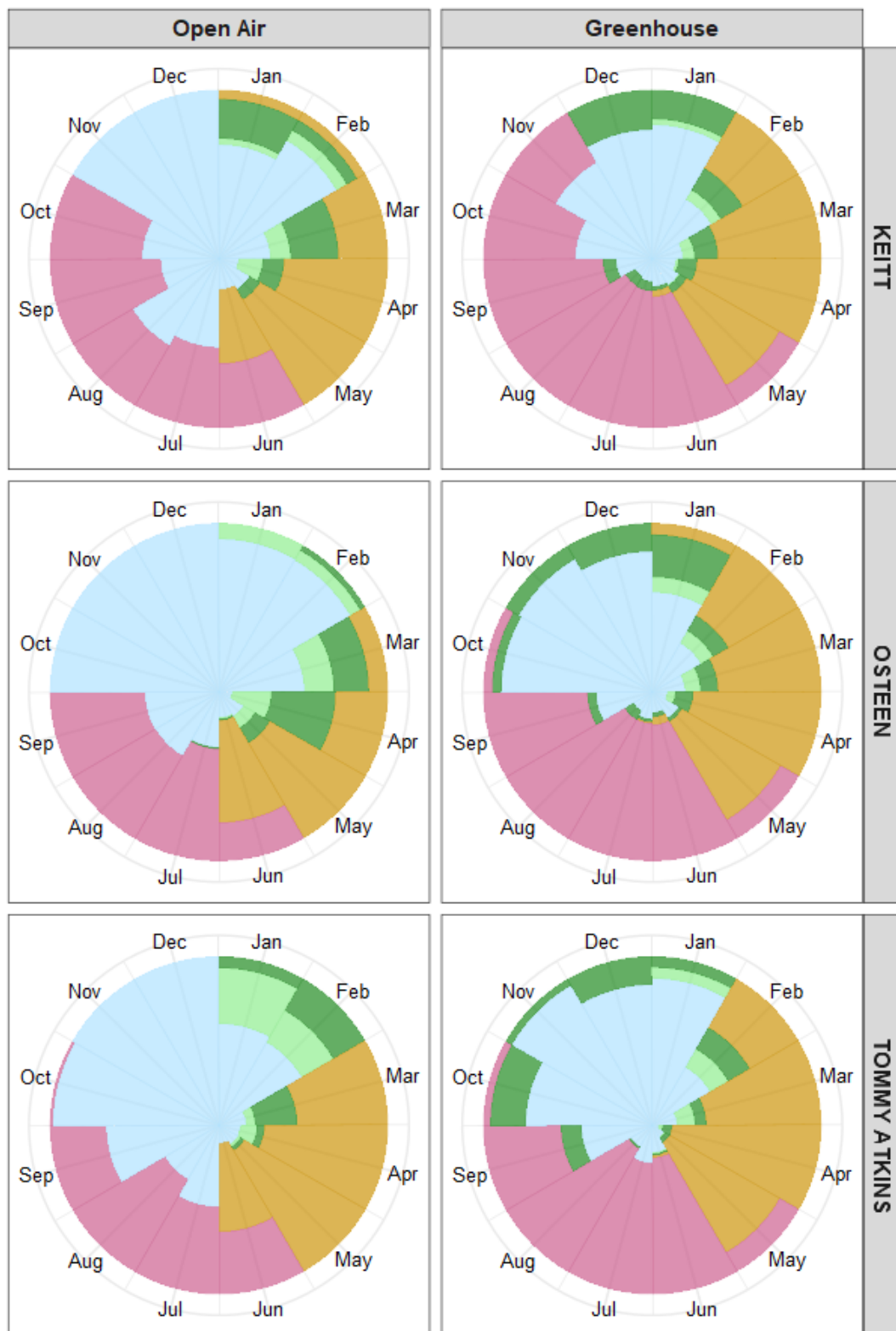# Phenological phases observed in the mango tree

Figure 2.10. Main phenological phases observed in the mango tree throughout the year. The radius of the coloured section represents the percentage of shoots found in each main phenological phase on the trees during each month.
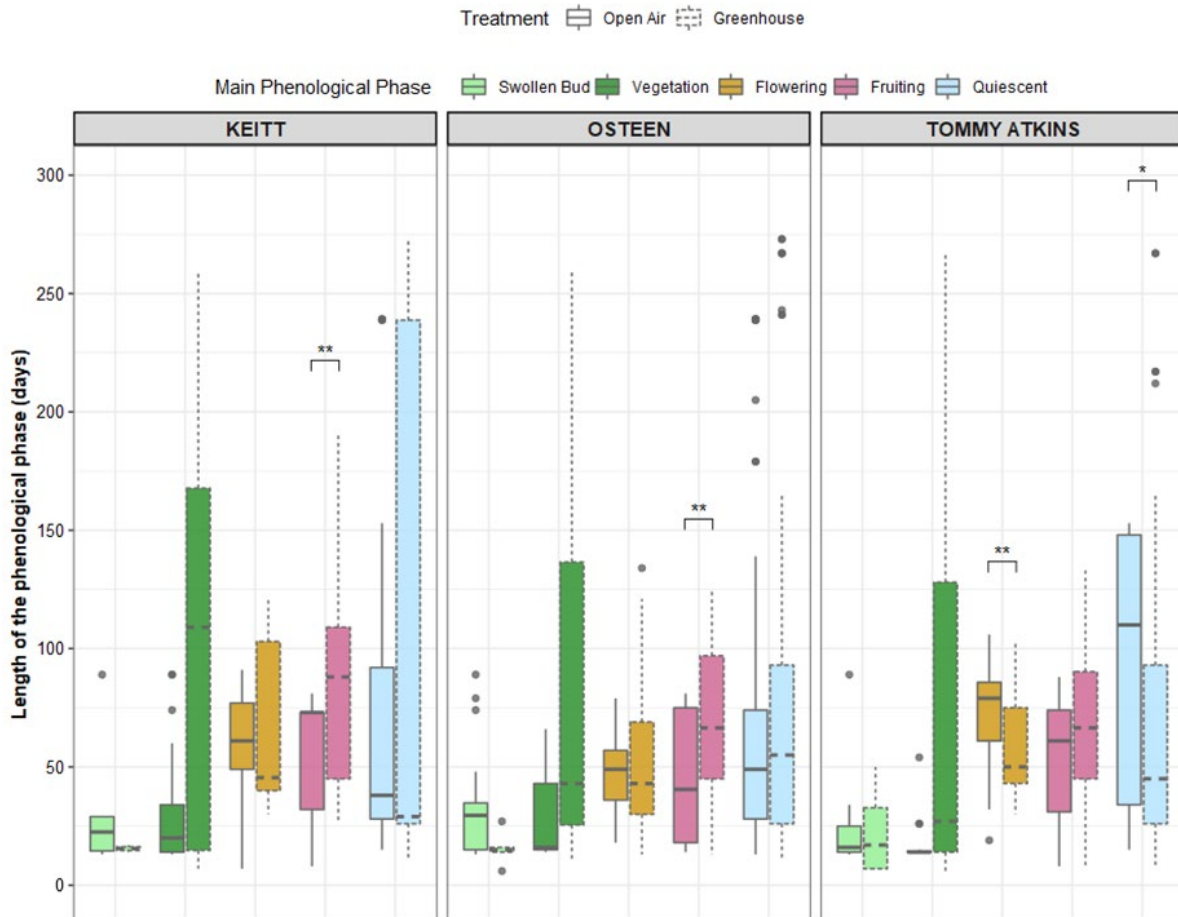
Figure 2.11. Length, in days, of each major phenological phase observed on the labelled shoots of the studied varieties. * and ** indicate significant differences between the cropping conditions for Kruskal-Wallis' nonparametric test at $p < 0.05$ and $p < 0.01$ respectively, with no significant difference otherwise.

2.2.3   *Discussion*

The mango phenological cycle observed in the open air in the Mediterranean differs from the one that is typically described in the tropics (Cull, 1989; Galán Saúco, 2009), in that only one vegetative flush is observed, with the shoots forming in January – February and expanding in parallel with the flowering during spring. Vegetative growth in mango is never continuous but occurs in separate flushes that terminate when the new shoots and leaves are fully expanded (Chacko, 1986; Normand *et al.*, 2009). The higher the temperatures at which it is exposed, the higher the number of vegetative flushes that the mango plant can produce (Whiley *et al.*, 1989). In the tropics, the strongest vegetative flush occurs after harvest during the hot and rainy season because in this period, temperatures are generally high and water is readily available (Normand *et al.*, 2015). In the Mediterranean, instead, harvest of the mango

fruit happens between September and October (**Figure 2.10**), just before the coldest period of the year, when the temperatures are not vegetation-inductive (**Figure 2.9**). In the greenhouse, on the other hand, vegetative growth begins in autumn after harvest, with several flushes following each other until the onset of the following summer due to the higher temperatures. As a consequence, no period of complete quiescence is observed in the plants growing in the greenhouse.

The most favourable temperature combination for flowering of mango has been observed to be 20°C day / 15° night (Núñez-Elisea and Davenport, 1991), while no flowering occurs when the minimum temperatures are below 10°C (Clonan *et al.*, 2021; Schaffer *et al.*, 2009). In both cropping conditions, flowering begins after the plants have undergone a period of cold temperatures, but in the greenhouse the panicles start forming earlier due to the higher average temperatures – up to 20°C – occurring during the day (**Figure 2.9**). This exposes the newly formed flowers to temperatures that may affect differently flower viability in the two cropping conditions. In fact, several authors conducted observations on the germination and growth of mango pollen tubes, finding that the optimum temperatures for these lie between 15 and 33°C (Issarakraisila and Considine, 1994; Sukhvibul *et al.*, 2000), while temperatures outside this range are highly unfavourable (Liu *et al.*, 2023). During the flowering period in the open air, temperatures fall constantly within the optimum range, while in the greenhouse, the maximum daily values almost always exceed it. This may lead to lower yields in the greenhouse compared to the open air.

The difference in the length of the fruiting phase between open air and greenhouse is probably a consequence of the higher temperatures and transpiration rates, which can limit the fruit growth rate (Léchaudel *et al.*, 2013). In other studies, in fact, transverse diameter of the mango fruit has been reported to increase until 19 weeks after full flowering in a greenhouse when measured in the open air (Carella *et al.*, 2021; Scuderi *et al.*, 2023). On average, on the

other hand, in cv. Tommy Atkins both the Flowering and Quiescent phases last longer in the open air than in the greenhouse: this is due to the fact that panicle development is the process that interrupts the resting period in mango (Normand *et al.*, 2017) – flowering starts earlier in the greenhouse, thus shortening the quiescence period.

2.2.4   Conclusions

The new depiction of the phenological cycle of the mango in the Mediterranean climate will represent an essential tool for crop management planning. In this synthetic work, growers can find information to consider at the moment of variety selection or the choice of the cultivation technique to adopt, in relation to the climatic conditions that characterise their territory.

Description of the mango phenology in a previously unexplored climate is important to better understand the environmental limits that the species can endure, and to assess the impact of climate change on its cultivation in different areas of the world. Moreover, the highlighted characteristics of the described phenological cycle in the Mediterranean climate offer new research insights that could be best addressed by an international network of researchers, institutions and firms interested in mango cultivation.

2.3 *Do mango phenology and development vary in the different climates?*

We have seen how the main phenological phases of the mango life cycle succeed to each other and when they are temporally localized in the Mediterranean. As we already pointed out, mango is found in a broad range of latitudes and climates: the environmental conditions at which it grows influence the occurrence, intensity and timing of the burst of vegetative or reproductive elements (Normand *et al.*, 2009).

The phenological cycle of mango in a subtropical climate is thoroughly described by Cull (1989) in **Figure 2.12**. From the depiction, it emerges clearly that reproductive and vegetative development happen in two well-separated moments of the year, and that the main vegetative flush, which constitutes the base for the flowering activity of the following year, occurs during the austral summer, which is normally characterized by high temperatures and high availability of precipitation water, the optimal conditions for the mango bud to undergo vegetative development (Capelli *et al.*, 2021; Schaffer *et al.*, 2009).
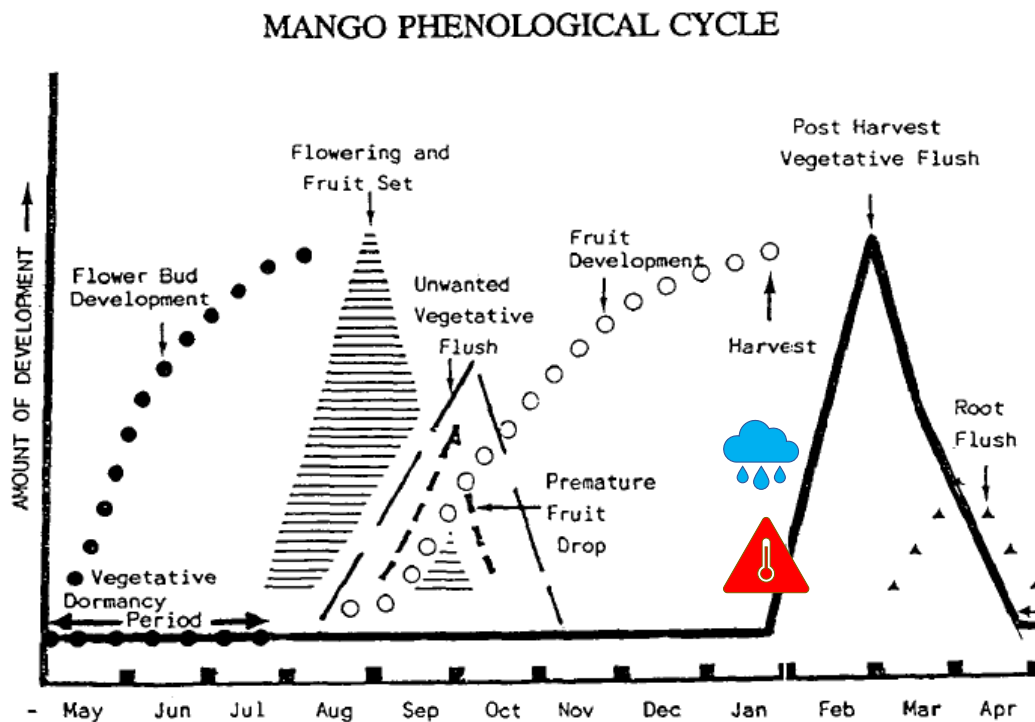

Figure 2.12. Phenological cycle of mango in a subtropical climate in the Southern hemisphere (Cull, 1989, adapted).

The simplest element constituting the architecture of the mango plant is the Growth Unit (GU): this is defined as a portion of the vegetative axis which is developed during an uninterrupted period of growth (Hallé, 1968). GUs appear in the mango canopy in flushes, and they follow a hierarchical structure: each GU can be seen as the "*mother*" or "*ancestor*" of the other GUs that sprout from it. The "*daughter*" or "*descendant*" GUs that appear on the same Growth Level (GL) can be seen as "*sisters*" (**Figure 2.13**).
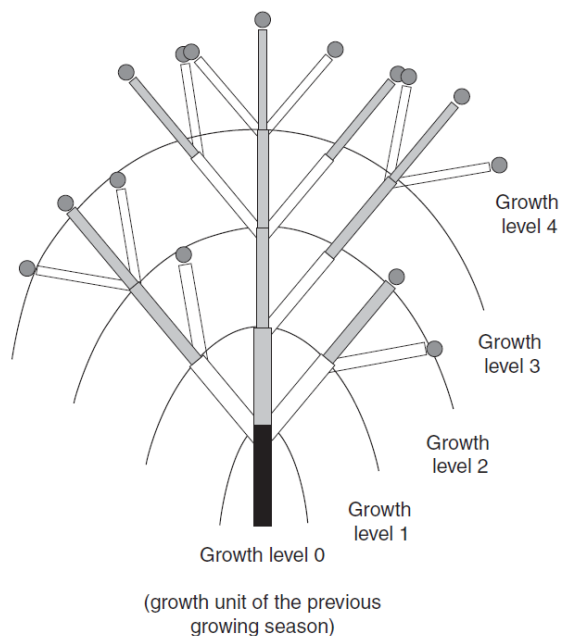

Figure 2.13. Schematic depiction of the GUs (rectangles) and GLs constituting the mango canopy architecture. The dots at the end of each axis represent terminal buds waiting to open and develop into new entities (Dambreville et al., 2014).

It is well acknowledged that environmental conditions and weather events play a key role in the alternation of the different phenological phases in all plant species (Dambreville *et al.*, 2012; Whiley, 1992), as well as endogenous genetic factors (Chacko, 1986). Still, a heavy degree of asynchrony is observed in the appearance of either vegetative or reproductive entities from the closed buds in the same mango plant's canopy, both temporally and spatially (Ramírez and Davenport, 2010).

Therefore, there must be more structural elements of each mango plant concurring to the differentiation of a mango bud into a GU or an inflorescence that have to be found at the branch or axis level. For the same species and variety, these factors should exert their effect regardless of the conditions where the plant is grown, unless the environment has a masking, limiting or contrasting effect.

The only way to be sure to actually understand and decipher the response of the same biological entity to different growing environments is to repeat the same experimental design

in different locations. To achieve this, we carried out observations on the architectural development of adult mango trees of the same varieties growing in the subtropical climate of Réunion island and in the Mediterranean climate of Sicily.

This study was carried out under the guidance of Dr. Frédéric Normand, and part of it was done on the premises of the UPR HortSys of Cirad – Centre de coopération Internationale en Recherche Agronomique pour le Développement, in Bassin Plat, Saint-Pierre de la Réunion, France.

2.3.1 *Material and Methods*

Three plants per variety of the mango varieties Kensington Pride, Nam Dok Mai and Tommy Atkins were selected randomly and labelled during their respective vegetative dormancy period in November 2021 in two experimental orchards located in the territory of Sant'Agata di Militello (38.06 N, 14.64 E) in Sicily, Italy and in July 2022 in one experimental orchard located in the territory of Saint-Pierre (21.32 S, 55.49 E) in Réunion island, France.

The tree canopy was divided into 4 expositions, corresponding to the cardinal points: in each exposition, 3 major branches were selected and for each branch, 5 terminal GUs were labelled, for a total of 15 GUs per exposition. Hence, on each plant, 60 terminal GUs were labelled and described around the tree canopy, amounting to a total of 180 GUs per variety, 540 GUs per location.

Figure 2.14. Experimental orchards where the study was conducted in Sicily (left) and Réunion (right).

2.3.1.1   Field data collection

The characters measured on each terminal GU besides its exposition (N-E-S-W) were its length (cm), using a flexible measuring tape, its base diameter (mm) using a digital calliper, its position with respect to his mother GU (A, Apical or L, Lateral), and the event that occurred to it in the last flush (if it flowered, F, if it did not flower, NF, or if it was pruned, P). These terminal GUs went to constitute the database of the "*mother*" GUs from which the new entities would originate. Each GU was assigned a code constructed as:

Variety (abbr.) **– Plant – Exposition – Branch – GU**

So, for example, the 3rd GU belonging to the 2nd branch exposed to the South of the 1st plant of the variety "Tommy Atkins" will be identified by the univocal code "**TA1S23**".

After completing the characterization of the mother GUs, the trees were monitored weekly for the appearance of new entities sprouting from the mothers. The new entities were recorded when they would reach stage C of the developmental stage of mango growth units and inflorescences proposed by Dambreville *et al.* (2015). For each one of them, the Date of Burst, Position (A/L), Type of entity (F – inflorescence; V – GU; FV – mixed inflorescence; VF – GU with flower panicles appearing) were recorded, as well as the code of the mother GU from which it originated and the Number of Leaves that it bore at the moment of the entity appearance. The "*daughter*" entities originating from any mother were assigned a code consisting of the mother GU's code followed by a dot and a sequential number: so, for example, the first and second entities to appear on the GU "TA1S23" will be named "**TA1S23.1**" and "**TA1S23.2**", respectively. The same naming was applied to the entities that appeared on the entities that were born during the course of the study, whose assigned code would therefore be "**TA1S23.1.1**" and so on. Therefore, the GL of each entity could be identified simply by the number of dots contained in the entity's code.

2.3.1.2   Data processing and statistical analysis

The data relative to the appearance of the new entities were inserted into an MS Excel database and then converted into a data frame to be processed with the statistical software R 4.2.0 (R Core Team, 2022).

First, a description of the dynamics of emergence and differentiation of new entities in the Mediterranean climate of Sicily was carried out, to obtain a reliable representation of the mango growing cycle in the new cultivation environment. The probabilities of flowering or producing new GUs were also calculated.

The inferential analysis focused on the probability of each mother GU to produce a new inflorescence or a new GU: to this end, two binary variables "Did It Flower" and "Did it Vegetate" were created, for which the only possible values were 0 or 1.

General Linear Mixed Models (GLMMs) were built for each variety in each location, using R package lme4 (Bates *et al.*, 2022) in each location to evaluate the effect of each architectural character of the mother GUs, and of their second-order interaction, on their probability to generate a reproductive or vegetative new entity. It was necessary to use a mixed model to account for the random error caused by the single individual plant, which constituted the smallest separation item of the experimental design (Kain *et al.*, 2015).

Statistical analyses to test the effects of the considered factors on each response variable were carried out proceeding step by step,: first, a model was built studying the additive effect of all factors on the response variable; then, a model with only the factors found to have a significant effect on the response variable was built and so on, until obtaining the simplest possible model for each response variable for each variety in each location (Persello *et al.*, 2019).

The response variables studied were, finally, the occurrence, timing and intensity of new entities bursting from the mother GUs population, translated into simple questions:

- Did it flower?

- Did it vegetate?

for which only a yes / no outcome was possible, and were therefore modelled to a GLMM of the binomial family with the link function; For the respective subsets of terminal GUs that flowered or vegetated, then, the subsequent questions were investigated:

- When did it flower?

- When did it vegetate?

for which the continuous date variable was converted into a categorical one – the month of appearance of the new entity – and therefore an ordinal regression analysis was conducted;

- How much did it flower?

- How much did it vegetate?

for which the count response variable was tested to belong to a Poisson or negative binomial distribution and then analysed accordingly.

The factors whose effect was researched on these variables were: the GU's exposition – it has to be considered that in the northern hemisphere, the southern exposition is the one which receives most sunlight and the northern one is the one that receives the least, and the opposite is valid in the southern hemisphere – its number of leaves, length (cm) and base diameter (mm), his position with respect to his own mother GU – apical or lateral – and if it had already flowered in a previous cycle – meaning the scar left by the abscission of the panicle could be seen on the apex of the GU – if it had been pruned or none of the previous. Only for the analysis of the vegetative activity, the variable "Did It Flower" was introduced, which could only assume values 1 or 0 in the cases that the GU had flowered in the same cycle or not, respectively. A correlation analysis was carried out after building the definitive dataset to avoid considering correlated variables in the statistical analysis.

The obtained models were plotted using R package sjPlot (Lüdecke *et al.*, 2023).

2.3.2   Results

2.3.2.1   Timing and probability of emergence of new entities

The dynamics of emergence of new entities in the first Growth Level (GL1) from the ancestor GUs labelled at the beginning of the study are depicted in **Figure 2.15**. The fact that the islands are located in opposite hemispheres must be considered when observing the moment of burst. In both islands, however, flowering occurs after the coolest months of the year, confirming that reproductive development of mango buds is inducted by a period at cool temperatures. Vegetative burst, instead, happens later in the season, when daily temperatures rise. It is interesting to notice that while a marked separation is seen between reproductive and vegetative burst in the subtropical climate in Réunion island, the two periods can be observed seamlessly in the Mediterranean climate of Sicily, with the beginning of the vegetative activity overlapping with the end of the flowering.

Moreover, it emerges that in Sicily, a higher number of entities is produced during the season. In particular, the varieties Nam Dok Mai and Tommy Atkins produced a very large number of inflorescences. In Réunion instead, the production of new entities, be them reproductive or vegetative, seems to occur compactly in clearly circumscribed flushes. Kensington Pride emerges as the variety with the strongest vegetative development: in facts, the vigour of this cultivar is well known in the literature, where the direct, positive relation between its vegetative activity and the temperature has been demonstrated (Whiley, 1992).
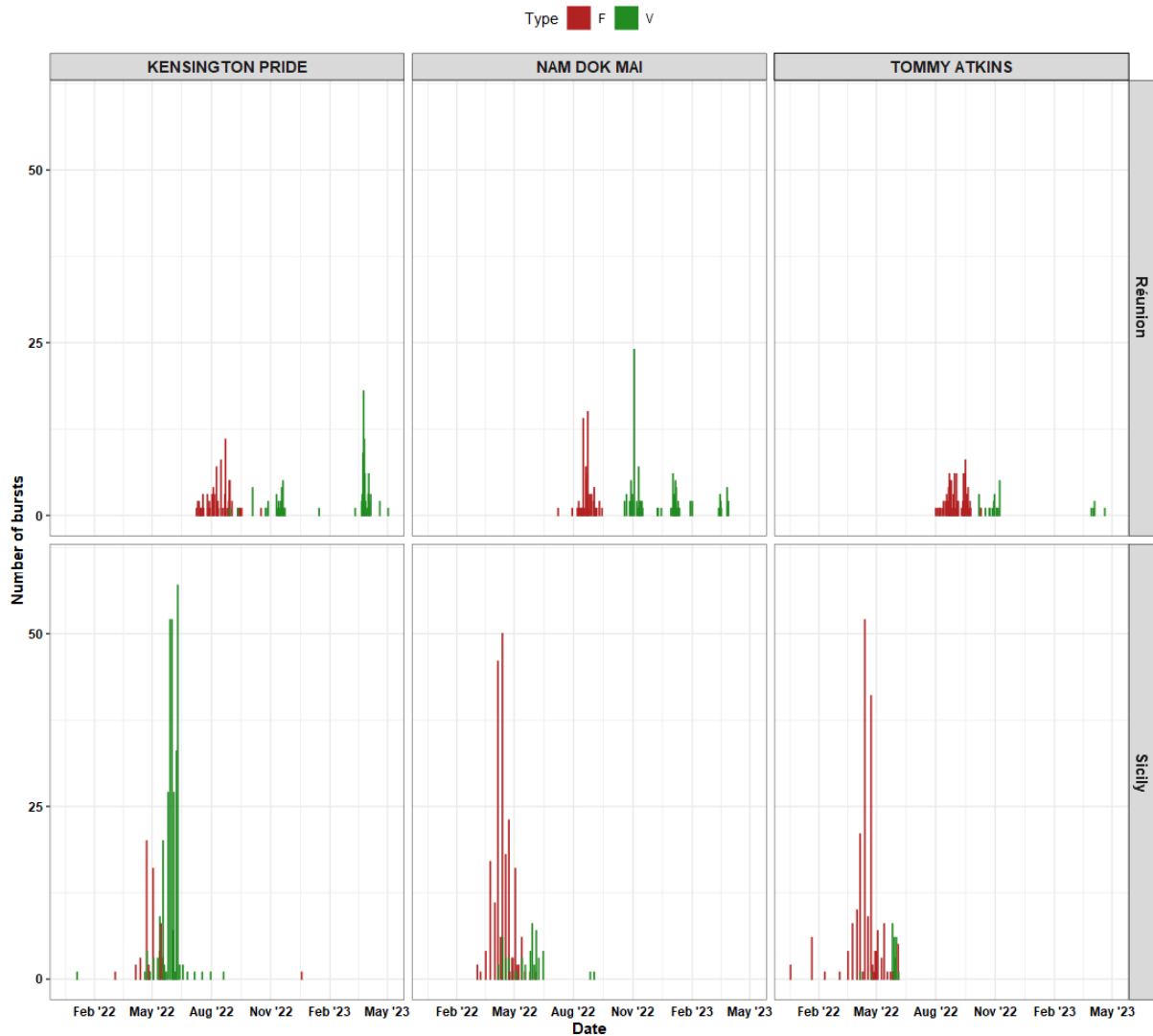
Figure 2.15. Number of burst events of each type (flowering or vegetative) on the 1st growth level (GL1) after the ancestor GUs observed on the mango plants in each island over the period of observation.

The probability for each ancestor GU to produce a new entity is reported in **Figure 2.16**.

Statistical analysis confirmed that Kensington Pride plants behaved differently than Nam Dok Mai and Tommy Atkins, with a very strong vegetative production and a low flowering. This could be a consequence of the plant's natural tendency towards alternate bearing (Normand *et al.*, 2017). Nam Dok Mai and Tommy Atkins plants in Sicily, instead, showed a very high probability of flowering, higher than 50% for both varieties, and significantly higher than in Réunion for Nam Dok Mai. For these two varieties, no differences were observed in the probability of producing new shoots between the two islands.
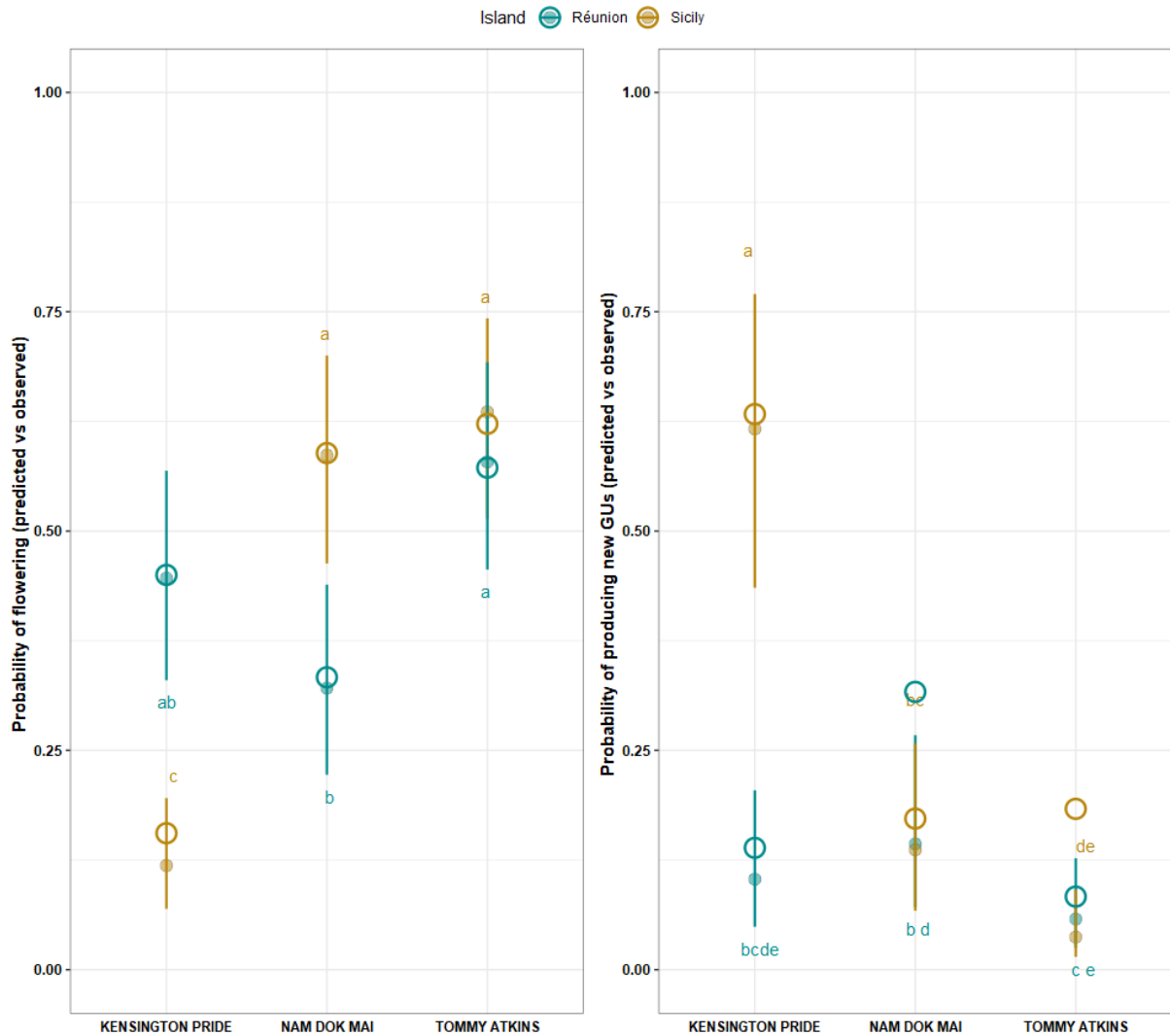
Figure 2.16. Observed (circles) vs. predicted (dots + error bars) probabilities of flowering and producing a new GU for the ancestor GUs of each variety in both islands.

These results suggest that in subtropical or Mediterranean climates, characterized by at least some months of cooler temperatures, mango probably finds conditions more favourable to its reproductive development compared to the tropics. In facts, poor induction and subsequent flowering is one of the hardest challenges that tropical producing countries have to face (Chacko, 1991; da Silva *et al.*, 2014; Núñez-Elisea and Davenport, 1991) and which is usually dealt with the use of chemical inductors such as Paclobutrazol (Rahim *et al.*, 2011; Tongumpai *et al.*, 1989; Upreti *et al.*, 2013; Yeshitela *et al.*, 2004), for which there is no need in the areas with consistent inductive periods.

2.3.2.2 Effect of the morphological and architectural variables on the occurrence, intensity, and timing of the production of new entities

2.3.2.2.1 Kensington Pride

On variety Kensington Pride, in Sicily, the base diameter of the ancestor GU had a significant effect on the probability of flowering of each GU, with the biggest GUs having a higher change of producing an inflorescence. On the other hand, in Réunion, it was both the position of the GU with respect to its mother that had a significant effect on each GU's probability of flowering, with the apical ones being more prone, just like the exposition of the ancestor GUs, with the ones exposed to the North side, which receive the most sunlight, that had the highest probability of flowering (**Figure 2.17**).
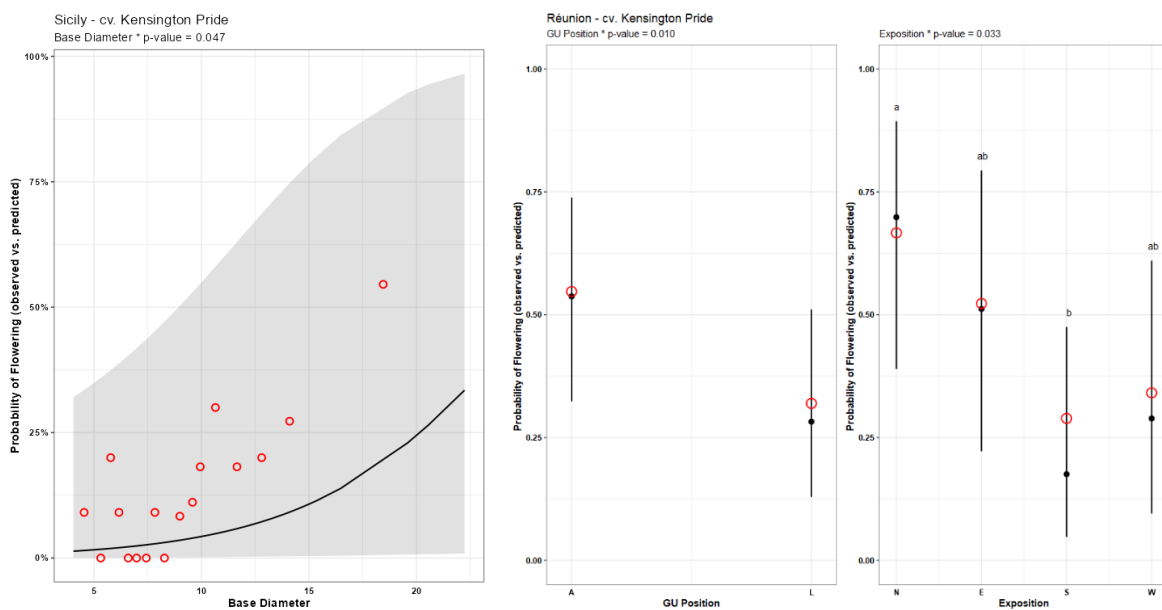

Figure 2.17. Observed (red circles) and predicted (black dots + error bars, lines) probability of flowering of terminal GUs of the variety Kensington Pride in Sicily (left) and in Réunion (right). In the plots where a continuous variable is the explanatory one, each circle represents at least 10 observations.

The number of inflorescences produced by Kensington Pride plants in Sicily was influenced by the length of the mother growth unit. The longer GUs had a higher probability of producing an inflorescence than the shorter ones (**Figure 2.18**). For the plants in Réunion, none of the observed variables showed a significant effect on the number of inflorescences produced by each GU. The base diameter of the GU was the variable whose effect had the lowest *p*-value, equal to 0.380 (data not shown).
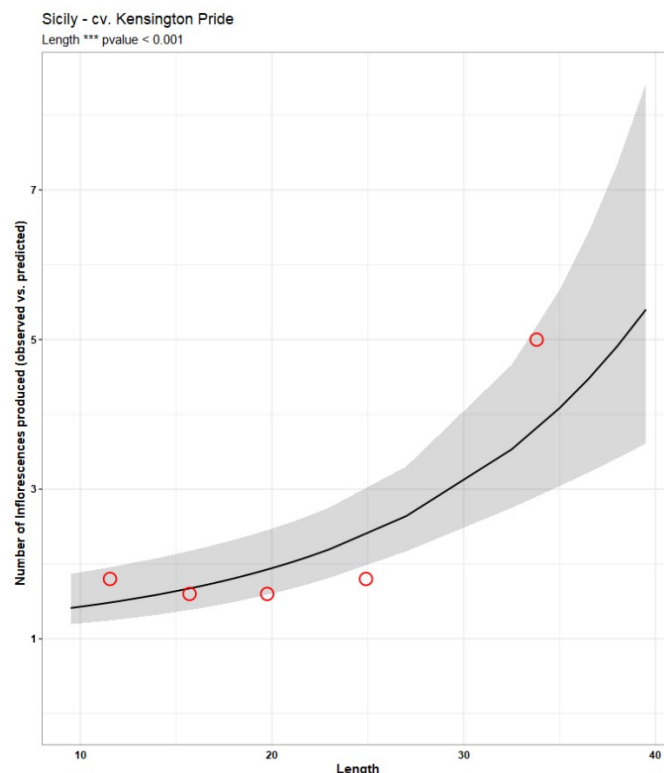
Figure 2.18. Observed (red circles) vs predicted (black line) number of inflorescences produced by the terminal GUs which flowered of the variety Kensington Pride in Sicily. Each circle represents at least 10 observations.

The timing of the flowering in Sicily was regulated by the position of the GU with respect to its mother: apical GUs had a higher probability of flowering in the month of April than the lateral ones, which instead had a higher probability of flowering in May. In Réunion, the earliness of flowering was linked to the number of leaves on each terminal GU: the GUs which carried the least leaves had a higher probability of flowering in the month of July; the opposite was true in the month of August (**Figure 2.19**).
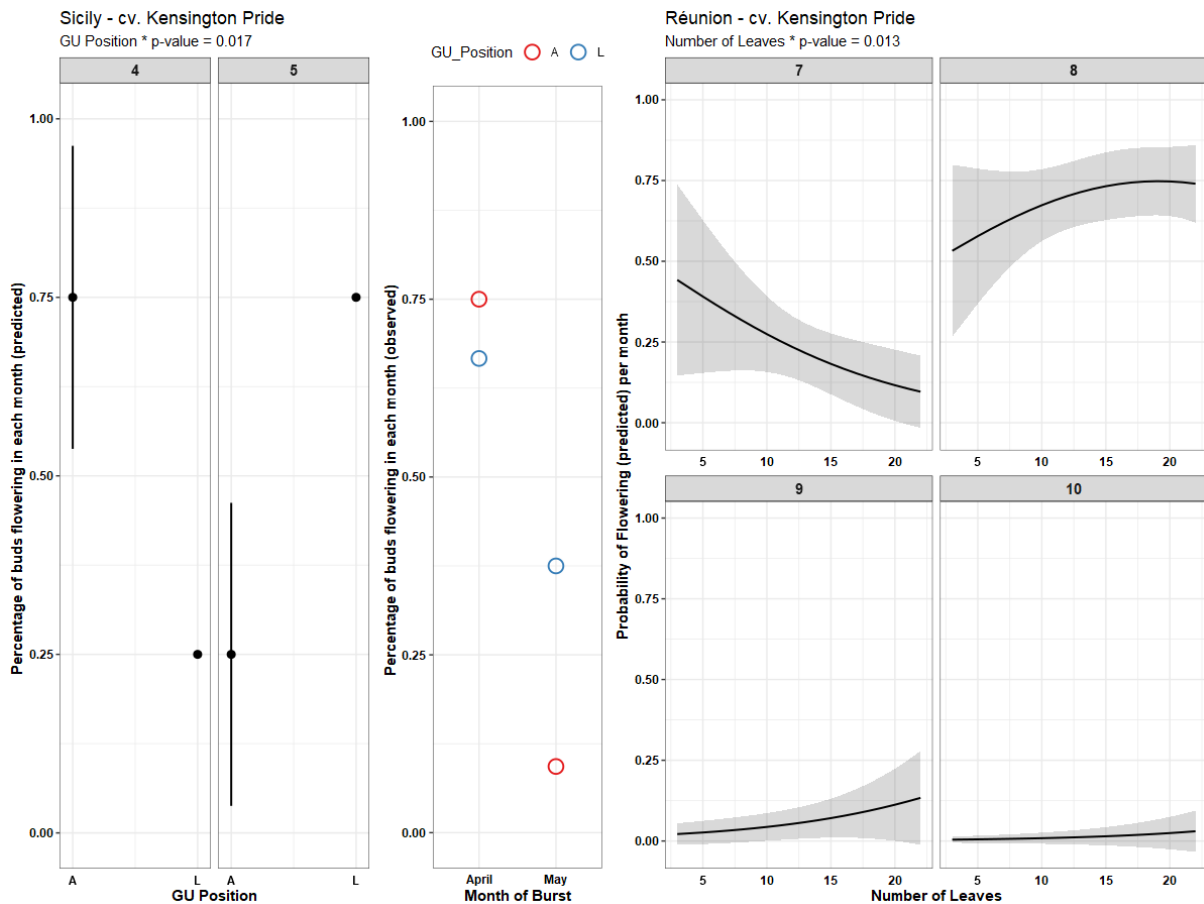
Figure 2.19. Probabilities of flowering in each month on the basis of the GU position for the Kensington Pride plants in Sicily (left) and on the basis of the number of leaves on the terminal GU for the plants in Réunion (right).

The probability of each GU to vegetate, therefore to produce new GUs, was regulated by two different interactions in Sicily and Réunion: in the Mediterranean island, it was the interaction between the GU's length and its exposition which increased the probability of each GU to vegetate. In particular, shorter GUs had a higher probability of flowering when they were exposed to the South or to the North, while GUs exposed to the East or West had a high probability of vegetating when they were longer than the average. In Réunion on the other hand, lateral GUs with a large base diameter had the highest chance of vegetating (up to 100%). A large base diameter meant a higher probability of producing new GUs also for apical terminal GUs, which however had a lower general probability of vegetating (**Figure 2.20**).
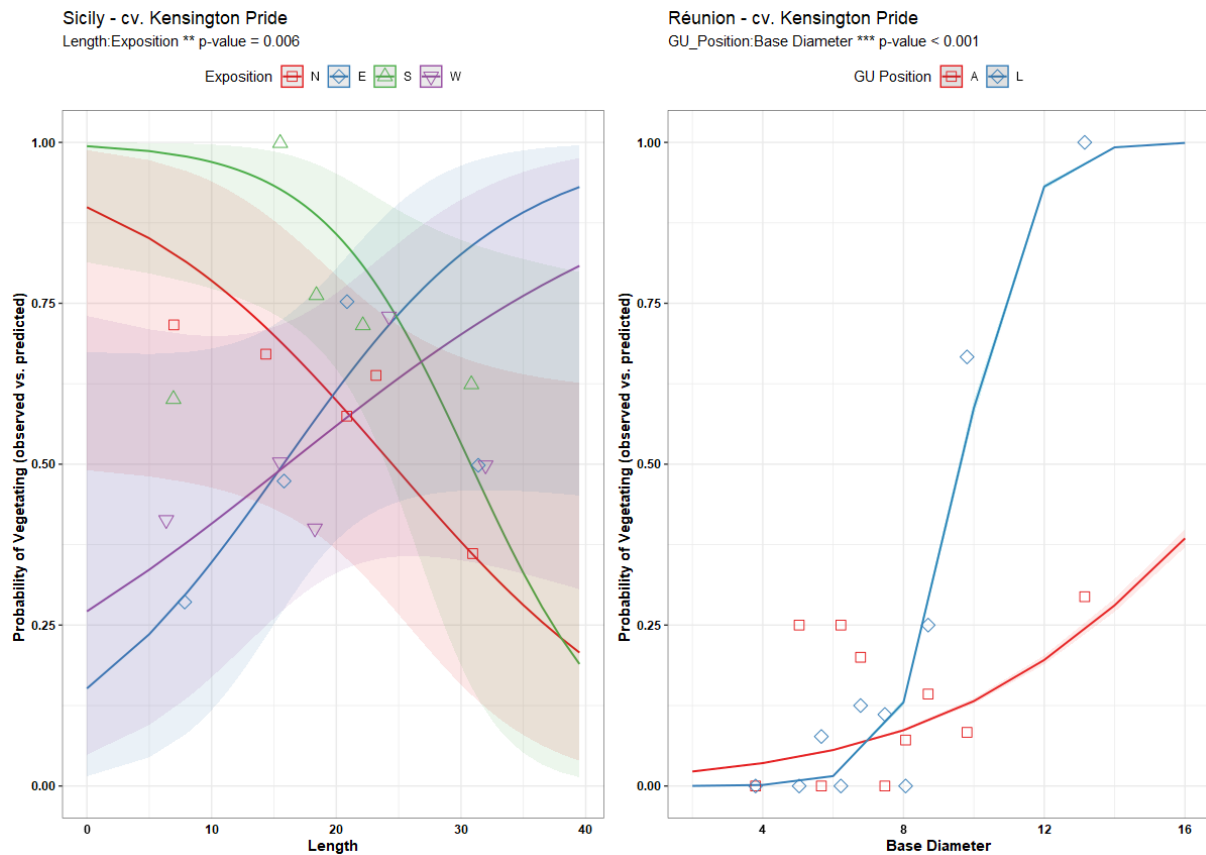
Figure 2.20. Observed (symbols) vs predicted (lines) probabilities of vegetation for the terminal GUs of Kensington Pride in Sicily (left) and Réunion (right) in relation to the respective most significant explanatory variables interaction. In the plots where a continuous variable is the explanatory one, each symbol represents at least 10 observations.

The number of new GUs produced by the terminal GUs of the Kensington Pride mangoes was significantly influenced by the axis' morphological characters in both Sicily and Réunion. In the first case, both the GU's length and base diameter had a significant positive effect on the productivity of each terminal GU, with the largest and longest GUs producing significantly higher offspring than the smaller ones. In Réunion, an equally significant effect was found only for the base diameter (**Figure 2.21**).

Finally, the base diameter of the GU had a significant effect also on the timing of its production of new GUs in both Sicily and Réunion. In both cases, larger GUs tended to produce earlier than the smaller ones. In Sicily, also the exposition of the GU on the canopy had a relevant effect on the timing of its production (**Figure 2.22**).
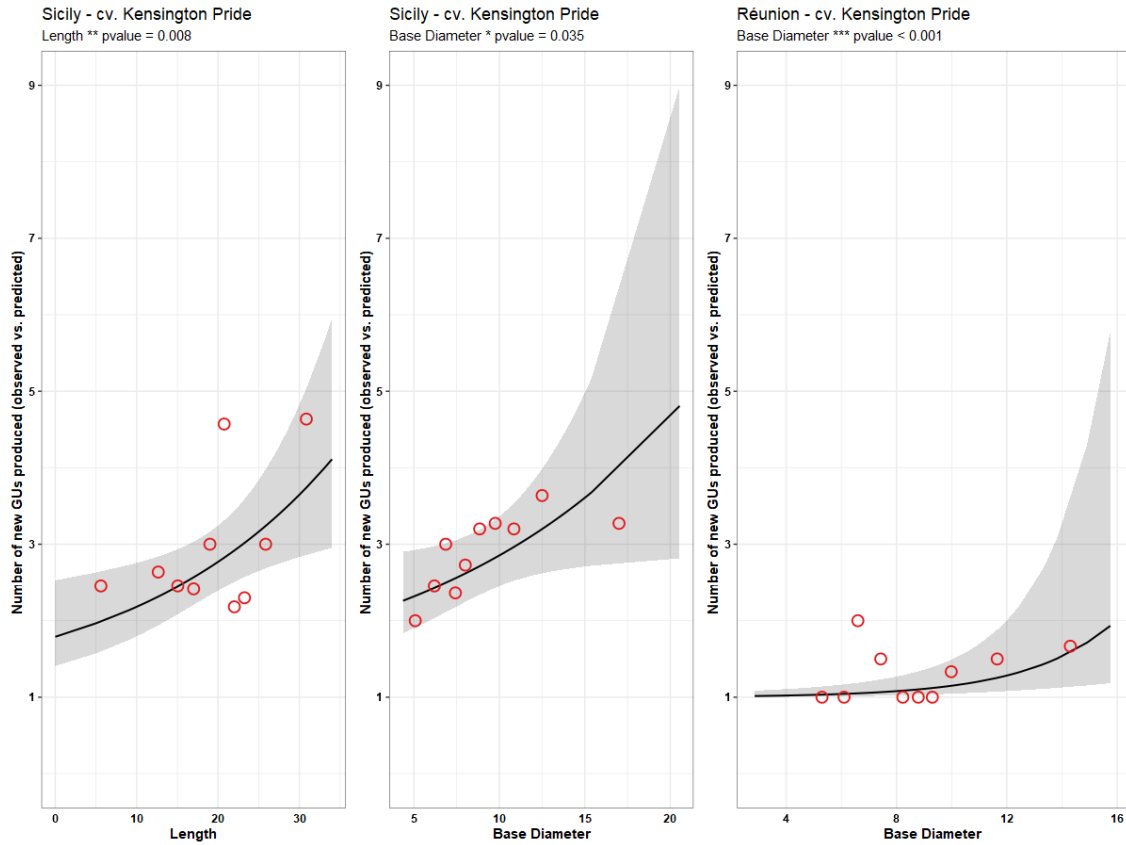
Figure 2.21. Observed (red circles) vs predicted (black lines) intensity of vegetation in the Kensington Pride mangoes in Sicily (left) and Réunion (right) in relation to the respective most significant explanatory variables. Each circle represents at least 10 observations.
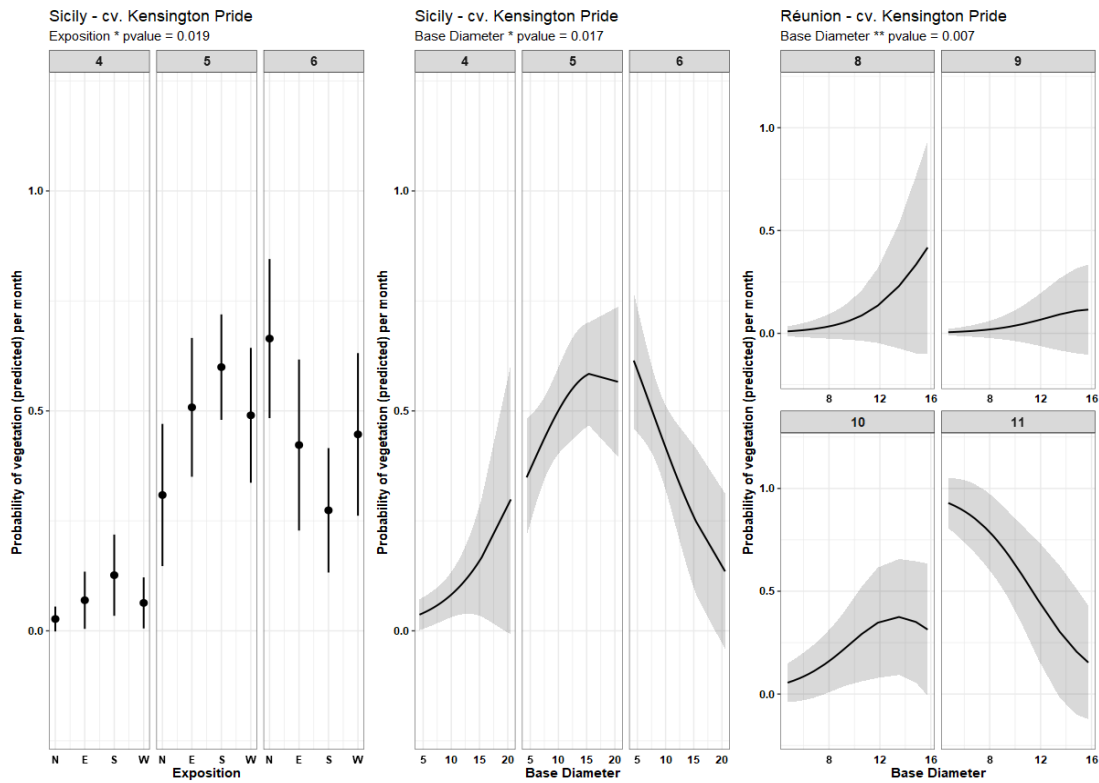
Figure 2.22. Predicted probabilities of production of new entities by the GUs that vegetated in each month of the year for the terminal GUs of Kensington Pride mangoes in Sicily (left) and Réunion (right).

2.3.2.2.2 *Nam Dok Mai*

As previously shown in **Figure 2.16**, a significant difference emerged in the probability of flowering for the plants of the Thai cultivar Nam Dok Mai between Sicily and Réunion. In the first case, this was influenced by the event that occurred to the GU in the previous cycle, meaning that GUs that had not flowered in the previous cycle had a significantly higher probability of flowering in the observed growing cycle compared to their counterparts; also the position of the GUs had a significant influence on their probability of flowering, this resulting higher in the apical ones. In Réunion, on the other hand, it was the interaction between this latter architectural character and the GU's position on the canopy that showed a significant effect on the probability of flowering. In facts, apical GUs tended to flower more when they were in the most sunlit parts of the canopy (North or East-oriented), while in the southern and western parts of the canopy it was the lateral GUs that showed the highest probability of flowering (**Figure 2.23**).
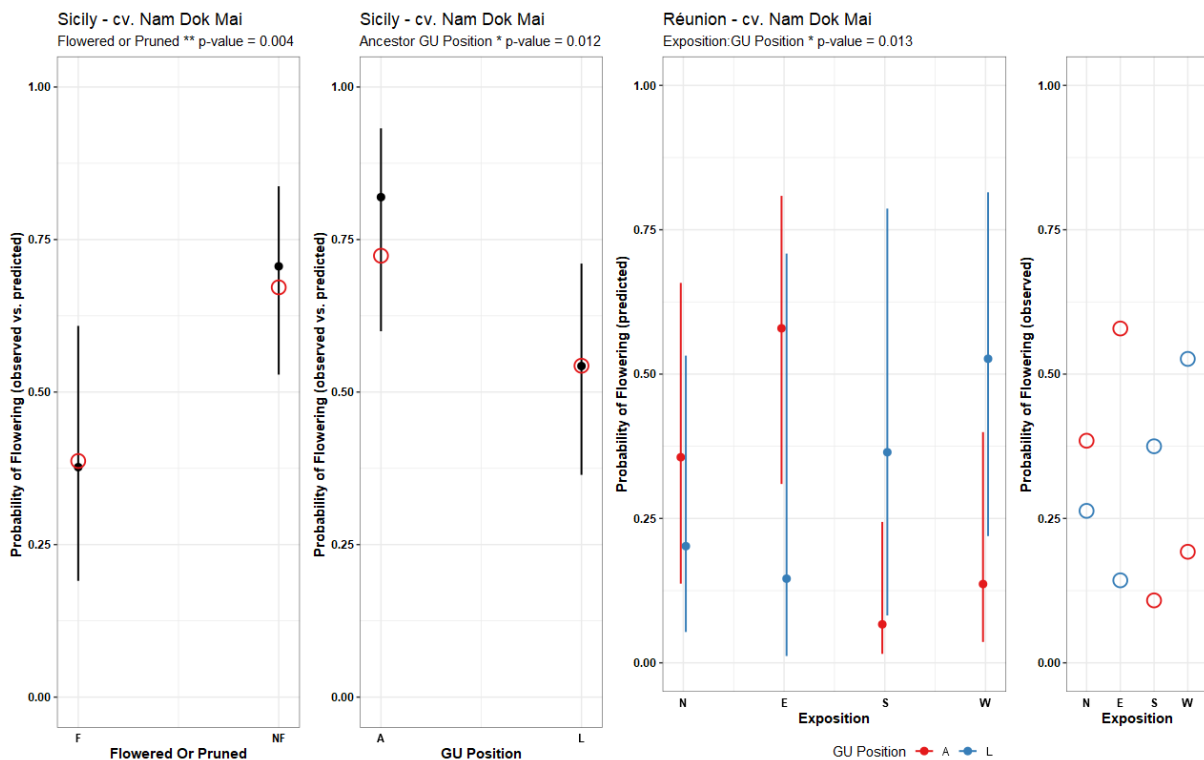
Figure 2.23. Predicted (dots + error bars) vs observed (circles) probability of flowering of the terminal GUs of the Nam Dok Mai in Sicily and Réunion, in relation to the respective most significant explanatory variables.

The same morphological character, i.e. the base diameter of the GU, was responsible for the flowering productivity of the terminal GUs of variety Nam Dok Mai in both Sicily and Réunion. **Figure 2.24** depicts the direct, positive relation between the GU's base diameter and the number of inflorescences that it produced.

The timing of flowering of Nam Dok Mai trees was influenced by the exposition of the GU in Réunion island, with the South-oriented GUs flowering significantly later than all the other ones in the canopy, and by the base diameter of the GU in Sicily. In the Mediterranean island, GUs with a larger diameter flowered as early as March; the ones with an intermediate diameter, between 6 and 10 mm, flowered mostly in April, while in May it was mostly the smaller GUs that produced new inflorescences (**Figure 2.25**).
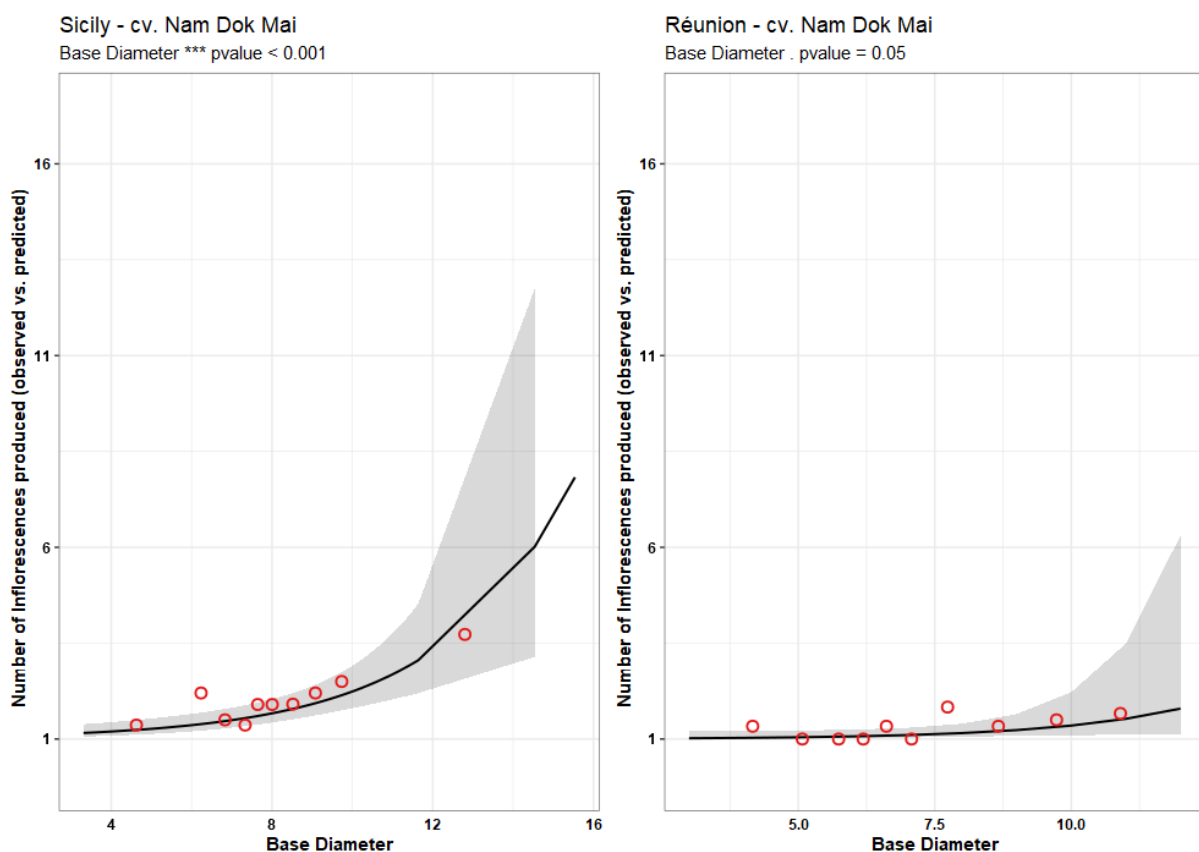
Figure 2.24. Observed (red circles) vs predicted (black lines) number of inflorescences produced, per classes of base diameter of the terminal GUs which flowered, in plants of the variety Nam Dok Mai in Sicily (left) and Réunion (right). In the plots where a continuous variable is the explanatory one, each circle represents at least 10 observations.

Figure 2.25. Predicted probabilities of production of new inflorescences in each month of the year for the sole terminal GUs which flowered of Nam Dok Mai trees in Sicily (left) and Réunion (right).

The GU position had a significant effect on the probability of the GU of producing new vegetation in both Sicily and Réunion (**Figure 2.26**). In the subtropical island, also the base diameter had a very significant, positive effect on the vegetation probability of the GUs, while in Sicily it emerged that the GUs that had not flowered in the same cycle had a much higher probability of producing a GU than those that did. However the probability of vegetation for any of them was in general below 0.3.
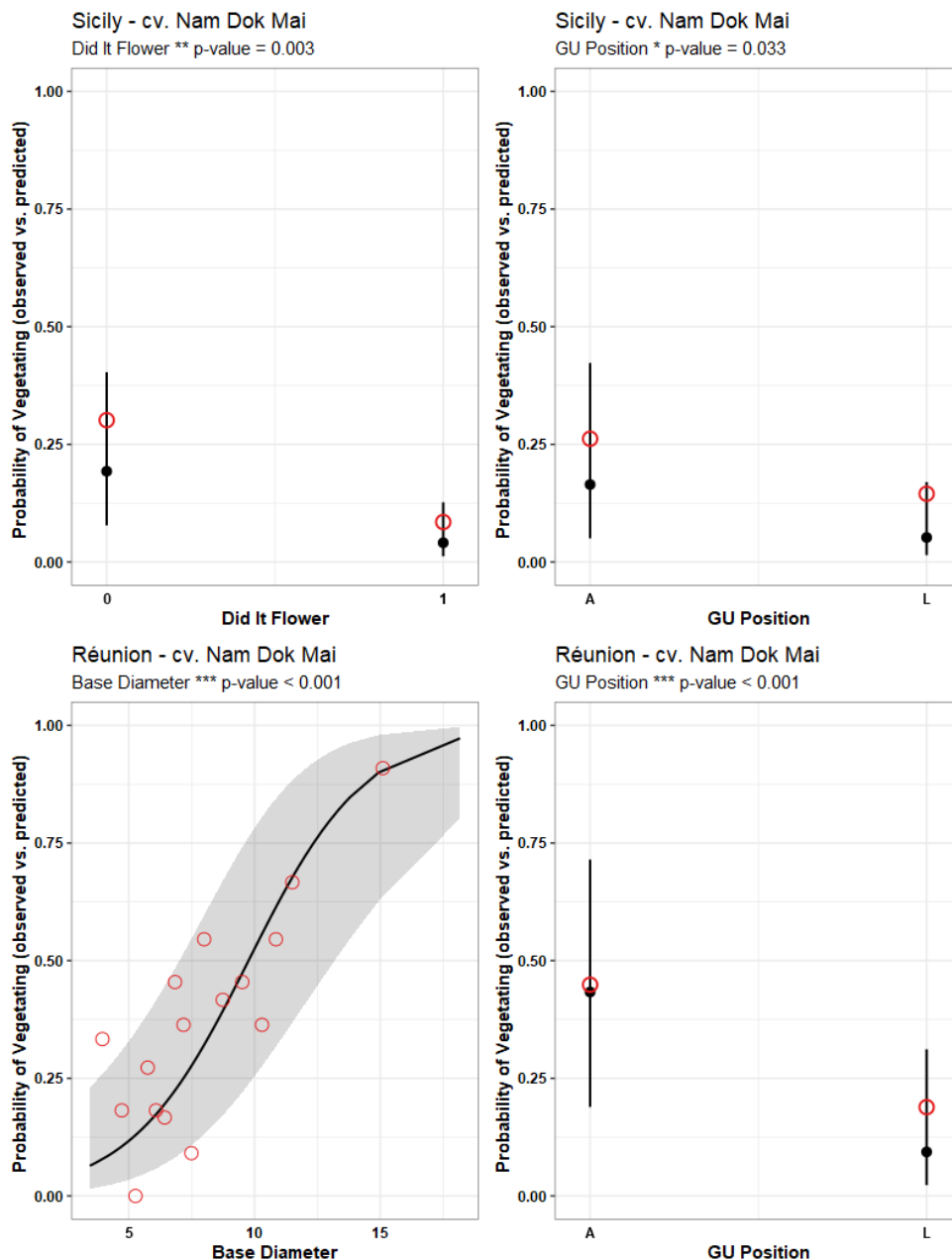
Figure 2.26. Predicted (black dots and lines) vs observed (red circles) probability of producing a new GU in relation to the most significant variables for the Nam Dok Mai plants in Sicily (upper row) and Réunion (lower row). In the plots where a continuous variable is the explanatory one, each circle represents at least 10 observations.

Even with regard to the number of inflorescences produced, at least one variable had a significant effect on the observed character in both the islands. In facts, the base diameter of the GU had a positive, significant effect on the number of new GUs produced by the terminal GUs. In Sicily, moreover, the fact that a terminal GU had flowered during the season was cause of a smaller number of new GUs produced (**Figure 2.27**).
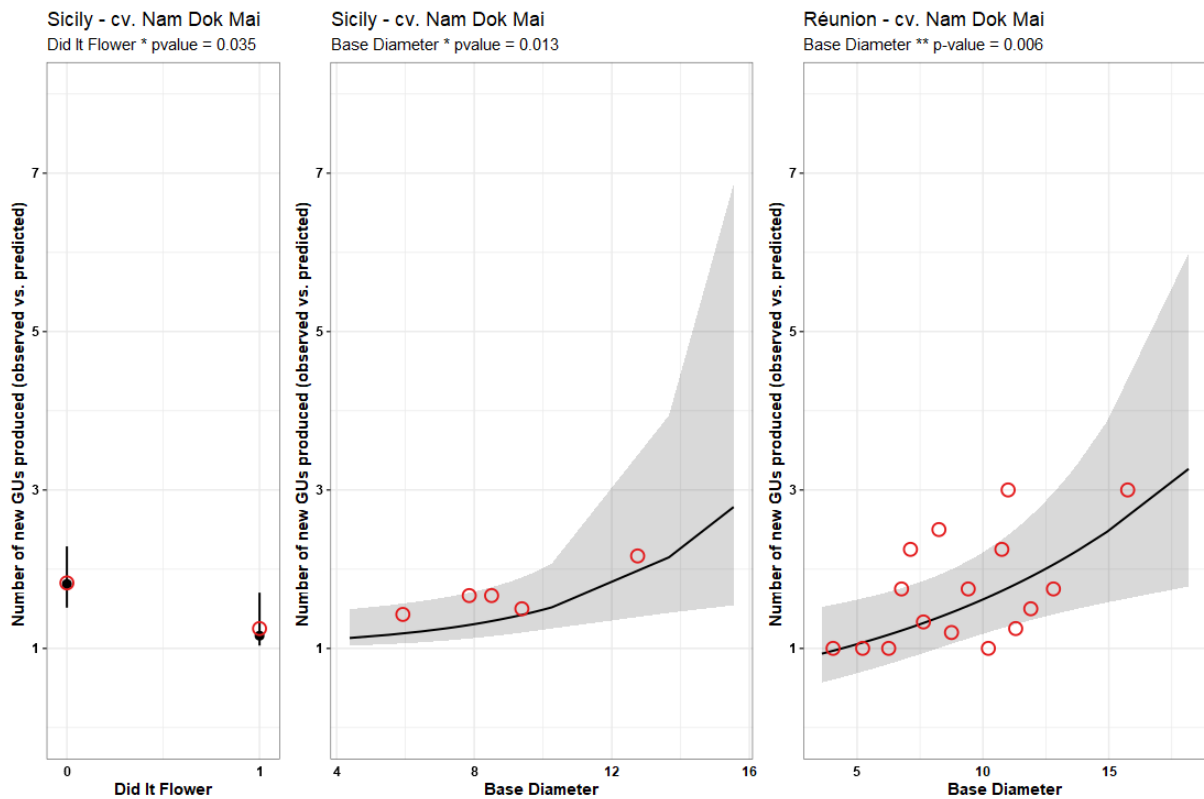
Figure 2.27. Predicted (black dots and lines) vs observed (red circles) number of new GUs produced by the Nam Dok Mai plants in Sicily and Réunion, on the basis of the most significant explanatory variables. In the plots where a continuous variable is the explanatory one, each circle represents at least 10 observations.

A significant effect on the timing of appearance of new GUs on the Nam Dok Mai trees could be attributed to various factors and interactions. In Sicily, GUs that had not flowered in the previous cycle were unaffected by the morphological characters. The ones that had flowered in a previous cycle, instead, vegetated in April if they had a large base diameter and a short length, and vegetated in June if they had the opposite characteristics. In Réunion, longer GUs tended to vegetate only as late as in January, and the long, East-oriented GUs in the canopy of the trees showed vegetative activity as early as in August. West and South-oriented GUs had the highest probability of producing new offspring in the month of January, especially if they were longer than the average (**Figure 2.28**).

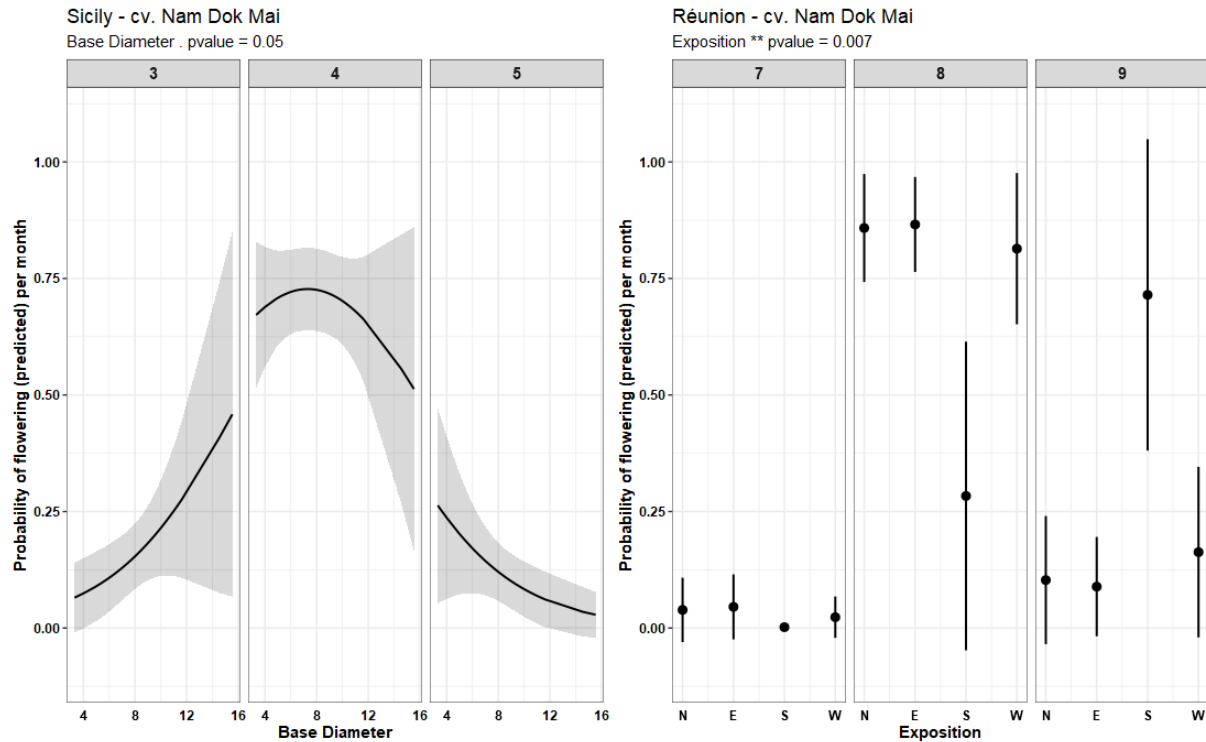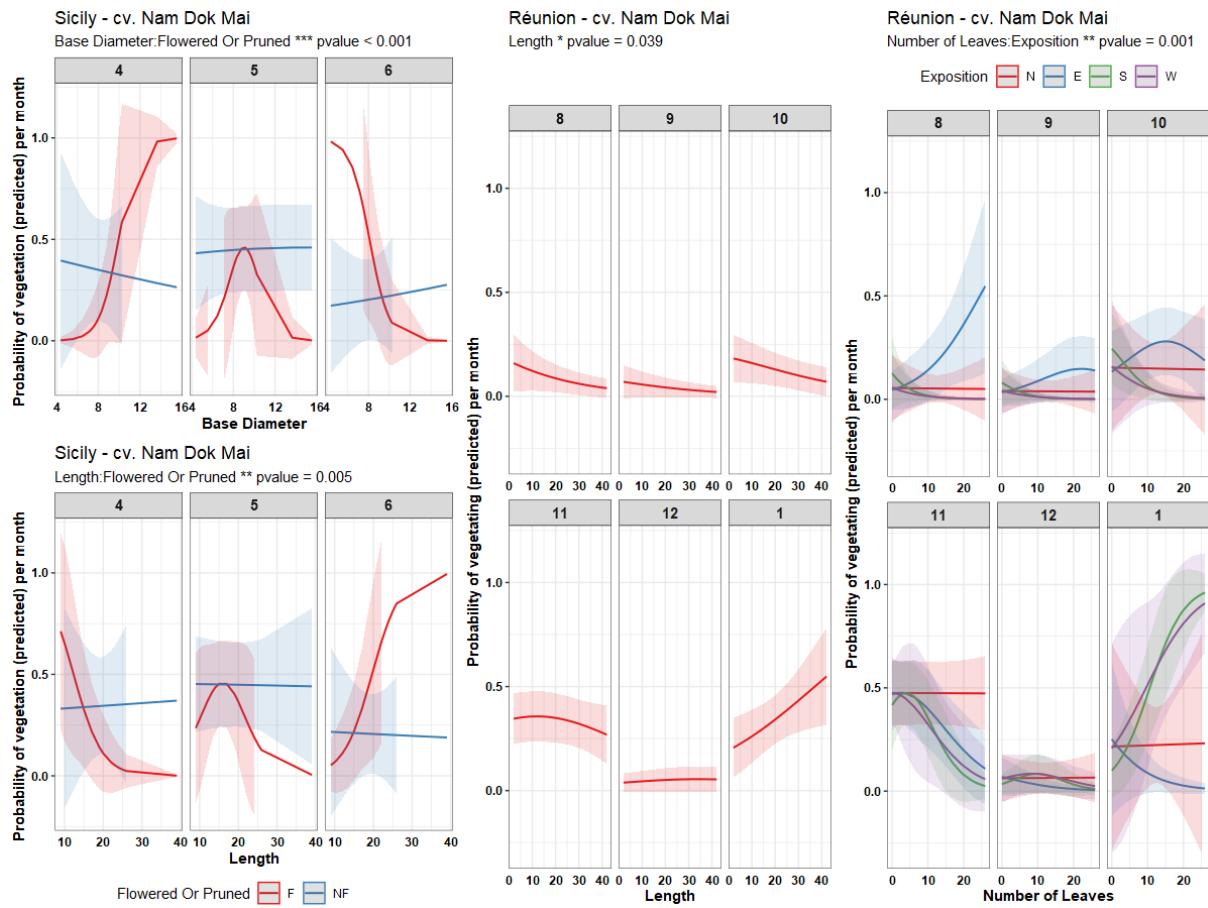**Figure 2.28**. Predicted probabilities of production of new GUs in each month of the year for the sole terminal GUs which vegetated of Nam Dok Mai trees in Sicily (left) and Réunion (right).

2.3.2.2.3 *Tommy Atkins*

In Tommy Atkins, both architectural and morphological variables exerted a significant effect on the probability of flowering of the terminal GUs (**Figure 2.29**). In Sicily, South-oriented GUs had the highest probability of flowering, while the North-oriented ones, which receive the least hours of direct sunlight, had the lowest probability. Also, the GUs that carried more leaves had a higher probability of flowering. In Réunion, both the GU's length and its base diameter exerted a similar, positive effect on the probability of flowering, and the GUs that had not flowered in previous cycles were also more prone to produce an inflorescence in the observed growing cycle.
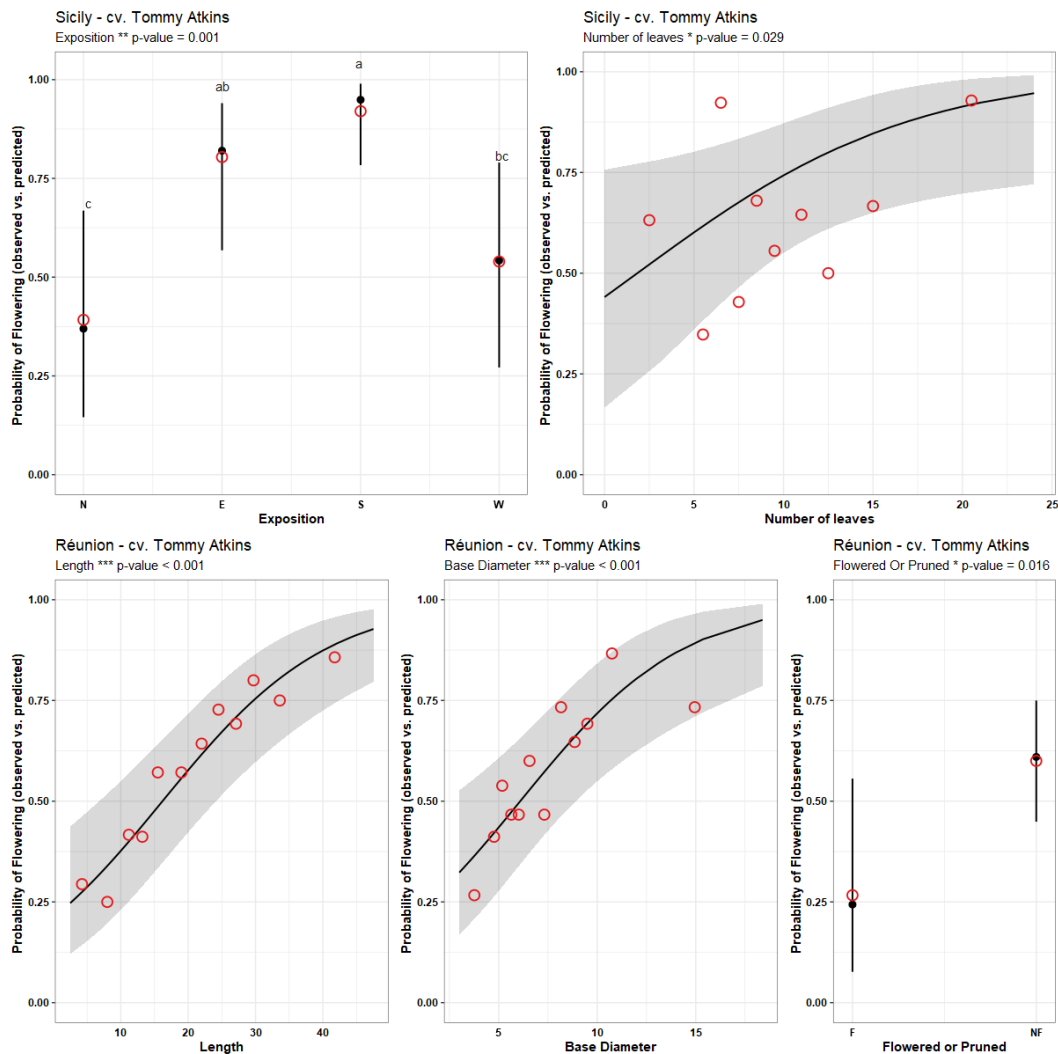
Figure 2.29. Predicted (black dots and lines) vs observed (red circles) probability of flowering on Tommy Atkins plants in Sicily (upper row) and Réunion (lower row) in relation with the most significant explanatory variables. In the plots where a continuous variable is the explanatory one, each circle represents at least 10 observations.

The analysis on the flowering productivity of the GUs of Tommy Atkins plants could not be carried for the dataset of Réunion island, since during the course of the study, no terminal GU produced more than 1 new inflorescence in the current cycle. In Sicily, instead, the longer GUs had a higher probability of producing a high number of inflorescences (**Figure 2.30**).
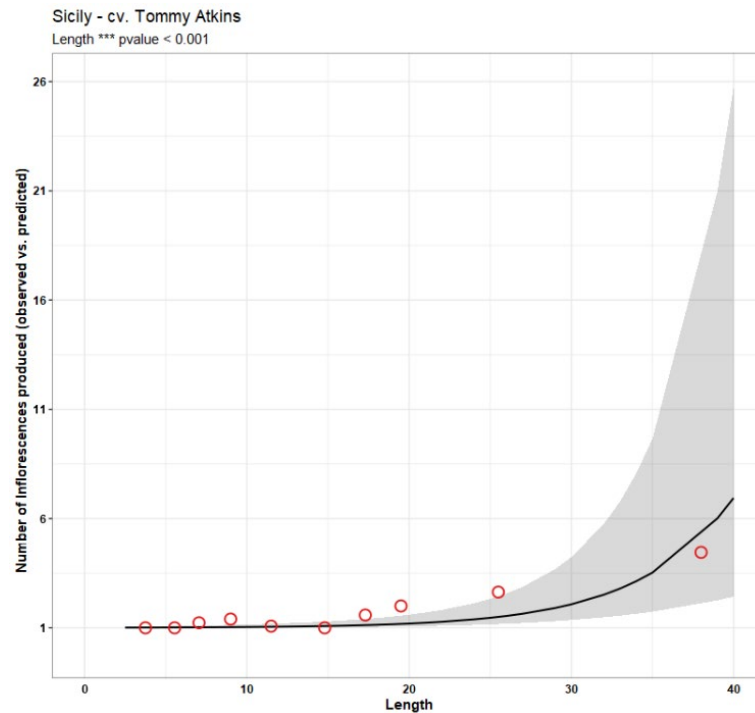
Figure 2.30. Predicted (black line) vs observed (red circles) number of infloresnces produced by the plants of Tommy Atkins in Sicily. Each circle represents at least 10 observations.

Two different morphological variables, finally, exerted their effect on the timing of flowering of the Tommy Atkins trees in the two islands. In Sicily, long GUs had a good probability (circa 0.5) of flowering as early as in January. Average length GUs would instead flower in April, while a percentage of the shorter GUs would flower in May. In Réunion, a significant effect on the moment of flowering of the terminal GUs was found in the number of leaves that each one carried. The GUs that carried more leaves, in fact, could more easily flower in the month of August, while the GUs that had a smaller number of leaves would more probably flower in September (**Figure 2.31**).
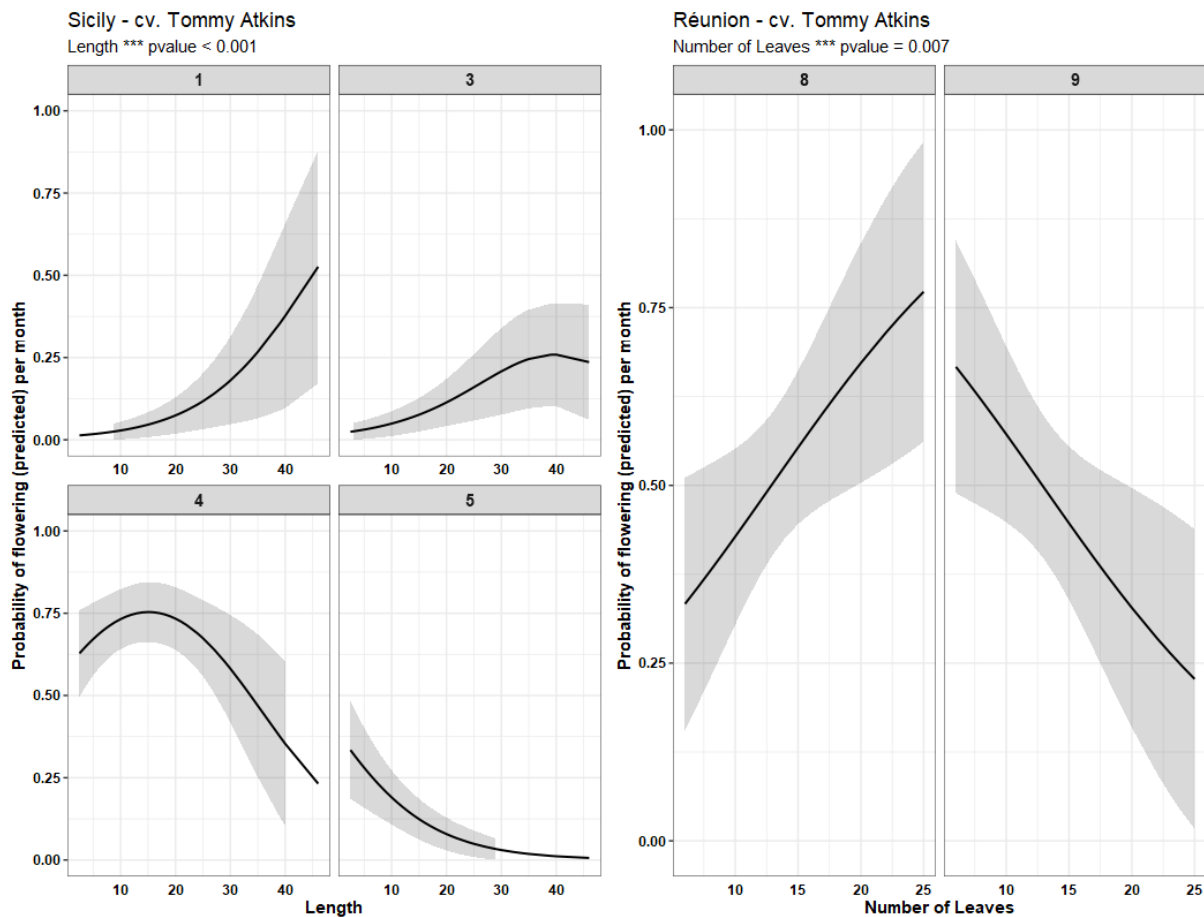
Figure 2.31. Predicted percentages of GUs flowering each month on the plants of Tommy Atkins in Sicily (left) and Réunion (right).

The analysis on the vegetative development of the Tommy Atkins plants could not be carried out in Sicily, because during the period of observation, only 10 new GUs were produced. In Réunion instead, both the GU's base diameter and whether it had flowered or not during the current cycle significantly influenced its probability of vegetation (**Figure 2.32**).

Just as with flowering, no terminal GU produced more than 1 new GU, therefore no analysis could be conducted on the intensity of vegetative activity. Similarly, no variable or interaction of any of the variables was found to exert a significant effect on the timing of appearance of new GUs on the labelled terminal ones.
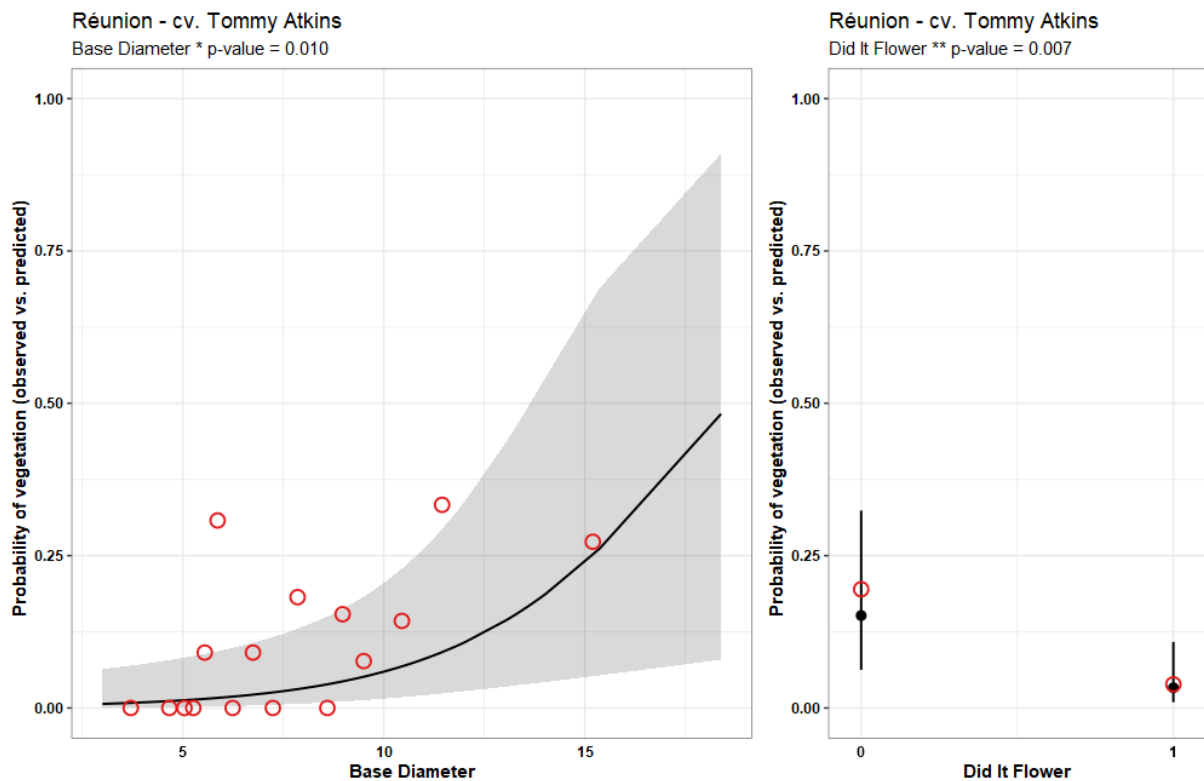
Figure 2.32. Predicted (black dots and lines) vs observed (red cirles) probability of vegetation for the Tommy Atkins plants in Réunion, on the basis of the most significant variables. In the plots where a continuous variable is the explanatory one, each circle represents at least 10 observations.

2.3.3 Discussion

The significant effects of each variable and their interactions on the probability, intensity and earliness of flowering and vegetation of the three varieties in each island is summarised in **Table 2.5**. The GU's base diameter was the variable that exerted significant influence on most characters across the varieties. It is interesting to notice that GUs with a large base diameter will more probably produce a higher number of both new GUs and inflorescences, in variety Nam Dok Mai, in both Sicily and Réunion. Therefore, this variable can be considered highly important for the productivity of this variety, regardless of the environment of cultivation. A large base diameter of the GU can be an index of higher availability of resources to use for architectural development.

Table 2.5. Table summarizing the effect of each of the studied variables on the probability, intensity and timing (earliness) of appearance of new entities on the plants of each variety in the two islands. Coloured cells within each row indicate that for the corresponding variety, the interaction of two variables has a significant effect on the given character, rather than each variable alone. "+" symbols indicate that the variable has a significant effect on the given character, while "-" symbols indicate that the variable has a negative significant effect on the given character.

Variable		Prob. flowering Sicily	Prob. flowering Réunion	Intensity flowering Sicily	Intensity flowering Réunion	Earliness Flowering Sicily	Earliness Flowering Réunion	Prob. Vegetation Sicily	Prob. Vegetation Réunion	Intensity Vegetation Sicily	Intensity Vegetation Réunion	Earliness Vegetation Sicily	Earliness Vegetation Réunion
Flowered in the same cycle	TA	-											
	NDM												
	KP	-		-									
Mother GU Position (Apical)	TA												
	NDM	+	+ (orange)					+	+				
	KP		+ (orange)			+			- (green)				
Exposition	TA	S-E-W-N											
	NDM		E-N (orange)					N-E-W					S-W (green)
	KP	N-E-W-S						S-N		S			
Non Flowered or Flowered	TA								+				
	NDM	+											
	KP												
Mother GU # of leaves (high)	TA		+										
	NDM												-
	KP					-							
Mother GU Length (large)	TA	+						+	+				
	NDM											-	- (green)
	KP			+				+		+			
Mother GU Base Diameter (large)	TA								+				
	NDM			+	+		+		+	+	+	+	
	KP	+							+ (green)	+	+		+

In general, the morphological characters base diameter and length of the terminal GU expressed a significant effect on the vegetative development of the bud, influencing positively the probability and intensity of new GUs production, for both Kensington Pride and Tommy Atkins. In Nam Dok Mai instead, a higher length of the GU corresponded to a later GU production.

Rather surprisingly, the fact that the GU had not flowered in a previous cycle, only had a significant effect on the probability of flowering of Nam Dok Mai in Sicily and on the probability of vegetation of Tommy Atkins in Réunion. Mango is generally considered an alternate bearing species in the tropics, subtropics and Mediterranean alike (El-Motaium *et al.*, 2019; Elsheery *et al.*, 2020; Goguey, 1997; Normand *et al.*, 2017), but our results suggest that flowering in one cycle is not the only (or main) reason limiting the possibility of flowering in the following one.

The exposition of the GU on the canopy significantly influenced the probability of flowering in both Réunion and Sicily, for at least one variety. In all cases, it was the most sunlit side of the canopy which saw a higher probability that the terminal GUs would flower and, in the case of Nam Dok Mai in Réunion, the earliness of the flowering. It is common, in facts, especially on mango trees growing in the wild, to observe heavy flowering concentrated in one section of the canopy, while no inflorescences appear on the other expositions (Davenport, 2006; Normand, 2023).

In Nam Dok Mai varieties, the architectural position of the terminal GU, or its position in relation to its mother GU, was a significant factor in the probability of both flowering and vegetation of the observed one, regardless of the cultivation environment. Apical GUs, in facts, had a significantly higher probability of producing new entities than lateral ones (Normand *et al.*, 2009).

2.3.4   Conclusions

Very few of the observed morphological or architectural factors of mango trees had a significant effect on their probability, intensity and timing of flowering and vegetation regardless of the environment of cultivation. Instead, most of these characters were influenced by different factors in the two observed island. This supports the hypothesis that the environmental conditions affect the behaviour of the mango plants to such an extent that the relations between architectural characteristics of the plant and its phenology are altered.

As previously shown in chapter 2.1, the climate in the two islands of Sicily and Réunion differs greatly. This reflects in the phenological cycle of mango in the Mediterranean basin shown in **Figure 2.10**, which in facts is significantly different from the one well-known for the subtropical areas (**Figure 2.12**).

As the cultivation of mango moves to new regions and territories, then, the paradigm used until now for its management cannot be used so firmly. This opens great opportunities and questions for new researches, that must cover all aspects of the plant's biology and cultivation.

2.4 *How to protect mango trees from environmental adversities in Sicily?*

In chapter 1 we mentioned that the most significant threats for the health of mango trees and fruits in the Mediterranean basin are represented by the bacterium *Pseudomonas syringae* pv. *syringae*, which causes the Bacterial Apical Necrosis (BAN) disease on buds and shoots, during winter, and the high solar radiation which, paired with the high summer temperatures, causes sunburn damage on the fruits. We conducted two preliminary studies on how to prevent these disorders on mango trees and fruits, using simple physical means. For BAN, we assessed the effect of the presence of windbreaks on the diffusion and dangerousness of the bacterial colonies (Vanneste *et al.*, 2013). We also evaluated the efficacy of Kaolin sprays in fighting the occurrence of sunburn damages on mango fruits (Baiea *et al.*, 2018).

2.4.1 *Material and Methods*

2.4.1.1 *Low temperatures and Bacterial Apical Necrosis*

The study was conducted in the winter of 2023 in a farm located in Acquedolci (ME, Italy, 38°05'N - 14°60'E) divided into sectors corresponding to the different plots, in which different types of planting and windbreaks can be found, in which 6-years old mango plants are present (**Figure 2.33**). Each plot contains plants of only one variety, and we selected three plots with cv. Keitt trees and three plots with cv. Maya. Environmental conditions that may be related to the development of bacterial disease were recorded during the winter from a weather station located in the centre of the farm under study. Specifically, minimum, average and maximum temperature, average wind speed and direction, and total precipitation were recorded at daily intervals. In each plot on the farm, 10% of the total plants were randomly selected. The severity of Bacterial Apical Necrosis was assessed on each of them as the number of shoots showing symptoms on the total number of shoots, and the incidence of the disease in the plot, understood as the number of plants showing symptoms out of the total number of plants observed, was also calculated.

Figure 2.33. Satellite view of the farm where the trial was conducted, with the plots taken into consideration highlighted and marked.

2.4.1.2 High temperatures and fruit sunburn

The study was conducted on the same farm from June to September 2022. Fifteen mango plants of the Glenn variety were selected and subjected to fruit observation during the entire production season using a specially designed empirical scale formulated to estimate fruit scald damage (**Figure 2.34**).

Figure 2.34. Scale of evaluation of the degree of sunburn (from 0 - no damage to 4 - maximum severity of damage) of mango fruit used during the study.

The progress of the disease was monitored during the growth and subsequent ripening of the fruit, in relation to the trend of temperatures and the degree of solar radiation to which they were subjected, as measured by a weather station located in the centre of the farm under study. The objective of the study was to evaluate the effectiveness of kaolin treatment in contrasting sunburn damage. Therefore, two different commercial kaolin products were applied after mango fruit set. Three treatments were prepared:

1) DustKAO: treatment based on powdered kaolin (DustKAO, AgriTrade, Melfi, Italy),

2) LiquidKAO: treatment based on kaolin dissolved in water (LiquidKAO, AgriTrade, Melfi, Italy),

3) CTR: control in which the fruits were not treated.

2.4.2   Results and Discussions

2.4.2.1   Low Bacterial temperatures and Apical Necrosis

Mango shoots are particularly susceptible to attacks by *Pseudomonas syringae* pv. *syringae* colonies when the swollen bud or vegetative stage, when the buds begin to open and provide sites for bacterial colonies to establish themselves.

The winter under study was not characterized by particularly cold temperatures: in fact, only on two consecutive days did the minimum temperature fell below 5°C. In general, the coldest days were those of the first decade of February. On the other hand, there has been a particularly wet winter. Out of 105 days of observation, from January 1 to April 15, as many as 41 days were rainy, which is a high value for the standards of the area. April 4 was the wettest day of the observed period with 33 mm of rainfall (**Figure 2.35**).
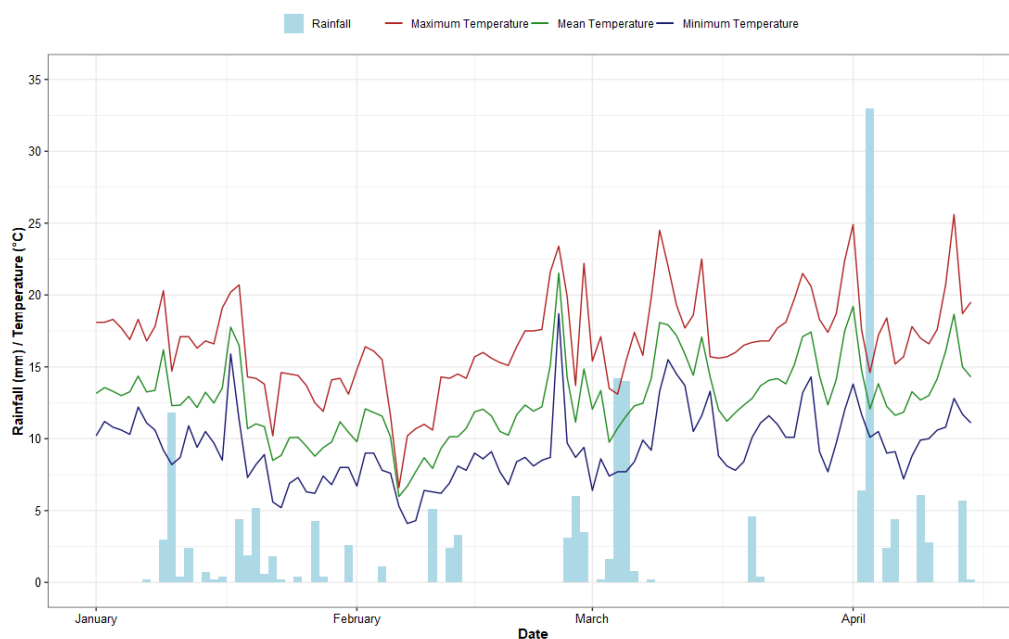

Figure 2.35. Maximum, mean and minimum daily temperatures (lines) and daily rainfall (columns) observed in the studied farm during the course of the winter.

During the observation period, the prevalent winds came mainly from the south-southeast and reached maximum speeds of 4.4 m/s (**Figure 2.36**). The strongest wind gusts came just from the southern sector. The weather station did not record winds coming from the North (0/360°).
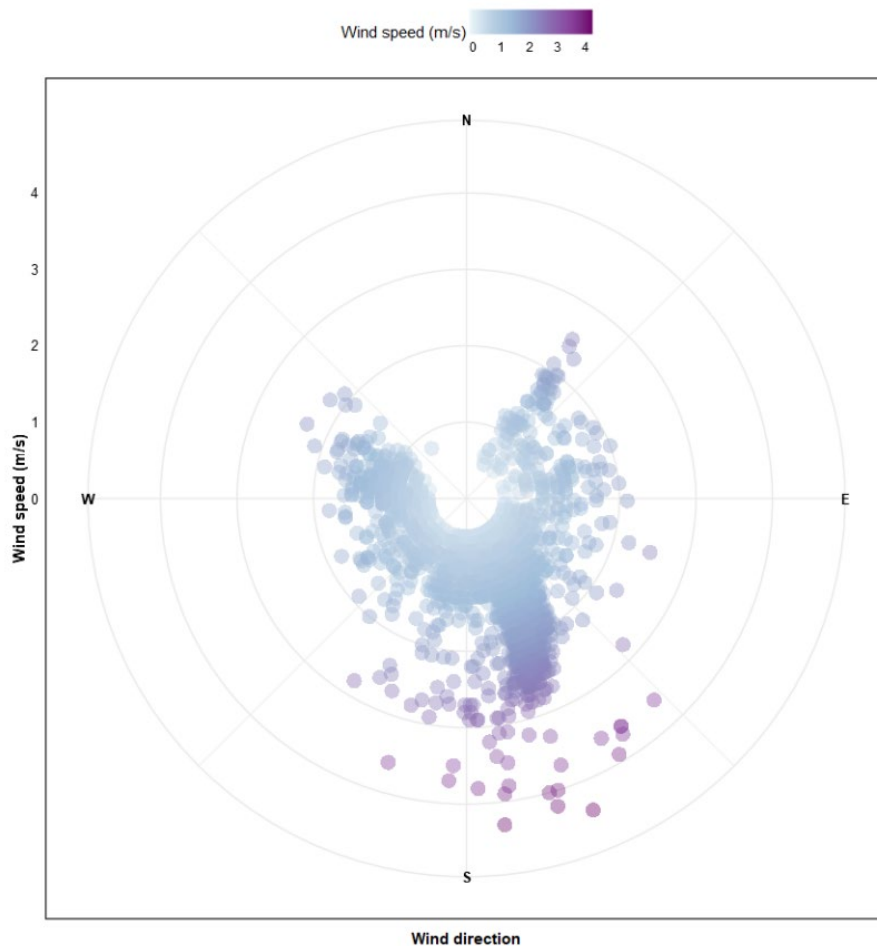
Figure 2.36. Prevailing direction and wind speed (m/s) recorded during the period under study by the weather station located at the centre of the farm.

The incidence and severity of damage from Bacterial Apical Necrosis on the various plots on the farm are shown in **Figure 2.37**. The incidence was high in all plots, with at least 60% of the plants affected by the bacterium. However, the value of damage severity varied greatly between plots in both varieties under study. In fact, this value ranged from 20% in plots D and M, up to 85-90% in plots A and B. Plots C and E showed average damage severity values around 60%, but with wide variability within the same plot, for plants of the Maya and Keitt varieties, respectively.
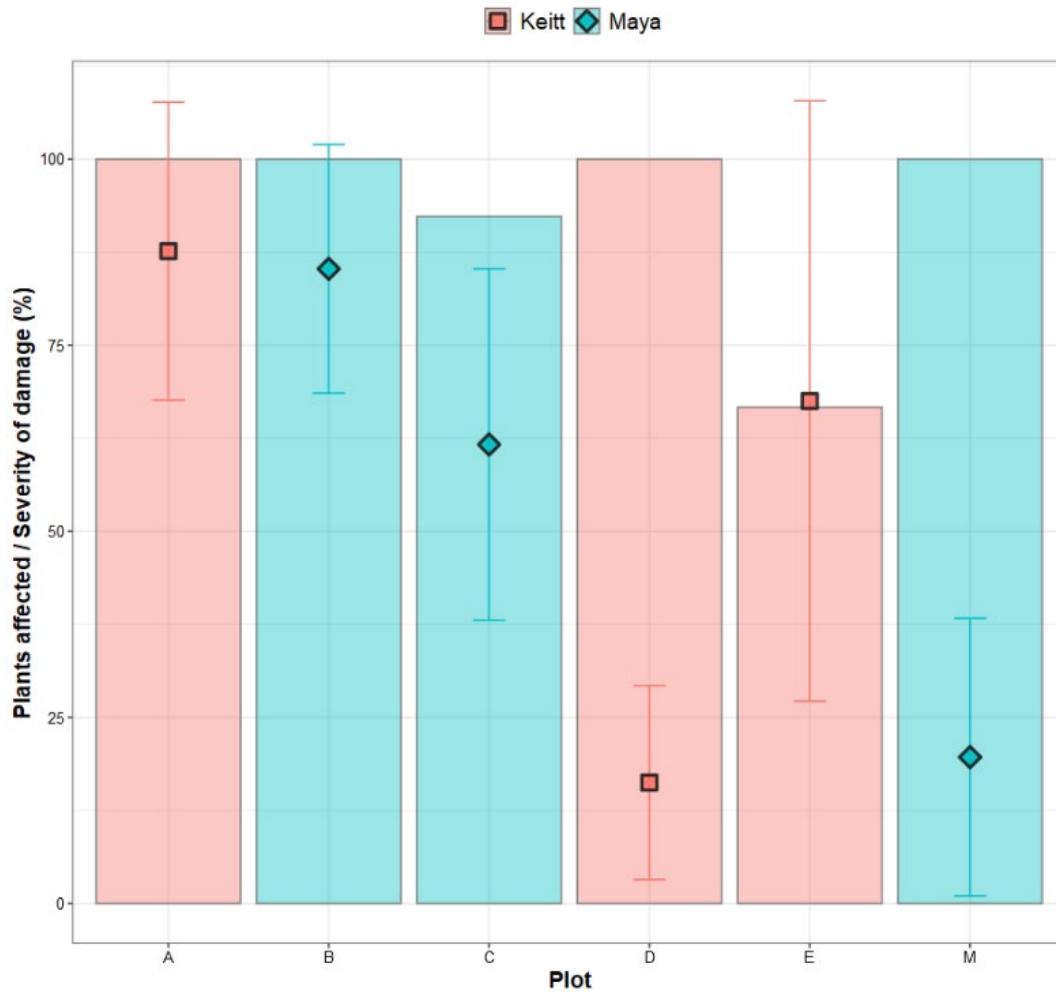
Figure 2.37. Percentage of incidence (columns) and mean severity (± std. dev.) (shapes + error bars) of damage on the plant in the different plots on the farm, for each variety.

The differences found in the distribution of the disease within the farm under study can be ascribed to the different heights and densities of the windbreaks, natural or artificial, delimiting the plots, since they are located within a radius of less than one kilometre and can be considered to be subjected to the same temperatures. In fact, while plots A and B, those where the greatest damage from Bacterial Apical Necrosis was recorded, are placed on the same level as the farm access road and are bounded only externally by windbreaks, plots D and M, where the least severity of damage was observed, are respectively sectioned off by windbreaks even within the same plot – so each plant is at most a few meters from the nearest windbreak – or 5 placed within a natural depression, with the wind flowing above the canopy of the plants, thus reducing the spread of pathogen inoculum. Plots C and E are in intermediate situations, and in

fact intermediate values of damage incidence and severity were observed in them. It can also be inferred from the results obtained from this study that Pseudomonas syringae pv. syringae, in the presence of physical injury on plants, can cause significant damage to mango plants even when temperatures are not particularly cold (> 5°C), as was the case during the observation period.

A further consideration can be made by looking precisely at the relationship between incidence and severity of the disease: regardless of the degree of the latter, the number of affected plants within the individual plot was still high. This indicates that once the pathogen is present within the field, it spreads very easily among the plants within it. However, proper plant protection techniques can make sure that the severity of damage on each plant can be limited, thus ensuring good production.

2.4.2.2 *High temperatures and fruit sunburn*

A problem that has emerged as mango cultivation has moved to latitudes farther from the equator is that of fruit sunburn (Hamdy *et al.*, 2022). In the temperate climate of the Mediterranean, summers are hot and dry and light intensities are high. This causes mango fruits to be exposed to particularly stressed environmental conditions during their growth, particularly a light/temperature combination that causes their peel pigments to degrade and result in sunburn (Abd-Allah *et al.*, 2013). Fruits affected by this condition result at the end of their development with black spots, almost always at the upper shoulder, and suffer severe depreciation until they are completely rejected by the market.

The data obtained revealed that temperatures during the fruit development period remained high, with daily lows above 20°C throughout the period and highs above 40°C. Significant rainfall events occurred only from August 13, when more than 45 mm of rain reached the ground (data not shown). Solar radiation incident on the fruits (**Figure 2.38**)

reached very high values, close to 1000 W/m^2/h, particularly in July, and then dropped to maximum values around 750 W/m^2/h in August.
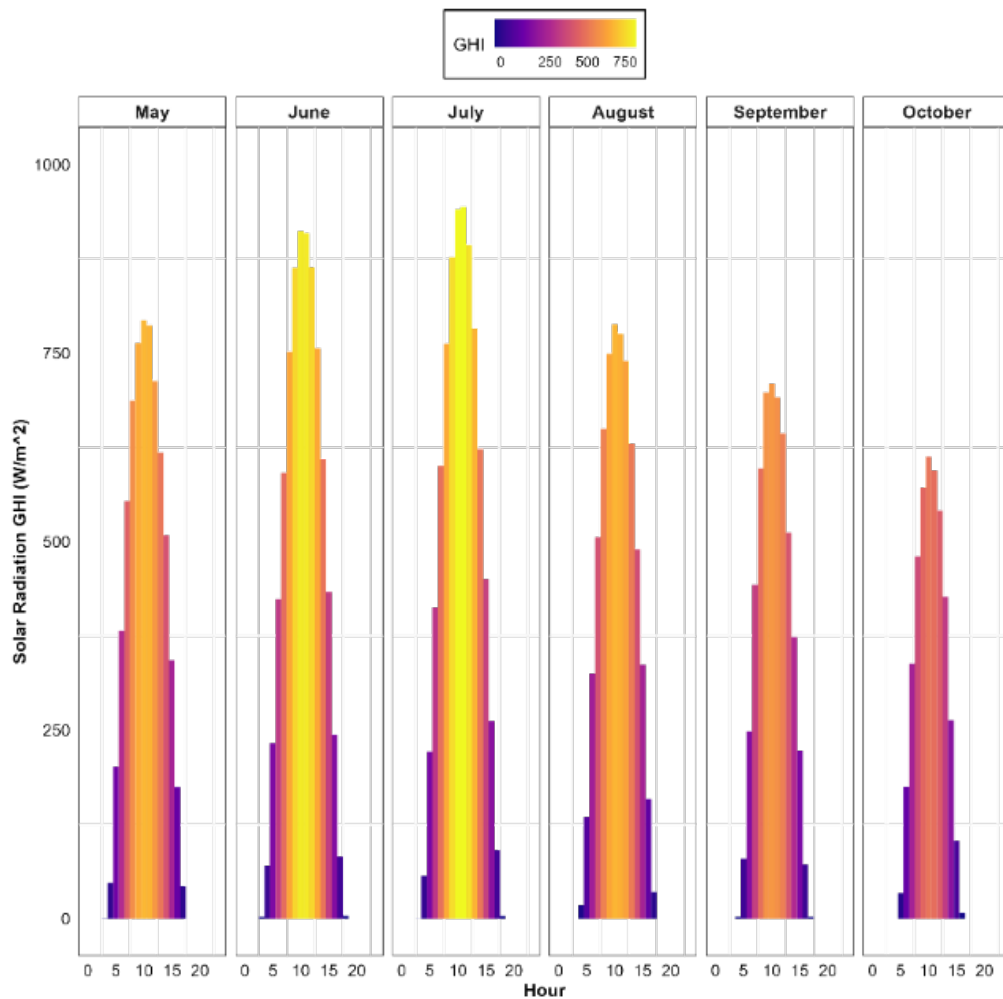
Figure 2.38. Evolution of average solar radiation (GHI, Global Horizontal Irradiance) at different times of the day during the development period of the fruits of the study.

The evolution of scald on mango fruits is shown in **Figure 2.39**. Fruits treated with kaolin powder or dissolved in water resisted blanching better than control fruits, despite being exposed to the same environmental conditions. In total, at harvest, 6 fruits out of 24 in the DustKAO treatment had a blanching degree of 1 and only 1 fruit had a blanching degree of 2, while the remaining did not show any blanching symptoms; 3 fruits out of 24 in the LiquidKAO thesis had a blanching degree of 1, 3 had a blanching degree of 2, and 1 fruit had a blanching degree of 3, for a total of 7 fruits affected by blanching in both treatments; in the CTR control

thesis, at harvest 7 fruits had a blanching degree of 1, 5 fruits had a blanching degree of 2 and

2 fruits had a blanching degree of 4, for a total of 14 fruits out of 24 affected by the pathology.
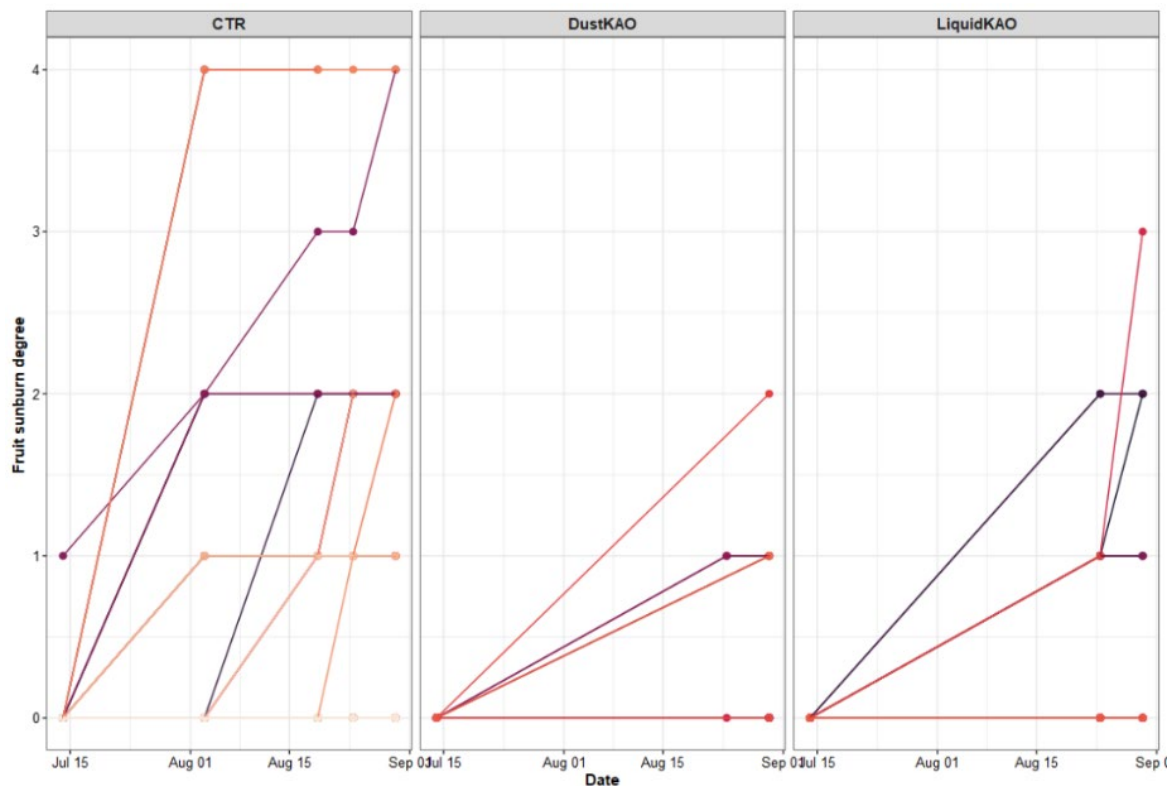
Figure 2.39. Degree of sunburn of mango fruits cv. Glenn affected by the disease during the observation period. Each line represents a single fruit and each box relates to a single treatment.

From the results obtained, it can be said that kaolin treatments have good efficacy in

limiting the problem of sunburn in mango fruits, even in an early and sensitive variety such as

Glenn, which precisely because of its speed of growth has a particularly thin skin and is

susceptible to damage caused by the high solar radiation occurring in the Mediterranean

environment.

2.4.3   Conclusions

Simple physical means like the instalment of windbreaks (be they artificial or natural)

or the use of Kaolin sprays can be effective in contrasting the two most important disorders on

mango trees and fruits in the Mediterranean climate. It is important to monitor consistently the

environmental conditions in each orchard, to be able to forecast the emergence of the disorder

and respond with the right timing, which will allow to prevent significant economic losses.

2.5   Do protected horticulture systems affect mango physiology and quality?

Systems of permanent protection of the orchards which have a more hybrid nature such as shading nets or partial plastic covers could be the right, efficient solution to meet the mango growers' needs. They can serve a double purpose, partially protecting the plants from cold temperatures and mechanical damage during winter (Chernoivanov *et al.*, 2022; Li *et al.*, 2019) and reducing solar irradiation of fruits during summer to prevent sunburn (Dayioglu and Hepaksoy, 2016; Gindaba and Wand, 2005; Manja and Aoun, 2019). Realization of fruit plantations within permanent greenhouses leads to a risk of extreme high temperatures during the summer and a sharp increase in investment costs.

However, questions that might arise regarding the use protection of tree crops are i) does the protection modify the orchard environment to such an extent that the phenology and photosynthetic activity of the plants are affected? ii) what are the effects of the protection system on the fruit's aesthetic and internal quality?

Several studies on different species have shown that shading increases the photo-synthetic activity of plants to different levels, or, when this does not happen, it does not reduce it in any case (Basile *et al.*, 2008; Jutamanee and Onnom, 2016; Kiprijanovski *et al.*, 2016; Medina *et al.*, 2002; Retamales *et al.*, 2008; Tinyane *et al.*, 2018). Shading nets, on the other hand, have been observed to slow down the colour development on the skin of fruits such as Cripps' Pink, Royal Gala and Fuji apples (do Amarante *et al.*, 2011; Gindaba and Wand, 2005)

In the first study, we observed the evolution of the skin colour of the fruit and the photosynthetic activity of mangoes (cv. "Kensington Pride") growing in two different protection systems and compared them to the ones growing in the open air, from the moment of full flowering until harvest, while assessing the relative environmental conditions. The aim of the second study was to evaluate the physiological and qualitative response of three international varieties of mango growing inside a plastic-roof greenhouse in south of Italy.

2.5.1   Experiment 1 – Physiology and fruit quality of Kensington Pride mango in hybrid protected cultivation systems vs open air

2.5.1.1   Material and Methods

2.5.1.1.1   Plant material and Environmental conditions

Five-years old Mango (*Mangifera indica* L. cv. Kensington Pride) plants of comparable size were monitored in a commercial orchard of the Papamango farm located in Sant'Agata di Militello (ME) (38°4'32''N – 14°39'1''E), starting at the full flowering moment – identified by the phenological stage 615 of the BBCH scale for mango (Hernández-Delgado *et al.*, 2011) - until harvest. The climate of the area is identified as Mediterranean in the Köppen classification (Balagizi and Liotta, 2019; Farina *et al.*, 2020b; Köppen, 1936) with average yearly temperature of 17-18 °C and average yearly rainfall between 800 and 1000 mm (Farina *et al.*, 2017c).

Plants were cultivated in open air and with two different covering systems: partial plastic covering (Cover) and Net covering (Error! Reference source not found.). Plants of the Open Air were used as control group, as they had no cover above them but were repaired by windshields on the sides similarly to the other blocks. Plants of the Cover treatment were below a partial covering placed above the external, productive part of the canopy leaving a small window in the central part of the plant and a larger windows between the rows.

The cover was realized with a Non-Thermal Diffusive (NthD) plastic film (OROPLUS UV+, Plastik Advanced, Bergamo, Italy – thickness 160 μm, visible light transmission 80%, haze 30%), meant to protect the young shoots and fruits from heavy rains damage and excessive solar radiation during the summer (Costa, 2019). Plants of the Net treatment were below and lateral continuous 30% shading white plastic net installed with the purposes of reducing sunburn damage on fruits, achieving an increase in temperatures during winter and reducing wind speed and consequent mechanical damage in the orchard. All plants of the experiment

were subjected to the same cultural routine and underwent the same management with regard to fertilization, irrigation, pruning and pest control treatments.

Temperatures in the plots corresponding to the three treatments were recorded with temperature data-loggers (Elitech RC-51, Elitech Ltd, London, UK) placed at the centre of each plot after being properly calibrated. The difference in the quantity of light available for the trees in each plot was assessed using a luxmeter (PCE LED-20, PCE Instruments, Meschede, Germany) at the same time of the same day, within an interval of ten minutes, under conditions of clear sky.

Figure 2.40. Views of the plots corresponding to the treatments Cover (a), Net (b) and Open Air (c).

2.5.1.1.2 Field measurements

2.5.1.1.2.1 Determination of the date of Full Flowering

Five plants per treatment were labelled. In each plant, three shoots per exposition were labelled and photographed at two-weeks intervals in order to identify the phenological stage according to the BBCH scale for mango (Hernández-Delgado *et al.*, 2011; Rajan *et al.*, 2011). Therefore, a total of 3 shoots*4 expositions*5 plants = 60 shoots per treatment were monitored, to determine the date of Full Flowering. This moment was identified in the date when a phenological stage between 610 and 630 was observed on more than 50% of the labelled shoots that had produced an inflorescence.

2.5.1.1.2.2 Gas exchanges measures

Gas exchanges were measured twice during the monitoring period, using a LI-COR 6400 portable system (LI-COR, Lincoln, NE, USA). Data were recorded on three plants per treatment. Four readings per plant were carried out, of photosynthetic activity A (μmol CO_2 / m^2 s) and stomatal conductance g_s (mmol CO_2 / m^2 s).on four different leaves, each exposed to a different cardinal point (N-S-E-W). Measurements were carried out at 12:00 in both dates. On date 2021-08-02 the conditions were: air temperature 35°C, PAR (Cover 900; Net: 800, Open air 1300 μmol m^{-2} s^{-1}); on date 2021-09-22 the conditions were: air temperature 31°C, PAR (Cover 800; Net: 7500 Open air 1200 μmol m^{-2} s^{-1}).

2.5.1.1.2.3 Fruit skin color evolution

After fruit set, 6 fruits per plant, located all around the canopy, were labelled and 4 different spots – A, B, C, D (**Figure 2.41**) – were marked on the surface of each labelled fruit, on five plants per treatment. By doing so, it was possible to monitor the evolution of color repeating measures on the exact same part of each fruit throughout its development. The color of the fruit skin below each marked spot was recorded using a Konica-Minolta CR-400 Chroma Meter (Konica Minolta Sensing Inc., Tokyo, Japan), starting at the first observation of color change and at two-weeks intervals until harvest. Values of L*, a* and b* read by the Chroma meter were then converted into hueangle (h°) and Chroma (C*) via the equations

$$h° = \begin{cases} \arctan\left(\dfrac{b^*}{a^*}\right) if\ a^* > 0\ and\ b^* > 0 \\ 180 + \arctan\left(\dfrac{b^*}{a^*}\right) if\ a^* < 0 \\ 360 + \arctan\left(\dfrac{b^*}{a^*}\right) if\ a^* > 0\ and\ b^* < 0 \end{cases} \tag{1}$$

and

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{2}$$

respectively (Hunter, 1942; McGuire, 1992; Mclellan *et al.*, 1995).

Figure 2.41. (left) Representation of the spots of the fruit surface on which skin colour was measured. Spot A corresponded to the shoulder of the fruit most exposed to sunlight, spot B was just above the fruit apex, spots C and D were on the two opposite cheeks. (right) One of the fruits observed during the study in the early stages of its growth.

Colour difference (Robertson, 1977) was then assessed between the protection systems and the open air control using equation

$$\Delta E = \sqrt{(L_o - L_t)^2 + (a_o - a_t)^2 + (b_o - b_t)^2} \qquad (3)$$

where x_o represents the parameter measured in the open air and x_t the parameter measured in any of the treatments.

2.5.1.1.3 *Fruit quality analyses*

Fruits were harvested on the same date – 124 days after full flowering – in all the treatments, using external color and Total Soluble Solids Content (TSSC) of a sample of fruits as an harvesting index (Ketsa *et al.*, 1991; Malevski *et al.*, 1977). After harvest, 12 fruits from each treatment were transferred to the post-harvest laboratory of the Department of Agriculture, Food and Forest Sciences of the University of Palermo where they were subjected to the measurements of weight – using a digital two-decimal precision scale (Gibertini, Italy) – and total soluble solids content– using a digital optical refractometer (Atago Co. Ltd., Tokyo, Japan). This was done in order to assess any difference among the degree of ripeness reached by the fruits of the three different treatments.

2.5.1.1.4 Statistical analysis

Statistical analysis was performed using R software (R Core Team, 2013). One-way ANOVA at a significance level of p-value < 0.05 was conducted to assess the existence of differences among the treatments.

2.5.1.2 Results

2.5.1.2.1 Temperatures

Data from the sensors placed in three treatment plots (**Figure 2.42**) showed that the protection systems had no significative impact on the minimum temperature: in facts, over the course of the observation period, weekly minimum temperatures were similar in all three treatments. However, from the onset of summer (21st of June), maximum temperatures in both Cover and Net were considerably higher than in the Open Air. The highest temperature, corresponding to 45.7°C, was recorded in the Net treatment plot on August 1st.
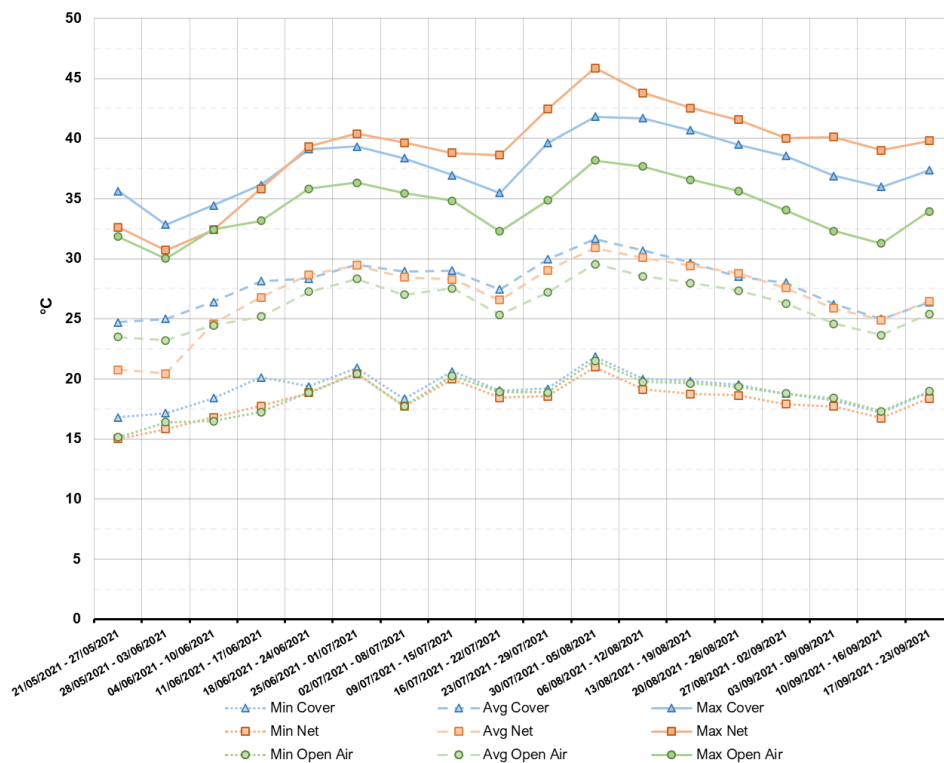
Figure 2.42. Weekly minimum, average and maximum temperature recorded in the three cultivation systems from the moment of Full Flowering until harvest of the fruits.

2.5.1.2.2 Light

Significant differences were found in the quantity of light available for the plants of each treatment (**Figure 2.43**). The plants growing in the open air were receiving 74.7 ± 4.93 kLux, while for the plants in the Cover and Net treatments this value was reduced by the 40% and 20%, respectively.
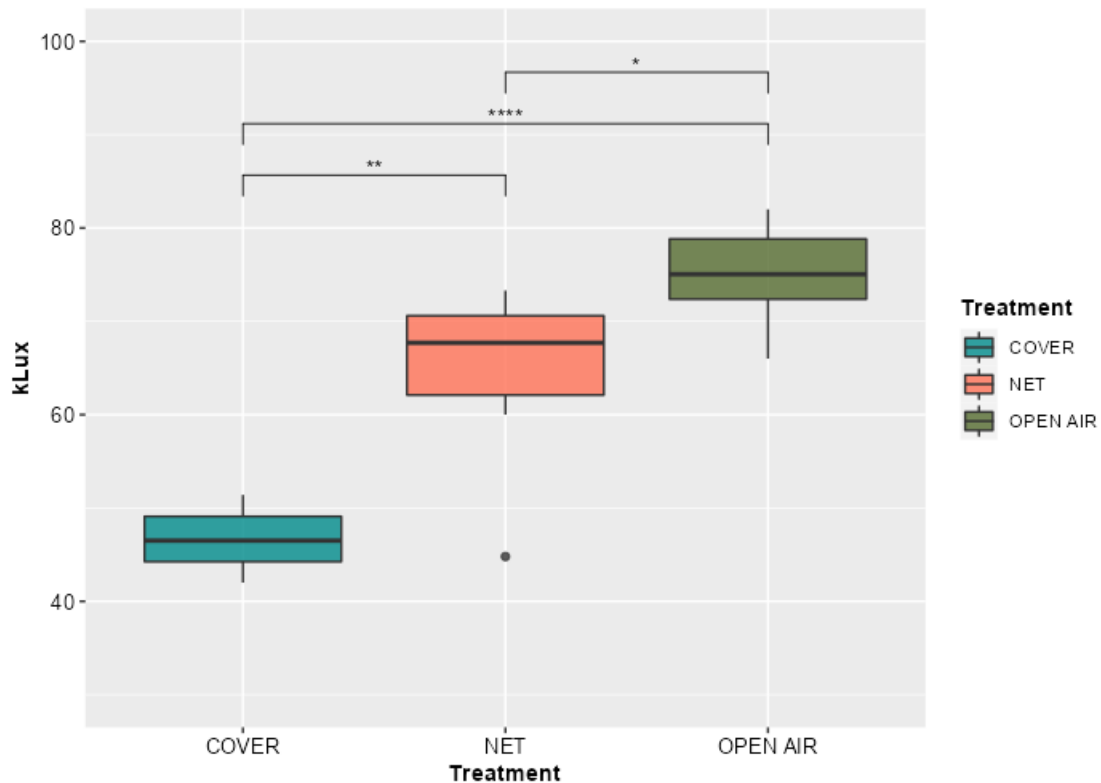
Figure 2.43. Values of kLux measured in the plots corresponding to each treatment. *, **, **** indicate statistically significant differences between any two treatments for Student's t-test at *p*-value < 0.05, < 0.01, < 0.0001 respectively.

2.5.1.2.3 Determination of the date of Full Flowering

Among all observed shoots of the plants of the three treatments that produced an inflorescence, full flowering was reached between 15th and 23rd of May. Therefore, the common date of the 20th of May was chosen as average date of Full Flowering. The protection treatments

did not cause any difference in the moment of reaching of full flowering.

Figure 2.44. Full Flowering (BBCH stage 615) observed in shoots of the Open Air (left), Cover (centre) and Net (right) treatments.

2.5.1.2.4 *Photosynthetic activity of plants*

Figure 2.45 reports the observed values of net photosynthesis of the plants for each treatment in the two measuring occasions.
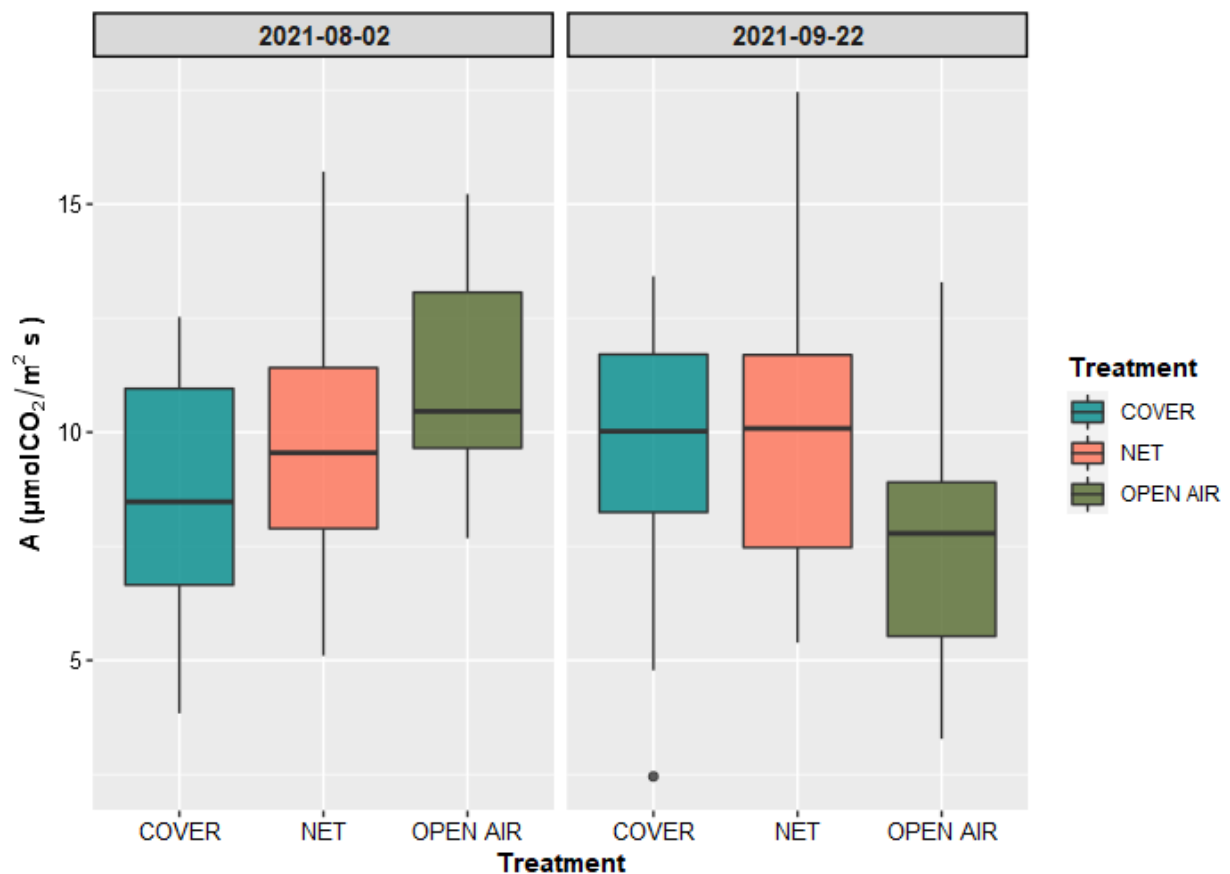
Figure 2.45. Box plots of the values of net photosynthesis A measured on the plants of the three treatments in the two recording occasions.

The values of A were between 5 and 15 µmol CO_2 / m^2 s in all the plants of the three treatments, that is the range normally observed in mango trees (Juntamanee *et al.*, 2013).

Statistical analysis confirmed that there was no significant difference among the treatments in any of the measuring occasions. Plants growing in the open air showed the highest rates of gas exchange in the first measuring occasion, at the beginning of august, while they showed a significant (*p*-value = 0.015) decrease of their A value on the second measuring date, which took place at the harvest moment. Plants growing in the two protection systems Cover and Net, instead, showed no significant difference in the values of A observed in the first and second measurement occasions.
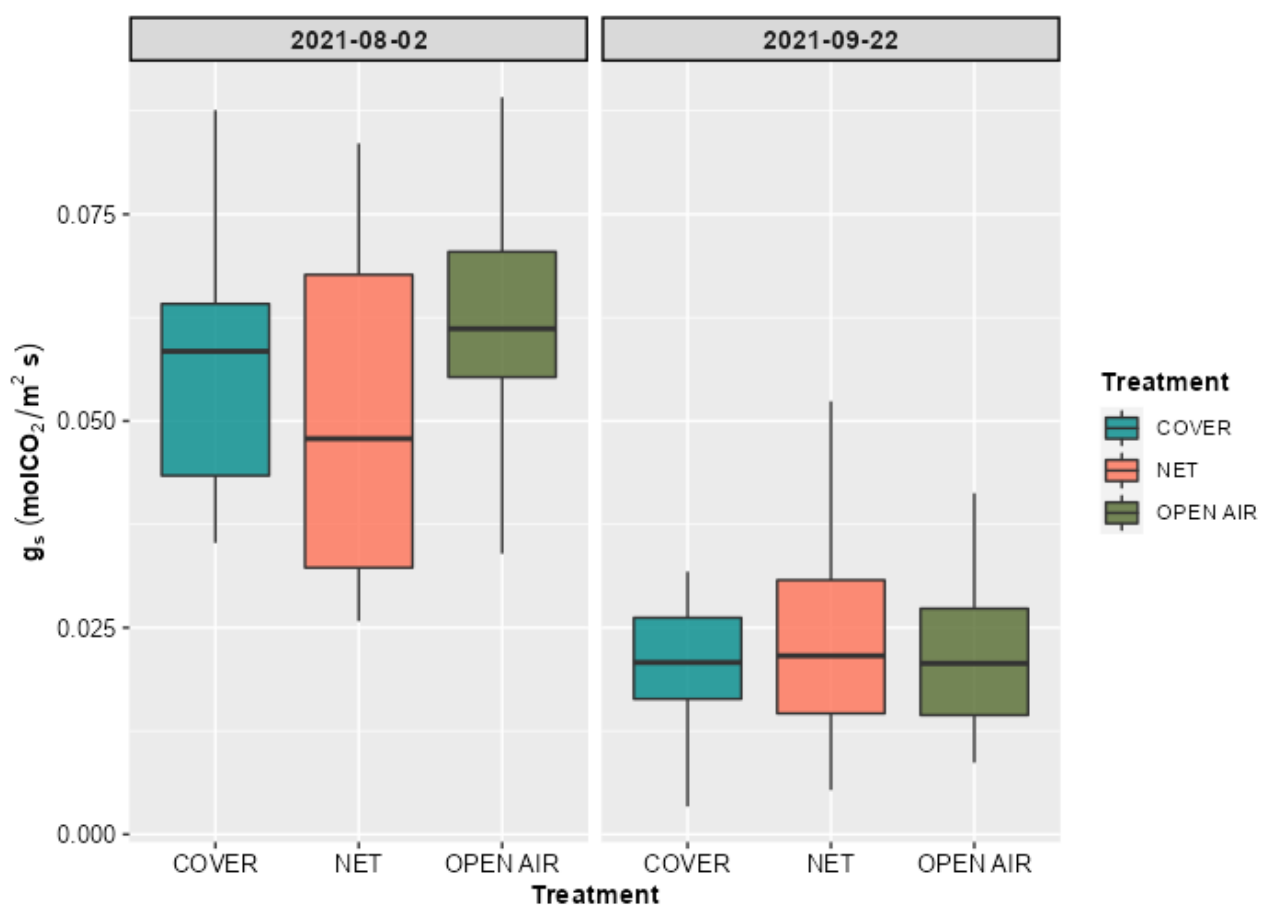
Figure 2.46. Box plots of the values of stomatal conductance g_s measured on the plants of the three treatments in the two recording occasions.

No difference was observed among the treatments with regard to the stomatal conductance g_s (**Figure 2.46**). However, all treatments showed a significant decrease of this value from the first to the second measuring occasion (*p*-value = 4.78×10^{-5}, 6.00×10^{-3}, 3.13×10^{-6} for Cover, Net and Open Air respectively).

2.5.1.2.5   Fruit skin color evolution

Figure 2.47 and **Figure 2.48** report the average colours observed on each spot of the skin of the Kensington Pride fruits from the moment when the first slight change of colour was observed – 73 days after Full Flowering – until harvest, which took place 124 days after Full Flowering.
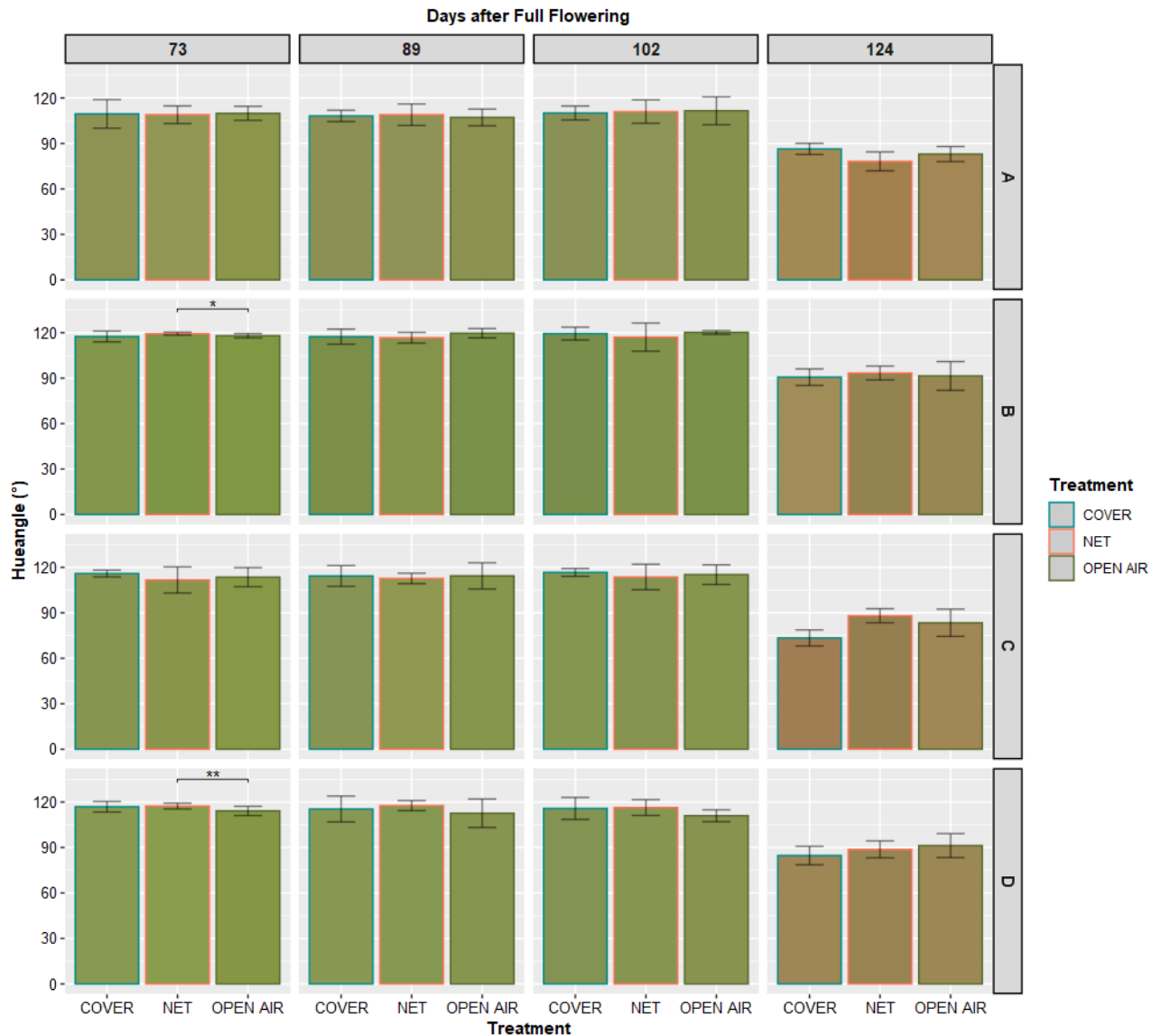
Figure 2.47. Mean values (n = 30) of hueangle (°) measured on the marked spots on the skin of the labelled fruits. The color of each bar corresponds to the average color of each spot (rows) at the given date (columns), transformed into visible RGB color space from the average values of CIE L*, a* and b* measured by the chroma meter, using the R package colorspace (Zeileis *et al.*, 2019). * and ** indicate significant differences between any two treatments for Student's t-test at *p*-value < 0.05 and < 0.01, respectively.
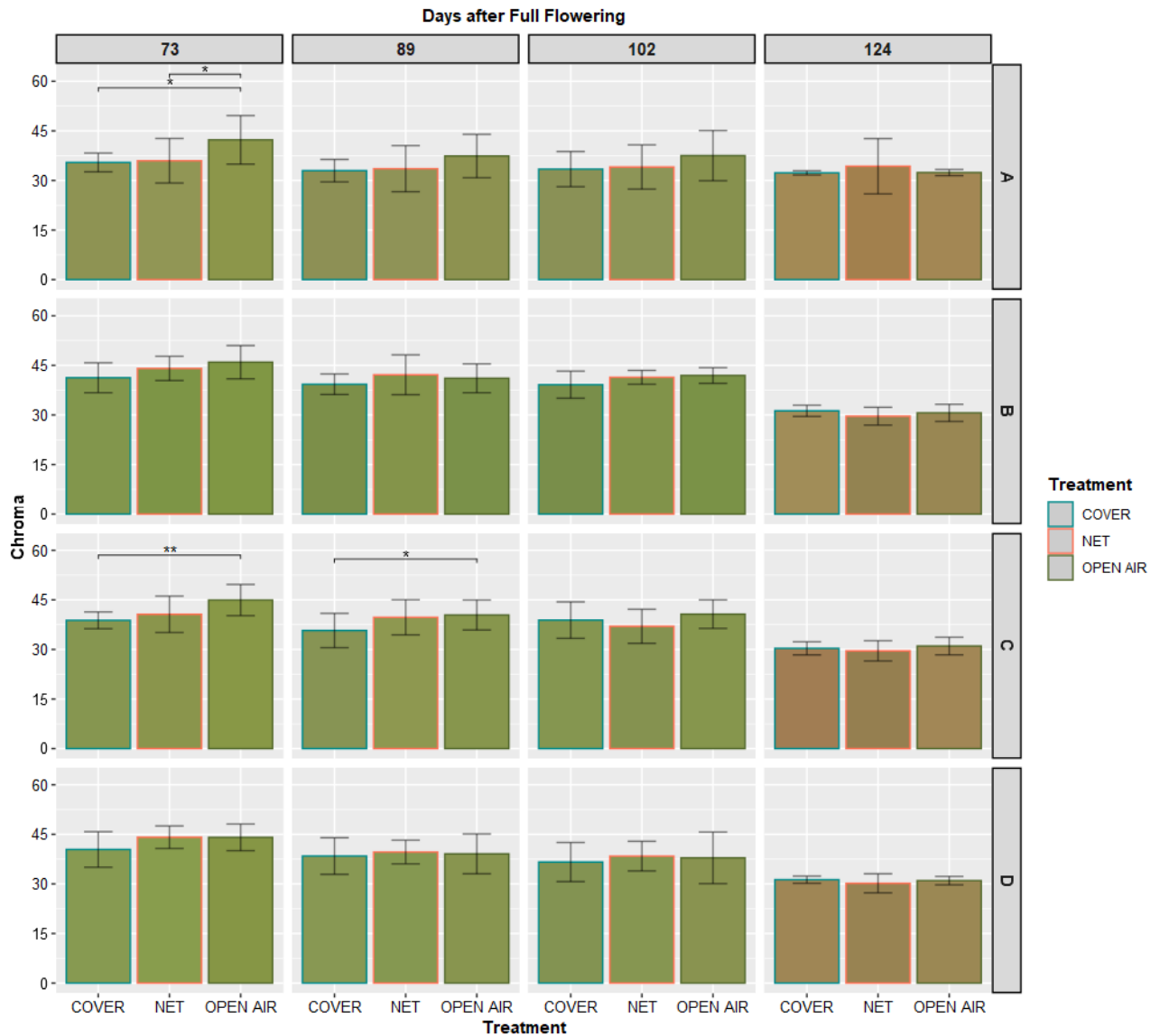
Figure 2.48. Mean values (n = 30) of Chroma (C*) measured on the marked spots on the skin of the labelled fruits. The color of each bar corresponds to the average color of each spot (rows) at the given date (columns), transformed into visible RGB color space from the average values of CIE L*, a* and b* measured by the chroma meter, using the R package colorspace (Zeileis *et al.*, 2019). * and ** indicate significant differences between any two treatments for Student's t-test at *p*-value < 0.05 and < 0.01, respectively.

As the figures show, no significant difference could be observed among the treatments after 90 days from Full Flowering. In facts, it can be stated that the protection treatments had no effect on the final appearance of the fruits.

Moreover, we could observe that the change of colour from green to yellow/golden occurs only in the final stages of the fruit development, 100 or more days after Full Flowering, and statistical analysis (**Table 2.6**) confirms that in variety Kensington Pride, which is one of the mango varieties where skin colour is most uniform over the whole fruit, there is no

significant difference in the values of hueangle (h°) and Chroma measured in any part of the fruit until the moment of harvest.

Table 2.6. F- and *p*- values of the analysis of variance of the effect of the Spot variable on Hueangle (h°) and Chroma parameters at harvest, for each treatment.

Treatment	Effect	d.f.	Hueangle (h°)		Chroma	
			F	***p***	**F**	***p***
COVER	Spot	3	1.46	0.262	1.55	0.241
NET	Spot	3	1.13	0.356	1.52	0.234
OPEN AIR	Spot	3	1.36	0.277	1.02	0.402

Finally, the obtained average values of ΔE color difference (**Table 2.7**) confirm the absence of diversity in the final appearance of the fruits: such values stayed between 1.28 and 5.24 at all measurement occasions.

Table 2.7. Average values of color difference ΔE between the protection systems and the open air grown mango fruits, at each measurement occasion.

ΔE	Days After Full Flowering			
	73	**89**	**102**	**124 (harvest)**
COVER / OPEN AIR	5.24 ± 1.65	2.66 ± 1.81	2.53 ± 1.12	1.70 ± 1.45
NET / OPEN AIR	3.07 ± 0.65	1.28 ± 2.74	1.86 ± 0.75	2.57 ± 3.89

2.5.1.2.6 Fruit quality analyses

No sunburn damage was observed on any fruit grown on the studied plants of any of the treatments.

Fruits of the three treatments showed no significant differences among them for the values of weight (*p*-value = 0.863) and TSSC (*p*-value = 0.145), which show that the Kensington Pride mangoes were harvested at mature-ripe stage, with a weight around 0.5 kilos and a content of sugars between 14 and 16.5 °Brix (Farina *et al.*, 2017a; Leneveu-Jenvrin *et al.*, 2021) (**Figure 2.49**). Even though the differences among the treatments were not found to be statistically significant, it can be observed that fruits in the two protection systems reached slightly higher TSSC values, an index of a greater sweetness of the fruit, which normally leads to a stronger appreciation by the consumer, than the fruits growing in the open air. This is probably a consequence of the higher average temperatures that were achieved during the fruit

development period in both Cover and Net treatments. Factors that enhance fruit transpiration, in facts, are positively correlated with the content of sugars in the pulp of the fruit (Léchaudel *et al.*, 2013; Rosales *et al.*, 2007).
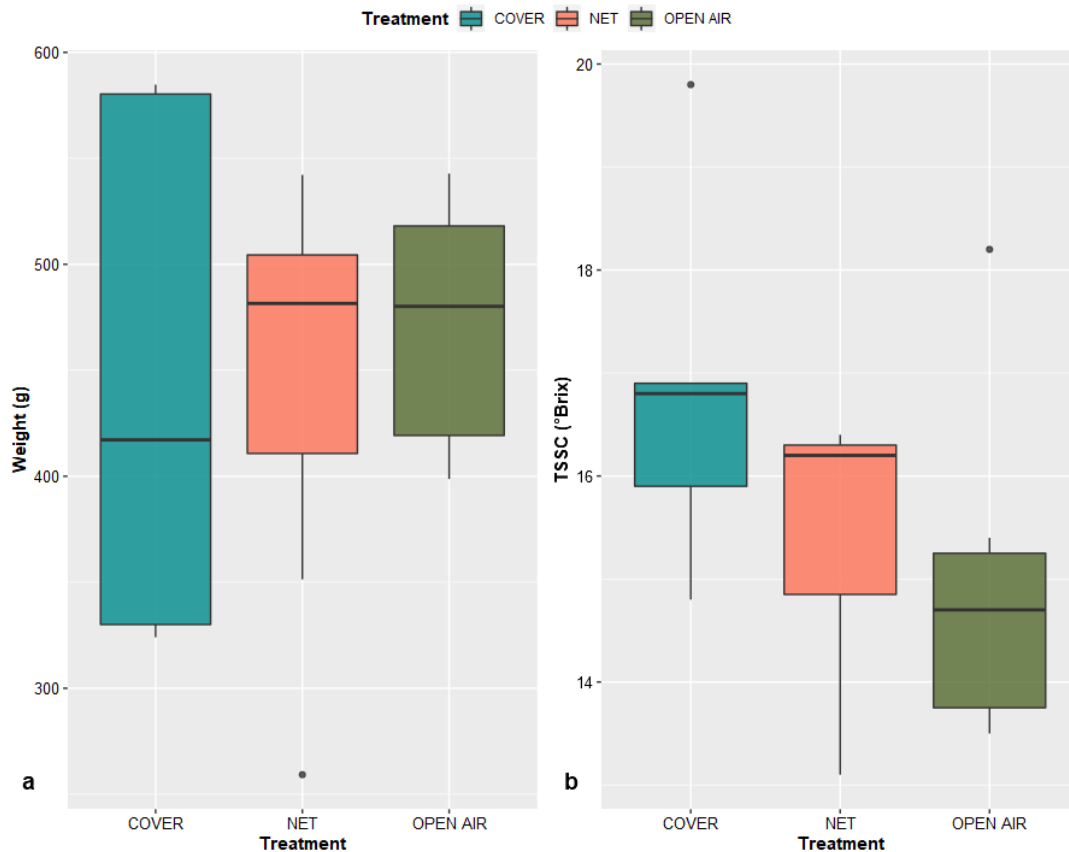
Figure 2.49. Box plots of the values of weight (a) and Total Soluble Solids Content (b) of the fruits of the three different treatments measured at harvest.

2.5.1.3   Discussion

Several studies available in the literature report contrasting effects of the presence of shading nets on the air temperature measured below them: Kurth *et al.*, Alaphilippe *et al.* and Mira-Garcia *et al.* (Alaphilippe *et al.*, 2016; Kührt *et al.*, 2006; Mira-García *et al.*, 2020, p.) all report of lower temperatures recorded below the shade netting covering orchards of different crops, while Gimeno *et al.* and Blakey *et al.* (Blakey *et al.*, 2016; Gimeno *et al.*, 2015) both found higher temperatures below the shade nettings, compared to the open air. Such different results are probably to be ascribed to microclimatic conditions and technical parameters – colour, shading intensity – of the netting material, as well as its installing technique (Mditshwa

et al., 2019; Mupambi *et al.*, 2018). The fact that the highest temperatures were reached inside the Net plot rather than in the Cover treatment, in facts, is probably due to the greater air circulation allowed by the latter, compared to the Net cover system which instead covers entirely the "ceiling" and "walls" of the plot (cfr. **Figure 2.40**). Overall, the partial plastic cover increased temperature no more than 5°C respect to open field, therefore not leading to exposure of plants to extreme high temperatures (Gugliuzza *et al.*, 2022).

It is also interesting to note that the dates of full flowering and harvest were not affected by the protection systems: this is due to their more hybrid nature that allows to protect plants without the great shift in environmental conditions that is seen in traditional greenhouses (Ueda *et al.*, 2000; Yonemoto *et al.*, 2018) and is of great importance to reduce the risk of exposing flowers to late frosts (Zohner *et al.*, 2020) and to allow the grower to easily plan routine cultural practices (Garcia *et al.*, 2021).

Fruit skin colour is an important feature of mango with fruit of many cultivars developing attractive pink to red coloration. It is genetically determined and typical of each variety (Schaffer *et al.*, 2009). Moreover, it is one of the factors that most attracts the consumers towards the fruit (Gianguzzi *et al.*, 2021). The fruits of the present study were uniformly colored in all the plants of the experiment, regardless of the protection treatment.

The protection systems that we studied did not cause any significant observable difference in the photosynthetic activity of the Kensington Pride mango plants, in spite of different temperature and light conditions. We can assume that satisfactory conditions for the photosynthetic machinery of mango were satisfied in all three treatments studied and the process is not enhanced substantially with an increase of temperature or light availability, after a certain threshold is reached (Nobel *et al.*, 1975; Pratap *et al.*, 2003). The difference observed on the measured photosynthetic activity and stomatal conductance values between first and second recording occasions can suggest a positive effect of the temperatures on the

photosynthetic activity of mango plants: in facts, as it can be observed in **Figure 2.42**, maximum temperatures in the Open Air were above 37.5 degrees Celsius in the first measuring occasion, while they had gone down to below 35 degrees in the second date. However, more frequent measurements over a longer period should be taken before considerations of this kind are taken. We will limit ourselves to observe that, in two measurement occasions over the course of fruit development period, photosynthetic activity of the Kensington Pride mango trees was not affected by the presence of a covering consisting of a shading net or a plastic film. This is in line with other studies that compared photosynthetic activity of the plants under shading nets at repeated occasions with different climatic conditions (Medina *et al.*, 2002; Mira-García *et al.*, 2020) and has been observed several times in mango (Burondkar *et al.*, 2012; Neluheni, 2006; Nir *et al.*, 1997).

2.5.1.4   Conclusions

Protected horticulture systems are usually evaluated on the basis of their effectiveness on preventing damage to the crops during the cold season. However, in light of the rapid climate change and extreme climatic conditions that occur around the world with increasing frequency, it is necessary to observe the effect – and possible side effects – of protection systems on fruit species during all the different topical moments of their growing cycle. Therefore, we have focused our attention on several parameters of the reproductive cycle of mango such as flowering moment, fruit colour development and harvesting indices, while assessing the environmental conditions and the physiological state of the tree.

Our results add more information on the subject, expanding the knowledge on the practice of horticultural shading and protection with particular regard to situations of high temperatures, like the ones that have been recorded in the Mediterranean in the last summers.

We could confirm that shading nets and plastic film covers, that are installed over several crops for many purposes, do not have negative side effects on the fruit skin colour or

the phenology and photosynthetic activity of mango during the fruit development period in the hot dry summer of Mediterranean climate. Both the protection techniques used in this study had no significant effect on the observed parameters, if compared to the open air control. No significant difference, either, could be observed between the shading net and the plastic film cover. Obtaining a synchronous reproductive period from flowering to harvest and a homogeneous fruit quality is an encouraging and positive result in the optics of diffusion of such protection techniques: in facts, they did not cause any damage on the final aspect of the fruit, therefore maintaining its value for the marketers and consumers.

More studies will certainly be needed to assess the effect of these protection systems during other critical periods of the mango life cycle in the Mediterranean climate, in order to determine which type of protection – if any is proven necessary – is more appropriate for the species in this area.

2.5.2 Experiment 2 - Fruit quality and photosynthetic performance of three cultivars of mango in a greenhouse in South of Italy

Cultivation of mango in a protected environment allows to reach all phenological stages of the plant's life cycle earlier compared to the open air, to better control pest incidence and to obtain high quality fruits (Akinaga and Hasbullah, 2000; Galán Saúco, 2015; Juntamanee *et al.*, 2013; Lionakis and Loxou, 1996; Ueda *et al.*, 2000).

Few information is present on actual advantages of mango greenhouse cultivation in Mediterranean areas.

2.5.2.1 Material and Methods

2.5.2.1.1 Plant material and cultivation environment

Adult plants of Mango, 8 years in age, of the international cvs Keitt, Osteen and Tommy Atkins, trained to an espalier shape to a 2.5×4 m planting density were cultivated inside a greenhouse located in Sicily Italy (38°06'N-14° 42' E). The climate of the area is Mediterranean according to the Köppen's Classification (Köppen, 1936). The greenhouse was realized with a metal structure, roof was covered with polymethylmethacrylate sheets and side walls in polyethylene film, and it was equipped with an automatic ridge and walls opening system activated by a temperature sensor. All the cultivars were grown according to the standard cultivation practices and irrigated and fertilized with the same protocols. Temperature was measured using sensors (RC-4, Elitech Ltd, London UK) equipped with a data logger, placed inside the greenhouse and just outside, to assess the thermal difference between greenhouse and open air.

2.5.2.1.2 Gas Exchange

Five plants per variety were chosen and marked in the greenhouse for a total of 15 plants. Three well-lit leaves per plant, all exposed to South and spread across the canopy, were monitored using a Li-Cor 6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Gas

exchanges were measured on the 2nd of August at 10 am with a temperature inside the greenhouse of 35 °C. Parameters measured were net photosynthesis, stomatal conductance, transpiration, water use efficiency and vapor pressure deficit.

2.5.2.1.3 Fruit physico-chemical and sensory quality

At the end of the season, fruits were harvested according to the farm's habits, using Total Soluble Solids Content (>11° Brix) of a sample of the fruits (Farina *et al.*, 2020b) as harvesting index. After harvest, fruits were brought to the Post-Harvest laboratory of the University of Palermo, where they were subjected to analyses of the following physico-chemical characteristics: weight, using a digital scale to the nearest 0.01 g (Gibertini, Novate Milanese, Italy); dimensions, using a digital caliper to the nearest 0.01 mm (Turoni, Forlì, Italy); firmness with an 8 mm probe penetrometer (Fresh Produce Instruments, Moerkapelle, Netherlands); total soluble solids content (TSSC; °Brix) using a portable digital refractometer (Atago, Tokyo, Japan); titratable acidity (M.U.) using an automatic titrator (HANNA Instruments, Woonsocket, RI).

Finally, a sensory analysis was performed by a panel of 20 semi-trained judges, 11 women and 9 men, all aged between 20 and 58 years (Farina *et al.*, 2016).

Where relevant, ANOVA was performed to compare group means using R (R Core Team, 2013).

2.5.2.2 Results and Discussion

2.5.2.2.1 Temperature

Figure 2.50 reports the daily minimum and maximum temperatures reached inside and outside the greenhouse. Records of temperature throughout the year inside and outside the greenhouse showed that a consistent heat gain was obtained during the day inside the greenhouse, while minimum temperatures were not affected by the plastic protection. Hence, the anticipation in phenological stages that is regularly achieved in greenhouse-grown mango

trees, is probably made possible by the shorted duration of cold temperature periods, compared to what happens in the open air. In facts, minimum temperatures in the two conditions are equivalent, but these persist below the plastic cover for a shorter period of time during the night (Körner and Challa, 2003; Kukla and Karl, 1993).

The highest temperature reached inside the greenhouse was 50.2°C, on June 29, while outside the greenhouse the maximum recorded value was 39.7°C. On the other hand, the absolute minimum temperature recorded in the greenhouse was 4.4°C, equivalent to the absolute minimum recorded outside the greenhouse of 4.6°C.

Mango is known to perform well on a wide range of temperatures, but it was observed that temperatures above 45°C can undermine the photosynthetic machinery of the plant (Litz, 2009; Normand *et al.*, 2015). However, the trees of our study did not suffer any apparent damage due to the thermal stress and the fruits developed normally.

Figure 2.50. Daily Maximum and Minimum temperatures reached inside and outside the greenhouse during the year of study.

2.5.2.2.2 Photosynthetic activity

Significant differences emerged among the three studied varieties with regard to the photosynthetic activity. Tommy Atkins proved to be the variety with the highest values of photosynthetic activity, conductance and transpiration; while no significant difference was found among the varieties regarding the Water Use Efficiency (WUE) or Vapor Pressure Deficit (VPD) of the leaf (**Table 2.8**).

The photosynthetic activity of both Keitt and Tommy Atkins varieties were noticeably higher than values reported in the literature, which are reported to stand between 4 and 12 μmol m^{-2} s^{-1} (Neluheni, 2006; Santos *et al.*, 2014) in trees growing in the open air. A similar result was observed by Juntamanee *et al.* (Juntamanee *et al.*, 2013) when studying mango trees growing under a plastic roof in tropical climate in Thailand.

Vapor Pressure Deficit of the leaf resulted to be higher than the one observed in the open air in the same territory in other studies (Carella *et al.*, 2021) or in greenhouse cultivated mango in other locations (Juntamanee *et al.*, 2013).

The high values of photosynthesis reached below plastic covers could be attributed to the CO2 enrichment occurring within the greenhouse, which decreases the oxygen inhibition of photosynthesis and increases net photosynthetic activity of the plants (Ioslovich *et al.*, 1995; Jung *et al.*, 2018; Panwar *et al.*, 2011). This effect is increased when VPD of the leaf does not exceed values of 5-6 kPa, which can be detrimental to plant health (Jiao *et al.*, 2019), as was the case in our study.

Table 2.8. Values of photosynthetic activity measured on the plants of the three studied varieties. Asterisks next to the column headers indicate significant difference for * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$. "ns" indicates no significant difference. Letters next to the values within a column indicate significant difference among the varieties for Tukey's HSD test.

Variety	Photosynthesis[***] (μmol(CO_2) m^{-2} s^{-1})		Conductance[**] [mmol(H_2O) m^{-2} s^{-1}]		Transpiration** [mmol(H_2O) m^{-2} s^{-1}]		Water Use Efficiency[ns] [μmol(CO_2) mol(H_2O)$^{-1}$]		Vapor Pressure Deficit[ns] of leaf (kPa)	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
KEITT	16.50[b]	2.34	68.38[b]	18.13	3.42[b]	0.88	5.05	1.13	4.82	0.05
OSTEEN	15.03[b]	2.59	72.28[b]	10.66	3.56[b]	0.51	4.24	0.50	4.92	0.04
TOMMY ATKINS	20.93[a]	1.76	88.66[a]	4.72	4.38[a]	0.22	4.78	0.44	4.84	0.04

2.5.2.2.3 Physico-chemical characteristics of the fruit

The biggest fruit in terms of weight, length, width and thickness were harvested on the cv Keitt (**Table 2.9**).

Although Osteen was the variety whose fruits had the highest content in Total Soluble Solids (°Brix), reaching a value of 17.68, Tommy Atkins proved to be the variety whose fruits reach the highest sugar/acid ratio (**Table 2.10**), one of the most important factors in consumer appreciation of the mango fruits (Farina *et al.*, 2020b; Gianguzzi *et al.*, 2021).

Fruits harvested in the greenhouse reached similar or higher values of TSSC than those grown the same territory in Sicily in the open air (Gentile *et al.*, 2019a).

Table 2.9. Physical attributes of the fruits harvested from the greenhouse. Results are shown as mean and s.d. of the evaluated sample (n=20).

Variety	Days to ripen		Weigth (g)		Length (mm)		Width (mm)		Thickness (mm)		Firmness (N)	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
KEITT	12.42	3.26	520.05	111.41	122.61	6.71	84.29	5.75	93.30	7.74	7.71	2.80
OSTEEN	8.50	2.81	363.49	118.47	114.38	15.26	71.29	10.12	79.64	8.91	9.89	4.35
TOMMY ATKINS	8.50	2.50	453.58	102.75	115.65	11.33	81.54	4.61	86.16	6.03	11.17	3.37

Table 2.10. Chemical attributes of the fruits harvested from the greenhouse. Results are shown as mean and standard deviation of the evaluated sample (n=20).

Variety	Juice content (g/100g)		TSSC (°Brix)		Titratable acidity (mg citric acid/g)		Sugar/Acid ratio	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
KEITT	29.25	1.09	16.25	1.94	0.33	0.02	0.54	0.01
OSTEEN	33.76	1.30	17.68	2.47	0.30	0.13	0.67	0.33
TOMMY ATKINS	30.69	0.75	16.79	1.23	0.14	0.02	1.22	0.17

2.5.2.2.4 Sensory analysis

All varieties were found to be visually attractive, with high scores reached by positive descriptors such as Exotic Fruit Odor, Sweet, Juiciness, Peach and Exotic Fruit Flavor. Descriptors such as Medicine, Sea and Off-odor, Medicine and Off-flavor were never highlighted by the panel members. Among varieties, Osteen was the one which was evaluated as the sweetest. No statistically significant differences were observed in the scores attributed by the panel members to the considered descriptors, among the three varieties (**Figure 2.51**).

Overall, the Sensory Analysis showed that the fruit grown in the greenhouse were appreciated by the judges and they presented themselves with an attractive appearance.
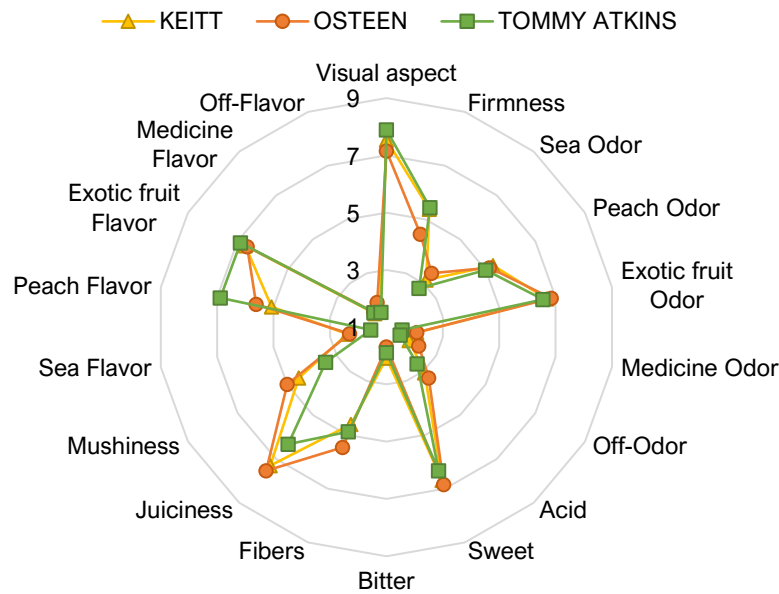
Figure 2.51. Sensory Analysis performed by the panel of semi-trained judges. Values represent the mean of the scores assigned by the judges (n = 20).

2.5.2.3   Conclusions

The cultivation of mango inside a greenhouse in Sicily subjected the plants to extremely high temperatures during the day, up to 50°C, while minimum temperatures reached during the night throughout the year do not differ from the ones recorded in the open air.

Photosynthetic activity of the plants, recorded during one summer day, showed high values of A, until 20µmol (CO_2)/s, which is one of the highest values ever recorded for mango, based on what is reported in the literature.

The obtained fruits were of high quality, showing no imperfections or traces of diseases and disorders. They had a high TSSC content and were fully appreciated by the judges which performed a sensory analysis.

These results suggest that greenhouse cultivation of mango in Sicily and, more generally, in the Mediterranean, allows to obtain quality productions with a good health status of the plant, in spite of the environmental factors such as the extreme high temperatures which can represent a source of stress for the plant.

2.6   *How does the mango fruit grow in a non-subtropical climate?*

As we have previously observed, the various phases of the mango phenological cycle in the Mediterranean climate are located in precise periods of the year, with the vegetative rest coinciding with the long and wet winter, flowering and, to a lesser extent, shoot development happening in the spring, and fruit set and development extending from the onset of summer until the next autumn. This means that, unlike in tropical and subtropical climates, the mango fruit develops in a dry climate, requiring that the water needs of the plant are met with irrigation (Rodríguez Pleguezuelo *et al.*, 2018).

Moreover, in the Mediterranean basin, mango is often cultivated under different growing conditions: besides open field cultivation, orchards can be found in greenhouses (Akinaga and Hasbullah, 2000; Galán Saúco, 2015; Gugliuzza *et al.*, 2022) or under shade netting (Jutamanee and Onnom, 2016; Lionakis and Loxou, 1996; Medany *et al.*, 2009; Scuderi *et al.*, 2022). Highly significant differences have been observed in the microclimatic conditions that occur in each of these cultivation systems, with the strongest discrepancies observed during the summer rather than in winter (Scuderi *et al.*, 2023). The question arises, then, about which system, if any, offers the best conditions for the mango fruit growth in the hot and dry Mediterranean summers, and what index can be used to assess the suitability of a location for this process.

Thermal time accumulation is considered an index for the development of a biological process, accordingly, the same value of such index should be needed to complete the process, regardless of differences in environmental conditions (Aguilera *et al.*, 2014; Hueso *et al.*, 2007; Normand and Léchaudel, 2004; Orlandi *et al.*, 2010). Growing Degree Days (GDD) are the most used unit for expressing the thermal time needed for the completion of a biological process in a way that is unequivocal and independent from growth environment, such as geographical latitude or open field vs greenhouse. To achieve a reliable estimation of the necessary value of

heat accumulation for the completion of a biological process, it is necessary to observe the behaviour of plants in a wide range of contrasting temperature conditions (Arnold, 1959; Bonhomme, 2000; Normand and Léchaudel, 2004). In facts, criticism has been moved to the GDD method (McMaster and Wilhelm, 1997; Yin *et al.*, 1995) since it ultimately proves effective only in small geographic ranges or for locally adapted varieties (Hodges, 1990).

This is due to the fact that the equation (3) widely used for GDD calculation:

$$GDD = \frac{T_{max} + T_{min}}{2} - T_{base} \times \delta \quad with \quad \delta = \begin{cases} 0 \; if \; T_{min} \leq T_{base} \\ 1 \; if \; T_{min} > T_{base} \end{cases} \quad (3)$$

where T_{max} and T_{min} are the maximum and minimum temperatures recorded in a day, respectively, and T_{base} a temperature value below which plant activity is considered to be null, relies on the model proposed by Arnold (Arnold, 1960), which does not consider the existence of an upper temperature threshold limiting biological activity (Baskerville and Emin, 1969). Other equations consider GDD accumulation as constant when the daily maximum temperature is above a certain threshold (Grigorieva *et al.*, 2010).

More methods to assess the effect of temperature on the rate of plant development have been proposed over the years. Growing Degree Hours (GDH) were introduced for the first time by Richardson *et al.* (1981). This index is based on the consideration that for each environmental factor there is an optimal set of values at which the development of the organisms occurs at the maximum rate. This set of values can be identified for each factor, *ceteris paribus*, by three critical limits: a lower one below which development is null; an optimum one where the maximum rate of development is observed; and an upper limit beyond which damage to the organism occurs or development is arrested. For GDH, this set of values is represented by the base (Tb), optimal (To) and critical (Tc) temperatures.

Over the course of the years, the GDH model has been improved and simplified (Anderson *et al.*, 1985; Richardson *et al.*, 1982, 1986), and a Beta equation (Abramowitz and Stegun, 1965; Mao *et al.*, 2018; Motisi *et al.*, 1994; Yin *et al.*, 1995) has been adopted: this has

since been validated on temperate and tropical crops such as peach, cherry or loquat (Imperiale *et al.*, 2022; Marra *et al.*, 2002; Scalisi *et al.*, 2018).

The aim of our study was therefore: i) to develop a model that could accurately predict mango fruit growth based on the temperatures experienced during the growing season, regardless of area of cultivation or growing condition; ii) to estimate the most suitable cardinal temperatures for the species (base, optimum and critical); iii) to compare the prediction capacity of the obtained model with others based on different time and thermal indices; iiii) to obtain reliable values of heat requirements for the fruit growth of different mango varieties.

2.6.1 Material and Methods

2.6.1.1 Experimental sites and plant material

The growth of mango fruits of the varieties Keitt (late ripening), Osteen and Tommy Atkins (average ripening) were followed through three different growing seasons in the years 2018, 2019 and 2021, in three different experimental orchards:

i) Cueva del Polvo, located in the island of Tenerife, Canary Island, Spain (28°13'42''N 16°50'01''W). Sixteen years old mango plants were grown in the open field and trained to a globe shape. Three plants per variety were selected and studied.

ii) Furiano, located in the island of Sicily, Italy (38°03'19''N 14°33'02''E). Twelve years old mango plants were grown in the open field and trained to a globe shape. Three plants per variety were selected and studied.

iii) Rocca di Capri Leone, located in Sicily, Italy (38°06'31''N 14°42'13''E). Four years old mango plants were grown inside a plastic greenhouse and trained to an espalier shape. Three plants per variety were selected and studied.

The climate in the island of Tenerife is classified as "tropical dry" or "tropical warm" according to Köppen or Papadakis classifications, respectively (Gianguzzi *et al.*, 2021; Köppen, 1936; Papadakis, 1966), while the climate in the island of Sicily is classified as Mediterranean in both classifications (Carella *et al.*, 2021; Gianguzzi *et al.*, 2016; M. C. Peel *et al.*, 2007).

2.6.1.2 Data collection

In each station, phenology observations of the selected plants were carried out at two-weeks intervals, from the beginning of spring of each year, to identify the moment of full flowering. Data from the station Cueva del Polvo were collected only in 2018, while data from stations Furiano and Rocca di Capri Leone were collected in 2019 and 2021. Therefore, 5 different growing periods were observed. The moment of Full Flowering for each of the observed plants was determined when 50% + 1 of a selected sample (n = 36) of terminal shoots on the canopy of the tree was found to be at stage 615 of the BBCH scale for mango (Hernández-Delgado *et al.*, 2011; Rajan *et al.*, 2011). After fruit set and the physiological fruit drop of mango fruitlets took place, 12 fruits per plant, located all around the tree canopy, were labelled and their length, corresponding to the longitudinal diameter, was measured at two-week intervals with a digital calliper with a resolution of 0.01 mm. Overall, 2048 fruit length data were collected and analysed.

2.6.1.3 Temperature data and Thermal time calculation

Temperatures in each station were recorded during each growing season with data-logger equipped temperature sensors, placed away from direct sunlight and protected from weather events (PCE-HT 71N, PCE instruments, Meschede, Germany). Temperature sensors were removed from the fields to download the data not before October 31st, after the last fruit of the studied varieties was harvested.

Growing Degree Days from Full Flowering until the harvest were calculated using equation 3 with a T_{base} of 10°C, on the basis of what was reported in the literature by numerous

authors (Barros *et al.*, 2010; Lucena, 2006; Mosqueda-Vázquez *et al.*, 1992; Rai *et al.*, 2003; Souza *et al.*, 2015)

Hourly temperatures and phenological data were used as input to run a Visual Basic program designed for the computation of Growing Degree Hours (GDH) using a Beta function based on equation 4 (Marra *et al.*, 2002):

$$Y = a + b \times \left(\frac{x - c + dm}{d}\right)^{e-1} \times \frac{\left(1 - \frac{x - c + dm}{d}\right)^{f-1}}{m^{e-1} \times n^{f-1}} \tag{4}$$

The program was set to randomly change the triplet of cardinal temperatures used to construct a Beta curve. A hundred cardinal temperature triplets, hence Beta models, were compared to determine the GDH accumulation between predicted and observed dates (Marra et al, 2017) to reach 90% of fruit final length for each variety pooling all years, locations, and treatments. The accuracy of the model was evaluated based on the root mean square error (RMSE). For equal or similar RMSE values, the best model was selected on the basis of the biological validity of the cardinal temperatures triplet (Gandar *et al.*, 1995; Paine *et al.*, 2012).

Ultimately, fruit growth was characterised simply by using the days after full flowering (DAFF) as a time variable.

2.6.1.4 *Fruit growth modelling and parameterization*

Fruit length data (a total of 2048 observations) were normalised (%) and plotted against GDHs from Full Flowering. They were later tested for their fit to a Logistic or Gompertz model via R function nls (R Core Team, 2013), using the equations

$$y = \frac{a}{1 + e^{\frac{b-x}{c}}} \tag{5}$$

and

$$y = ae^{\left[-e^{(b-cx)}\right]} \tag{6}$$

respectively, where *y* is the normalized fruit length, *x* is the thermal time expressed in GDHs from Full Flowering, *a* represents the asymptote of the function, *b* represents the value of *x* at which the function reaches 50% of its asymptotic value (Mid-point) and *c* is a parameter defining the shape of the curve (Slope, dimensionless) (Archontoulis and Miguez, 2015; Marra *et al.*, 2009).

The chosen equation was used to model the evolution of fruit length in relation to three indices: Growing Degree Hours (GDH), Days After Full Flowering (DAFF) and Growing Degree Days (GDD). The three models were applied to each studied variety and compared on the basis of their Bayesian Information Criterion (Schwarz, 1978). Data belonging to the different observation years, locations, and treatments were pooled for each variety, and the parameters for each equation with the best correlation between seasonal indexes and fruit length were obtained. Double-check using R packages sicegar (Caglar *et al.*, 2018) and parameters (Lüdecke *et al.*, 2020) was also performed.

2.6.1.5   Heat requirements determination

After choosing the best fitting model and relative parameters for each index, heat requirements for the fruit growth of each studied variety were calculated. Since mango fruits reach their final size (100% of the fruit length) some time before their actual maturity and maintain it for a variable period of time (Davenport, 2009; Singh, 1954), the final growth threshold value was identified as 90% of the fruit's final length. For each variety, the Coefficient of Variability (C.V.) among the time and heat requirements in the different years and locations was calculated and then compared.

2.6.1.6   Statistical analysis

Statistical analysis was conducted using R statistical software (R Core Team, 2013).

2.6.2   Results and Discussion

Full Flowering dates across the varieties, locations, and treatment are reported in **Table 2.11**. Within the sampled shoots, a variability of ± 3 days was observed to estimate the precise date of Full Flowering. Therefore, a common central date was chosen for each variety.

Table 2.11. Dates at which the moment of Full Flowering was reached in the different years, locations and treatments of observation.

| | 2018 | 2019 | | 2021 | |
| | Tenerife | Sicily | | Sicily | |
Variety	Field	Field	Greenhouse	Field	Greenhouse
Keitt	18/03/2018	30/05/2019	17/04/2019	22/05/2021	30/03/2021
Osteen	20/03/2018	30/05/2019	30/04/2019	31/05/2021	13/04/2021
Tommy Atkins	13/03/2018	17/05/2019	17/04/2019	22/05/2021	13/04/2021

Minimum and maximum daily temperatures recorded in the stations are reported in **Figure 2.52** along with the date of Full Flowering for each variety. It can be observed that temperatures in Tenerife (year 2018) have in general a smaller variability over the fruit development period, with maximum daily temperatures that did not exceed 30°C and minimum daily temperatures that never fell below 12°C. In Sicily, on the other hand (years 2019 and 2021), a greater variability is observed in the temperatures that occur over the year and between open field and greenhouse condition. In facts, a much wider thermal amplitude occurs every day, with respect to the subtropical climate, and higher maximum temperatures are reached during summer. In both years of observations in Sicily, a maximum temperature of at least 40°C was reached in the open field. In the greenhouse, the high maximum temperatures were far above 45°C in both years of observation, for several days, and an absolute maximum temperature of 50.2°C was reached in 2021 – on June 28th, precisely. No significant difference could be observed in the daily minimum temperature values recorded in the open field and in the greenhouse.
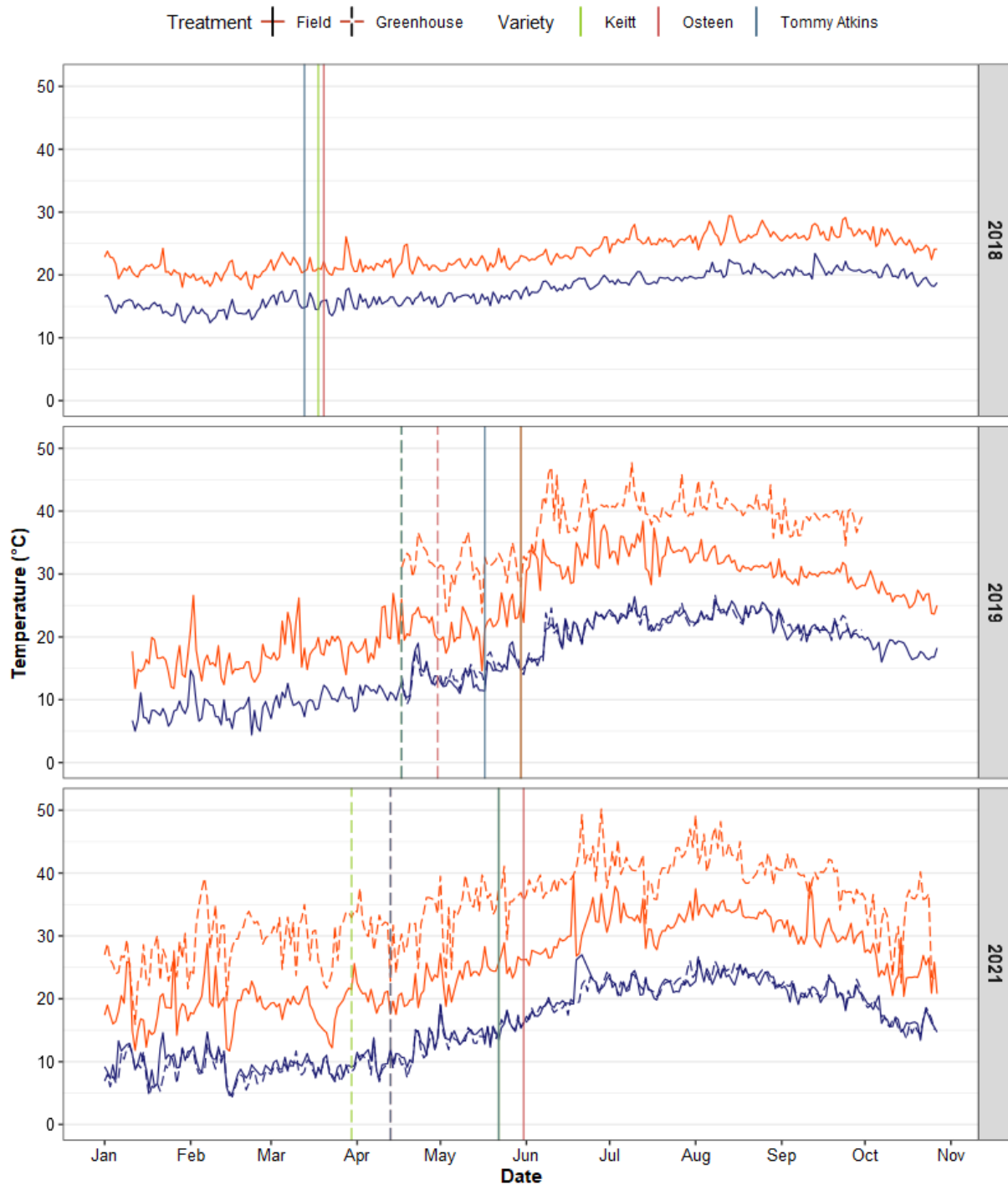
Figure 2.52. Minimum (blue) and maximum (orange) temperatures recorded in the 5 growing seasons in Tenerife (year 2018) and in Sicily (years 2019 and 2021) in the field (solid lines) and in the greenhouse (dashed lines). The vertical lines represent the date when the moment of Full Flowering has been identified for each studied variety.

2.6.2.1 GDH parameterization

A hundred temperature triplets were tested against each variety's growth data by the software and ranked on the basis of the RMSE they produced in estimating the GDHs needed to reach 90% of the final length of the fruit.
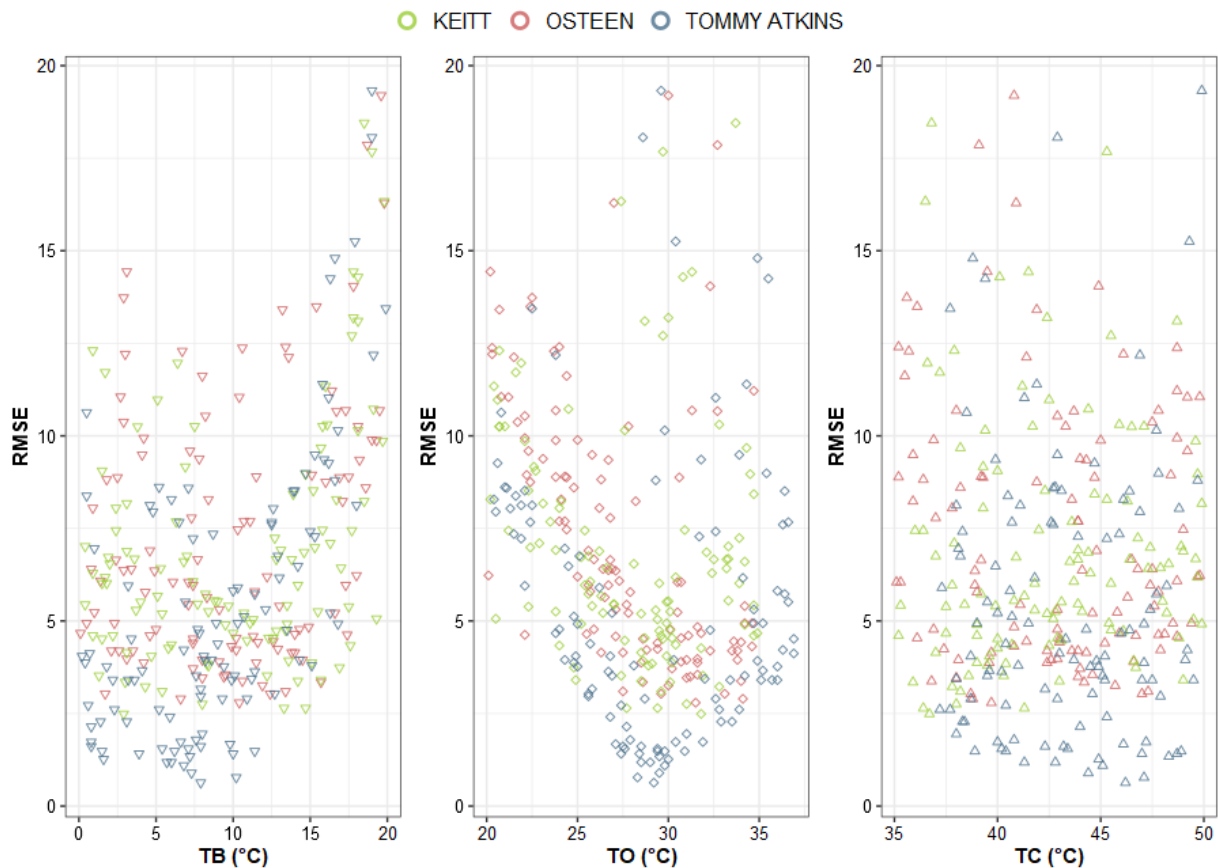
Figure 2.53. RMSE values obtained in relation to the tested cardinal temperatures.

Figure 2.53 shows the effect of each cardinal temperature – base temperature T_b, optimal temperature T_o and critical temperature T_c – on the model's estimated RMSE. While a trend is seen for T_b and T_o, with the lowest RMSE values obtained in the 5-10°C and 27-32°C ranges, respectively, T_c values have a much smaller effect on the final RMSE.

Several triplets allowed to reach a low RMSE value between 2.5 and 3.5 (**Table 2.12**), but among them, the triplet formed by 8.0°C as base temperature, 31.0°C as optimal temperature and 37.9°C as critical temperature was chosen as the best one, being the only temperature triplet that figured among the 10 best-predicting for all three varieties. As it can be observed from the table, some temperature triplets allowed to reach better RMSE values in each variety. However, the goal of our study was to find a common model which accurately predicted mango fruit growth, regardless of the variety, rather than finding the best model for each variety.

When tested against the pooled data from all varieties, the chosen temperature triplet allowed to reach an RMSE value of 2.66.

Table 2.12. Values (°C) of the temperature triplets tested by the software which produced the 10 lowest RMSE values and relative GDH accumulation calculated based on the triplet.

KEITT					OSTEEN					TOMMY ATKINS				
Tb	**To**	**Tc**	**GDH**	**RMSE**	**Tb**	**To**	**Tc**	**GDH**	**RMSE**	**Tb**	**To**	**Tc**	**GDH**	**RMSE**
9.4	31.2	43.9	33660.54	3.4928	12.2	27.4	40.3	34057.30	3.5214	**8.0**	**31.0**	**37.9**	**30797.89**	**1.4494**
8.0	**31.0**	**37.9**	**31837.21**	**3.4641**	15.7	24.9	49.0	32704.10	3.4059	10.0	27.4	48.7	34322.33	1.4142
10.6	34.0	36.8	27528.44	3.3764	14.2	28.5	43.0	32786.56	3.3764	7.2	29.5	48.3	34197.21	1.3416
15.7	28.6	44.2	31329.66	3.3466	3.0	30.1	35.9	32459.91	3.3466	1.6	30.0	44.9	34503.52	1.2649
11.9	30.0	45.7	33721.00	3.2558	4.3	32.8	37.8	31564.78	3.2249	6.0	28.5	42.8	34227.46	1.1832
13.4	27.5	47.3	34129.62	3.0984	5.4	29.6	38.2	34091.44	3.0984	5.7	29.0	41.3	33741.59	1.1832
12.4	29.6	47.0	33836.92	3.0332	**8.0**	**31.0**	**37.9**	**32472.22**	**2.7568**	6.8	29.8	45.1	33798.48	1.0954
1.7	33.1	38.7	31639.79	3.0332	14.7	27.7	41.3	32244.87	2.6458	7.3	29.5	44.4	33742.93	0.8944
6.6	34.1	38.8	30130.07	2.8983	13.3	29.4	36.4	30354.08	2.6458	10.2	28.3	47.1	33752.77	0.7746
10.4	31.5	39.7	31814.76	2.7928	2.9	31.8	36.7	31815.03	2.4900	7.9	29.2	46.2	33967.19	0.6325

The selected temperature triplet was used to calculate the GDHs accumulated from the moment of Full Flowering until each recording occasion for all fruits of each variety in each treatment, location, and year, using equation 4.

2.6.2.2 *Fruit growth modelling and parameterisation*

Pooled normalised fruit length data were plotted against DAFF; GDD and GDHs from Full Flowering and they were observed to be following a Logistic rather than a Gompertz distribution, the Logistic one having a smaller residual sum-of-squares (9.27 versus 9.48, respectively) (Kobayashi and Us Salam, 2000). The obtained parameters for the logistic model fitting fruit length to the different indices used and their statistical values are reported in **Table 2.13**. No significant difference was observed in the parameters obtained among the three varieties for any of the indices: therefore, a single set of parameters was used in the continuation of the study. The model comparison performed on the basis of Bayesian Information Criterion (BIC) to select the most correct model among a set of candidates (Aho *et al.*, 2014) confirmed that the model based on GDH was the one that best predicted the pooled fruit growth data.

Table 2.13. Values ± s.e. of the parameters of the logistic regression equation obtained for each index and statistics of the relative model.

	Asymptote	Mid-point	Slope	R^2	BIC
DAFF	0.997 ± 0.005	47.673 ± 0.335	16.256 ± 0.322	0.917	-1932.344
GDD	0.952 ± 0.005	465.596 ± 4.789	183.175 ± 4.413	0.866	-931.750
GDH	1.000 ± 0.004	17822.616 ± 112.477	6153.834 ± 106.602	0.966	-2057.242

Figure 2.54 synthetises the goodness of fit of the models obtained using the different indices. In general, average fruit length data points always follow the logistic distribution. However, in the GDH model, the variability in the observed values among the years of observations and growing conditions is much smaller than in the other models, and the observed data are more adherent to the predicted ones. In particular, in the GDD model, open field data are significantly shifted to the left of the model prediction while the greenhouse data are shifted to the right, indicating a systematic error of the prediction.
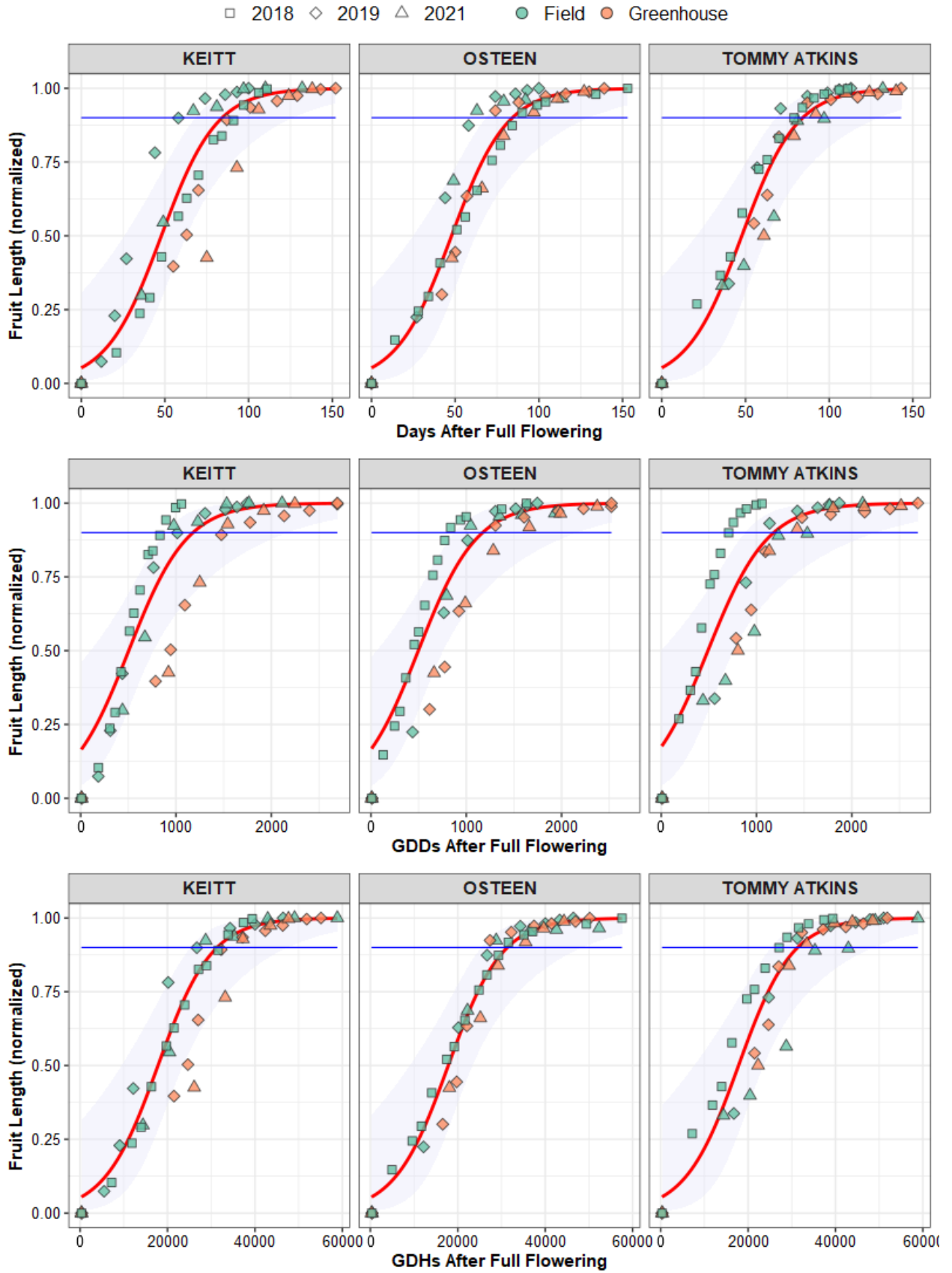
Figure 2.54. Curves representing the fit of the obtained models (solid red line: general GLM obtained for each variety) to the average measured fruit lengths (points) for each time index, namely Days After Full Flowering (top); Growing Degree Days from Full Flowering (centre); Growing Degree Hours from Full Flowering (bottom). The grey area below the curves represents the 95% C.I. from the general growth GLM. The blue horizontal line represents 90% of the fruit's final size.

GDH was the index that best predicted the moment at which fruits reach 90% of their final dimension, with the smallest Coefficient of Variability values in all varieties (**Table 2.14**). Analysis of variance confirmed that no statistically significant difference existed between the total GDHs needed by the three varieties to reach 90% of the final fruit length for any of the observed index (data not shown).

Table 2.14. Mean and standard deviation values of each index needed by each variety to reach 90% of its final dimensions among the 5 observation periods, and relative Coefficient of Variability (C.V.). The values of the index Days After Full Flowering are based on the actual observed data rather than on those predicted by the model.

Variety	Days After Full Flowering		GDD		GDH	
	Mean ± s.d.	C.V. (%)	Mean ± s.d.	C.V. (%)	Mean ± s.d.	C.V. (%)
Keitt	83.40 ± 9.35	11.21	1164.72 ± 262.22	22.51	31434.14 ± 969.74	3.08
Osteen	80.00 ± 9.14	11.43	1195.88 ± 283.37	23.69	30981.02 ± 2030.99	6.55
Tommy Atkins	79.00 ± 4.56	5.77	1112.46 ± 224.26	20.15	30604.74 ± 727.08	2.37

On average, the daily GDH accumulation in the experimental stations during the months from May to October – from flowering to harvest – ranged from 330 to 460 GDH (**Figure 2.55**). Therefore, looking at the standard deviation of the values predicted by the GDH model in Table 5, this corresponds to an uncertainty of only 3 to 5 days in the moment when fruits of the three varieties reach 90% predicted by the model. On the other hand, the daily mean GDD accumulation in the same period was 17.0 in September and 12.2 in October. Therefore, the GDD model predicts the date of reaching 90% of the final fruit dimensions with an error of 16 to 23 days.

2.6.2.3 *Difference in daily GDH accumulation between the field and greenhouse*

Figure 2.55 illustrates that in the two years of observation in Sicily, where observations on the fruit growth in both open air and greenhouse cultivation were available, on the basis of the obtained model, the GDH daily accumulation is higher in the greenhouse than in the open air until the month of May: during the summer months, instead, more GDHs are accumulated

in the field than in the greenhouse, until October when the mean daily GDH accumulation coincides in the two cropping conditions.
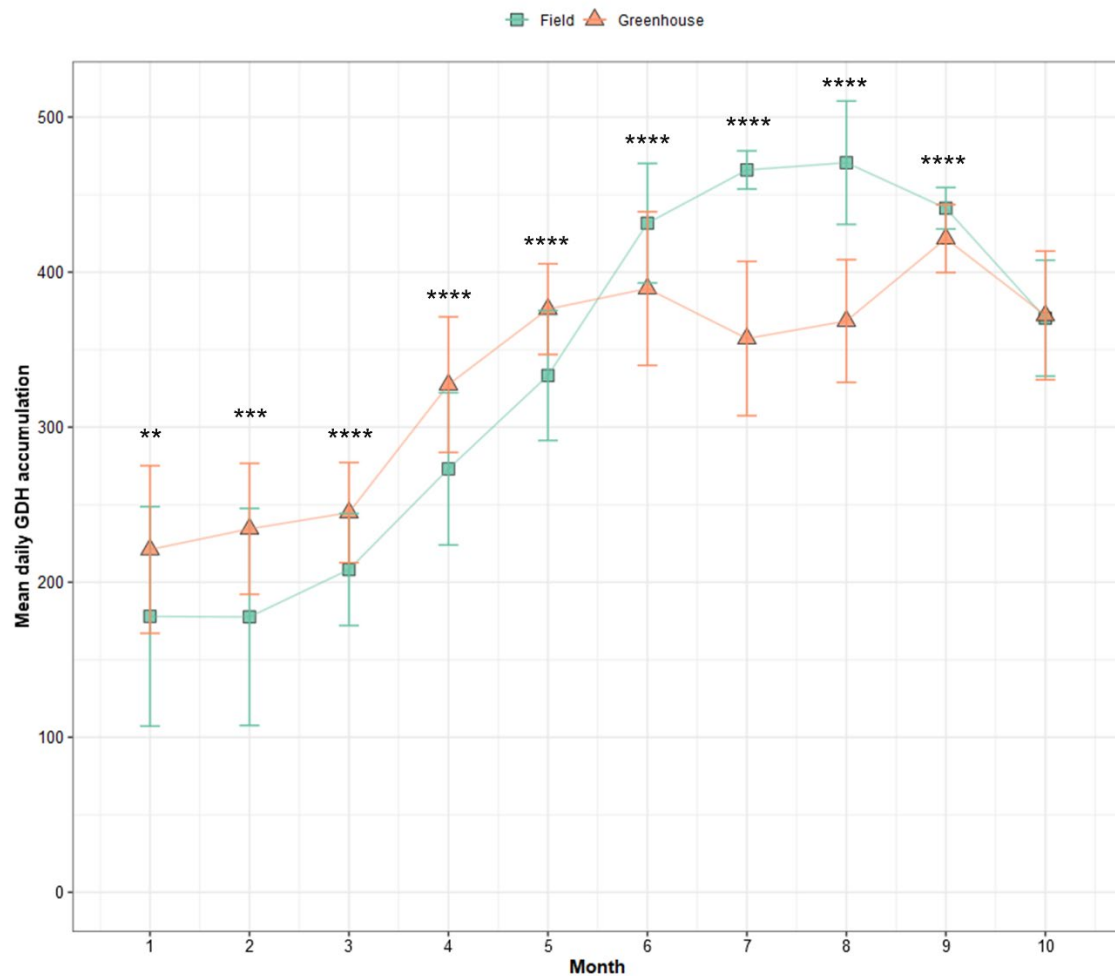

Figure 2.55. Average daily GDH accumulation in the field and greenhouse during each month over the course of the observation in the island of Sicily. **, *** and **** symbols indicate significant difference between the cropping conditions for Student's t-test at $p < 0.01$, $p < 0.001$, $p < 0.0001$ respectively, with no significant difference otherwise.

This is due to the extreme high temperatures that are reached inside the greenhouse during the Mediterranean summer months, with maximum daily values that are up to 10°C higher than in the open air and regularly exceed the T_c value of 37.9°C (Figure 1). When this happens, 0 GDHs are accumulated, resulting in the observed depression of the mean daily accumulation value observed in Figure 4. This explains what was observed in a previous study by Scuderi *et al.* (2023), where mango fruits growing in the greenhouse are seen growing in size until 125 days from full flowering, while they reach their final dimensions within 110 days from full flowering in the open air.

2.6.3 Conclusions

The GDH-based model that we propose shows some highlights:

- It allows to confidently establish cardinal temperature values for the mango fruit growth process. No significant differences emerged between the three observed varieties, of which one (Keitt) is among the latest ripening. The model attests that fruit growth takes place in a range between 8°C and 38°C. These temperatures exceed those that are normally observed in the mango producing areas during the reproductive period and can allow to extend the cultivation area to a wider range, provided that all other eco-physiological requirements of the species are satisfied.

- Once T_b, T_o and T_c have been calculated, with the triplet $8.0 - 31.0 - 37.9$°C which turned out to be the most reliable, it is *simple*: it is only necessary to add the hourly temperatures and the dates of phenological events (Full Flowering) in the model equation and calculate a simple sum.

- It works *regardless of the variety or the cultivation environment*: the model was tested in three very different environmental conditions – subtropical dry climate, Mediterranean climate, and greenhouse cultivation – and we obtained non-significant differences among the values required by each variety to achieve final fruit size. A value of about 31000 GDH allows to reach 90% of the fruit's final size in all the observed varieties, with reasonable uncertainty. Therefore, varieties can be compared only on the bases of the final thermal time requirements, *ceteris paribus*.

Undoubtedly, temperature is not the only factor regulating fruit growth. It must be remembered that, despite the clear relationship between fruit growth and GDH accumulation, fruit development is definitely influenced by other factors, such as cultivation techniques, water

availability, fruit competition, leaf-to-fruit ratio, light environment, and photosynthesis, which were all observed to affect mango fruit growth (Léchaudel, 2004; Léchaudel *et al.*, 2007, 2002). We can however rightfully assume that these factors were considerably different among the 5 different observation periods. The fact that the model achieved a good fitness to the measured data across such different fruit growth periods, however, reinforces its value and is particularly meaningful from a practical point of view. In fact, keeping the model as simple as possible facilitates its application in agricultural conditions, allowing growers to use it for managing field work and optimising harvesting times, reducing waste of labour and product and allowing to offer high quality fruits to consumers.

The information obtained from the study, finally, should be taken into account when planning a protected horticulture system for mango in the Mediterranean climate: we showed that the anticipation of flowering that is obtained in the greenhouse is neutralised by a longer fruit development period caused by the extremely high temperatures during summer. A smart solution would probably be represented by hybrid protection systems to be put in place during the colder months of the year and removed at the onset of summer.

Future studies will focus on the application of the protocol used to model fruit growth in other biological processes of the species, such as flowering and fruit ripening.

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3 Coffee

Coffee (*Coffea* L.) is a genus of plants belonging to the family of the Rubiaceae and native to the highlands now corresponding to South-Western Ethiopia (Lashermes *et al.*, 1999). Coffee trees in their original habitat can normally grow to be as high as 10 meters. The root system is superficial and reaches depths no deeper than 30 cm, depending on the soil characteristics (Cannell, 1971; Saiz del Rio *et al.*, 1961). Leaves and branches are always opposite, sprouting from a single central trunk (**Figure 3.1**). The largest horizontal branches are the furthest from the apex of the main trunk and their size decreases going towards the top of the plant. As a result, the plant respects a pyramidal development, which provides a complete covering of the ground below the canopy. Leaves are of a deep, dark green colour at maturity, but they can vary from light green to yellowish to bronze at the moment of their emergence. The flowers appear on the leaves' axils. They appear on mature branches in inflorescences with a common peduncle attached to the leaves' axils, and the corolla of the single, pentamerous flower is white (DaMatta *et al.*, 2007; Teketay, 1998).

Figure 3.1. Coffee plant growing in a greenhouse in Sicily, in consociation with papaya (*Carica papaya*) plants. Photo by the author.

The coffee fruit is an elliptical drupe, with a growth following a double sigmoidal curve taking on average 290 days from the moment of full flowering, red or yellow at maturity, depending on the variety, which contains two seeds (Fernandes *et al.*, 2017). Coffee drupes have a peculiar composition: the epicarp corresponds to the fruit skin, the mesocarp is the pulp while the endocarp is represented by the so-called "silver skin" which, at maturity, detaches from the pulp and adheres to the seeds. The coffee seeds, commonly referred to as "coffee

beans", are convex with a typical longitudinal furrow. The active substance for which they are sought after is the caffeine, which they contain in values between 0.5 and 3%, depending on the variety (Charrier and Berthaud, 1985; Walsh, 1894).

The two most common species of the genus *Coffea* are *Coffea arabica* L. and *Coffea canephora* L., the latter also known as "robusta". The two are grown mainly in different areas of the world, with arabica coming mostly from Africa and South America, while robusta coffee is mainly grown in India and South-East Asia (Vegro and de Almeida, 2020). The two coffee species thrive at different environmental conditions: arabica is mainly cultivated at higher altitudes, between 600 and 2000 m above sea level, while robusta can be grown also in the lowland (Bunn *et al.*, 2015). Both species require at least 1200 mm of yearly rainfall (Bote and Vos, 2017; Kath *et al.*, 2021). The caffeine content of the beans is double in robusta than in arabica (Bicho *et al.*, 2013).

Many recent studies are focusing on the impact of climate change on the global coffee production (Adhikari *et al.*, 2020; Camargo, 2010; Eitzinger *et al.*, 2013; Grüter *et al.*, 2022) as well as the plant's adaptation and responses to the new climatic conditions (DaMatta *et al.*, 2019; Gomes *et al.*, 2020; Läderach *et al.*, 2017; Schroth *et al.*, 2015). The research highlights that while some current production areas might become less suitable for the cultivation of coffee, with the correct crop management it will be possible to grow the species in new areas and countries.

Until the present day, only very limited information is available about attempts of cultivating coffee in countries of the Mediterranean basin. A literature research conducted with the keywords "coffe" + "cultivation" + "*Country_name*" resulted in only three isolated studies, located in Italy (Riccobono, 1912), the Levantine coast (Gindel, 1961) and Egypt (Saleh *et al.*, 2013). Some experimental work is being carried out at research centers across the Mediterranean sea (J.M. Losada, personal communication, 2023). However, the commercial

cultivation of coffee in Sicily, Italy, has started in the last years and was reported in specialized and mainstream media outlets (Miller, 2022; The Guardian, 2021).

Despite the coffee plant being native to tropical areas distant from the Mediterranean Sea, in this part of the world the beverage has risen to the status of a cultural symbol like few others. The first coffee consumption houses were established in Istanbul and Venice after the diffusion of the beans by Arab merchants (Crawford, 1852) (**Figure 3.2**).

Figure 3.2. Miniatures of coffeehouses in Istanbul in the 16th century (left) and Budapest in the 18th century (right). Anonymous authors.

Nowadays, every Mediterranean country has its own peculiar fashion of preparing, serving and consuming coffee and the rite of coffee unites all these different populations (Topik, 2009; Vegro and de Almeida, 2020). Thus, exploring the possibility of growing coffee beans in Mediterranean territories is an idea that tickles the landowners and transformers alike, who could offer a unique product to a very affectionate market, especially since this region has one of the highest consumption ratios per capita (Carvalho *et al.*, 2016; Grigg, 2002; Quadra *et al.*, 2020). As a result, any future plantation project will benefit local farmers while also boosting the region's economy, and evoke meaningful cultural suggestions.

3.1 Behaviour of coffee in the Mediterranean climate

Observations of precise moments of the coffee plant's life cycle were carried out in separate moments, spanning from the autumn of 2021 until the spring of 2022, and then again in the spring of 2023. In these separate study periods, we assessed the tolerance of coffee plants to the cold temperatures that can be reached in the Mediterranean and the moment of their awakening after the vegetative rest that occurs during winter.

Coffee can be cultivated in the shade of forestry trees or in full sunlight (DaMatta, 2004). In many cases, smallholder coffee farms consist of plants growing in forest hills, with little light available, while bigger, industrial farms are made up of specialized orchards where the coffee plants are in full sunlight with no shading coming from higher trees (Moraes *et al.*, 2010). Light is considered to influence the plant's vegetative and reproductive development and fruit quality (Salazar *et al.*, 2019; Somporn *et al.*, 2012). The tropical and subtropical conditions of the countries of the so-called coffee belt, however, differ greatly from the Mediterranean basin, with regard to duration of the day, intensity of incident light and cloud cover (cfr. **Figure 2.6**).

In the second period, we located the moment of flowering of coffee plants in the Mediterranean climate and the architectural position of the buds undergoing reproductive development. Coffee buds which produce new inflorescences are generally located in the central part of the plagiotropic branch (de T. Alvim, 1973) and are influenced by the presence of developing fruits on the same branch (Majerowicz and Söndahl, 2005). We analysed the timing of emerging and position of inflorescences over one flush in the Mediterranean spring, to assess the behaviour of the plant in the new cultivation environment and evaluate the similarities and differences with the flowering phenology of the plant in its traditional cultivation areas.

3.1.1   Material and Methods

Both experiments were carried out in an unheated greenhouse located in the municipality of Terrasini, Palermo (38.08° N 13.09° E, 0 m above sea level, 1.2 km from the coast). The greenhouse has a metal structure, with the roof and side walls made in PVC film. The coffee plants of the varieties Caturra and Catimor were grown in promiscuous cultivation among trees of banana (*Musa* spp.) and papaya (*Carica papaya* L.) and were 2 years old at the beginning of the first experiment.

The observations on the plants' vegetative growth were carried out on a sample of 8 plants per variety and consisted in the repeated measurement of the plant's height and of the length of selected shoots, from the month of November 2021 to April 2022. Contextually, the environmental conditions inside the greenhouse were monitored with a thermo-hygrometer equipped with a data logger (Elitech Ltd, London, UK) and a lux-meter recorder (TekCoPlus, Hong Kong). At each measuring occasion, the quantity of light reaching each plant was assessed by placing the luxmeter sensor above the plant's apex and noting down the instantaneous reading.

The observations on the flowering phenology of the coffee plants were carried out on the same plants during one flowering flush, from March to June 2023. Four plagiotropic branches per plant were selected randomly around the tree canopy, the number of nodes in each branch was counted and the phenological stage at each node was determined using the BBCH scale for coffee (Arcila-Pulgarín *et al.*, 2002). In case more phenological stages could be found on the same node, the most advanced one was assigned to it.

3.1.2   Results and Discussion

3.1.2.1   Vegetative growth

The conditions of temperature and light available for the plants during the observed winter are represented in **Figure 3.3**. The greenhouse allowed to keep minimum temperatures

above 5°C throughout almost all the observation period, even in January during the coldest days of the year. However, a late cold spell happened at the end of the month of March, where temperatures approached 0°C after a period where maximum daily temperatures had already risen to values around 35°C. This enforces the need to cultivate tropical crops in protected cultivation systems, at least for the first years of their lives, in the Mediterranean basin, which is subjected to late cold returns happening routinely (Bachofen *et al.*, 2016).
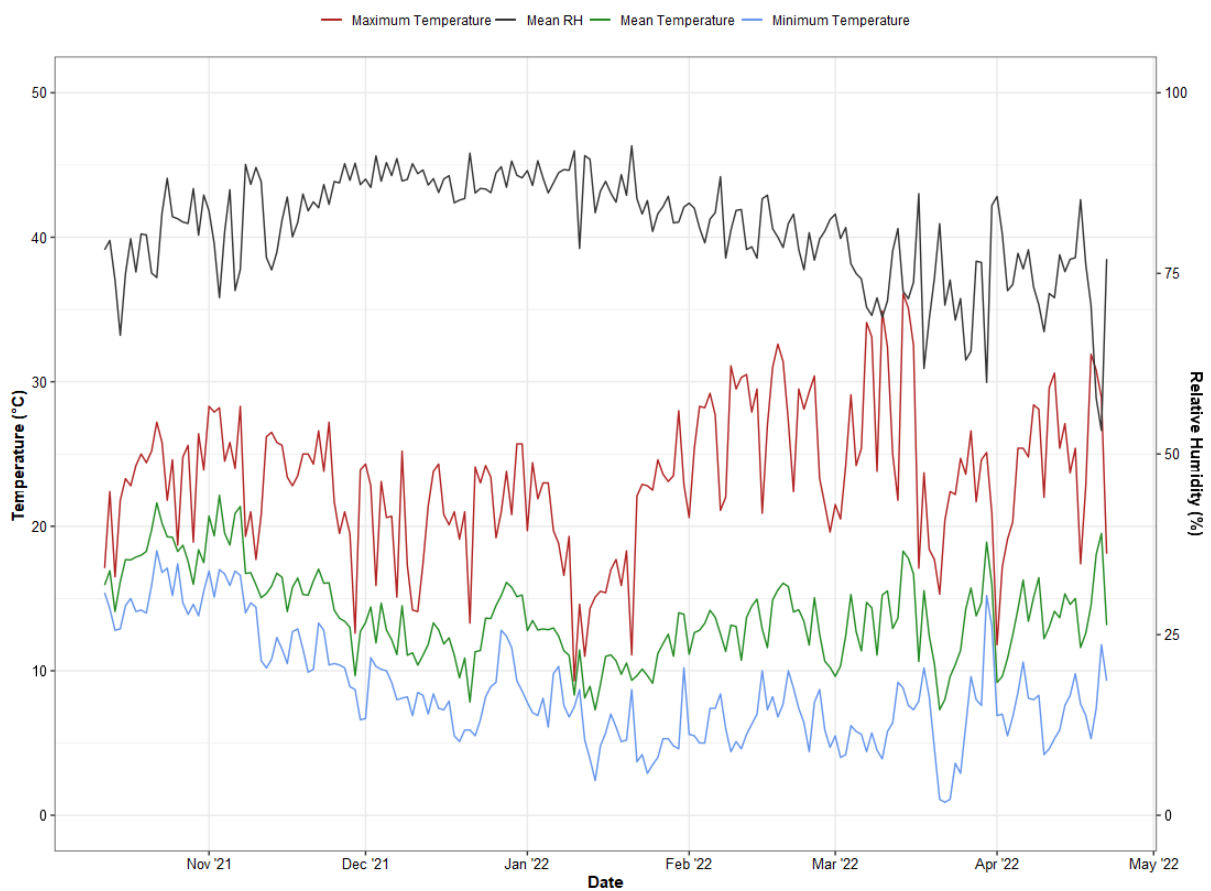
Figure 3.3. Minimum (blue line), mean (green line) and maximum (red line) daily temperatures and mean relative humidity (black line) recorded inside the greenhouse during the period of observations.

The average light availability for the plants inside the greenhouse, instead, showed an impressive increase from the month of October, where values did not even reach 5000 Lux, which is the light normally available in an office room, until the month of April where such values where 10 times higher (**Figure 3.4**). This is a peculiar characteristic of the Mediterranean and temperate climates, to which tropical plants are not adapted to. Moreover, also the length

of the day has a significant variation in latitudes further from the equator, while in the tropical belt the daily hours of light vary less throughout the year (Borchert *et al.*, 2005).
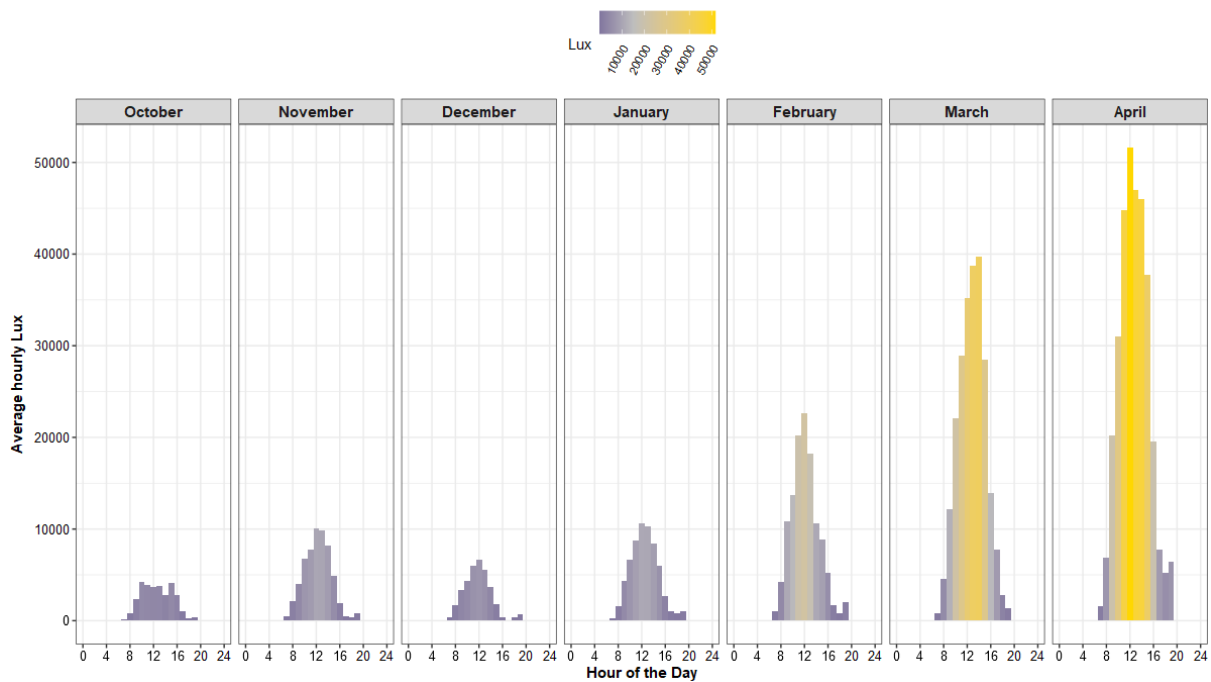
Figure 3.4. Average hourly values of Lux intercepted by the coffee plants inside the greenhouse, throughout the months of observation.

The vegetative growth of the plants in response to the described conditions is depicted in **Figure 3.5** and **Figure 3.6**. Plant height growth is generally slow through the winter months, with daily growth rates between 0 and 0.5%. However, an increase in both the plant's absolute height and its growth rate is observed after the end of the cold period, between the months of May and June. Regarding the shoot length as well, the daily growth rate only rises to significant values between the months of May and June, after the temperatures have risen consistently to average daily values above 15°C. Most plants of the study, however, especially of the variety Caturra, showed an impressive elongation from one year to the next (**Figure 3.7**), with some plants doubling their size. This shows that vegetative development in coffee occurs in flushes happening throughout the year (Boss, 1951; Browning and Fisher, 1975; Cannell, 1971) even in Mediterranean conditions, provided that protection from the open air minimum temperatures and weather events is given to the plants.
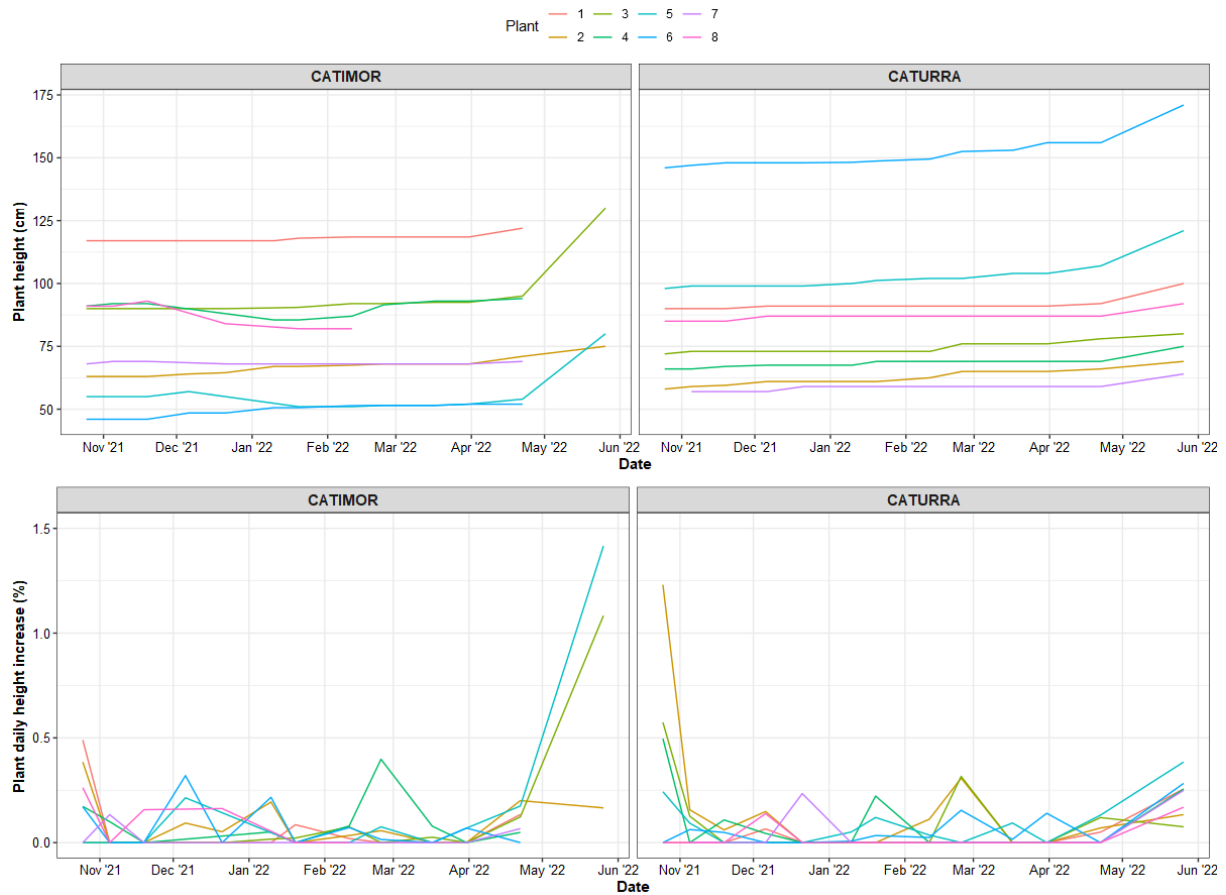
Figure 3.5. Absolute plant height (upper row) and daily plant height increase (lower row) of the coffee plants in the greenhouse over the observation period.
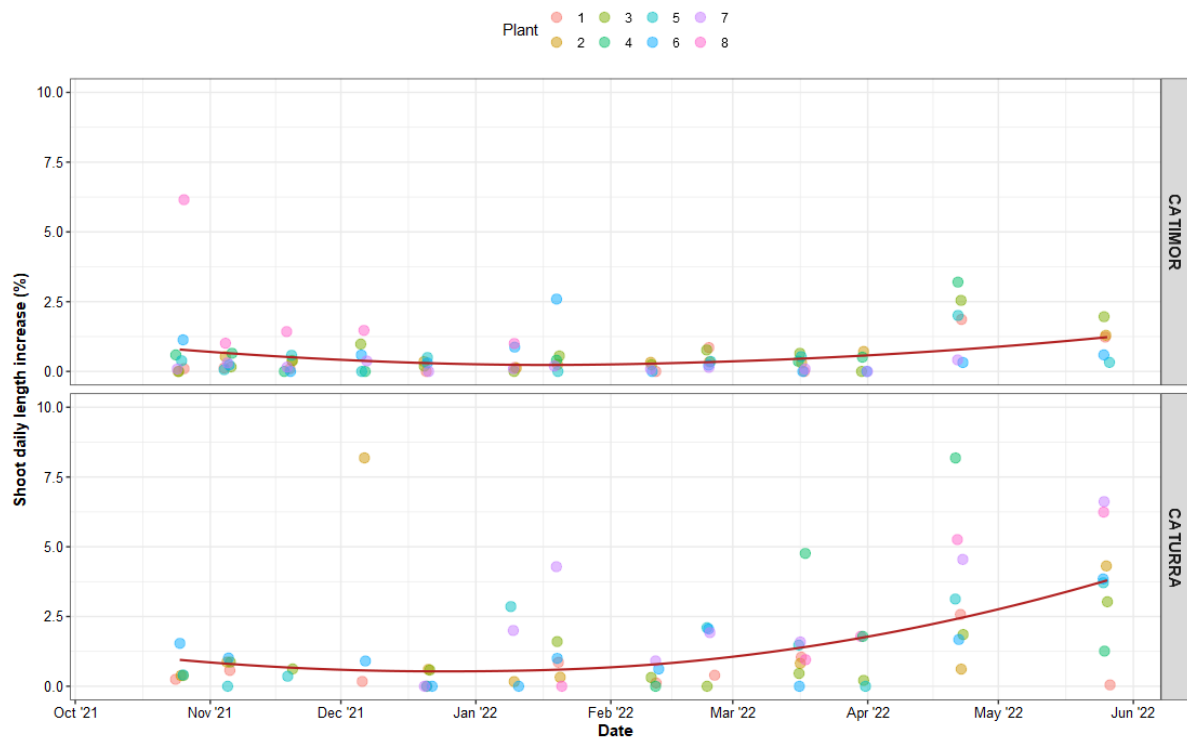
Figure 3.6. Daily percentage length increase of the selected shoots on the coffee plants inside the greenhouse over the observation period. The red lines for each variety represent the smoothed mean of all observed shoots.
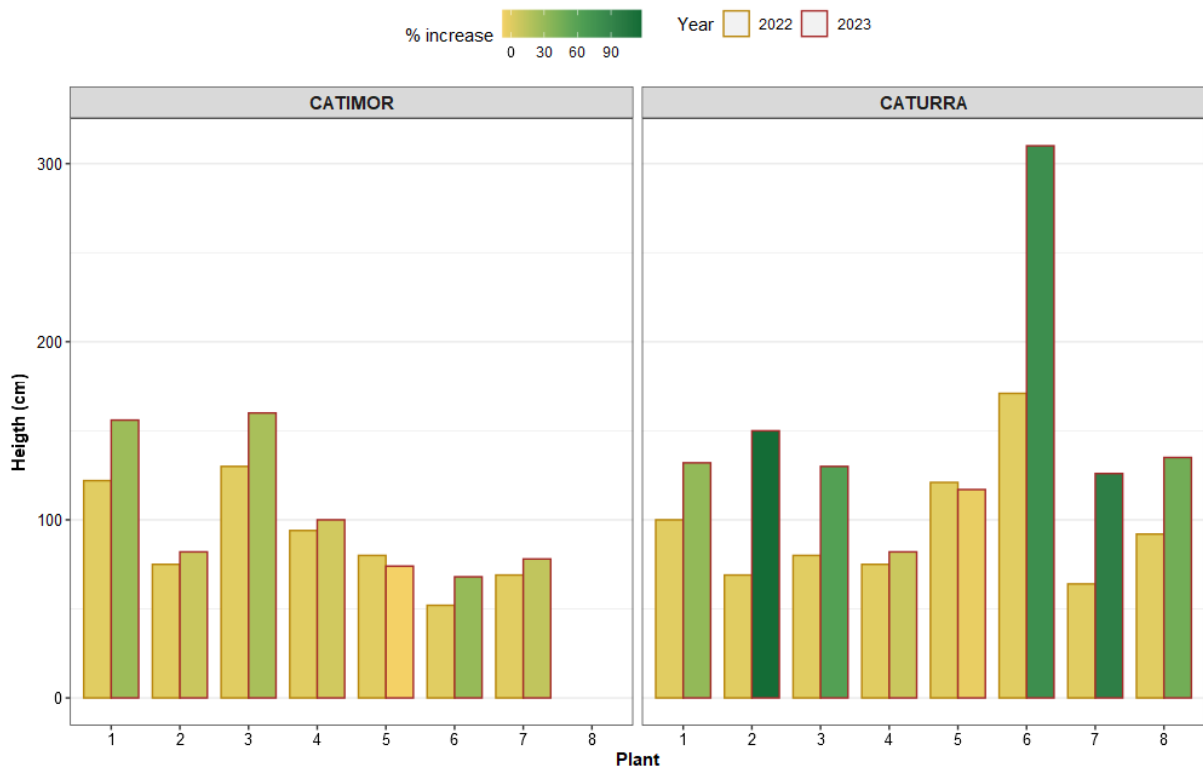
Figure 3.7. Heights of the coffee plants of each variety, measured in the spring of 2022 (gold border) and 2023 (red border).

3.1.2.2 Flowering Phenology

The number of vegetating, flowering and fruiting buds and their relative node position on the coffee plant's branches is depicted in **Figure 3.9**. Flowering starts earlier than vegetation in both Catimor and Caturra varieties. In all observed plants, the number of buds producing new inflorescences was higher in the nodes closer to the main axis of the plant or in the central section of the branch. The most distal nodes of the branch are exclusively devoted to vegetative development (Bote and Vos, 2016). This suggests that the age of the node is a factor in regulating if the buds will undergo reproductive or vegetative activity (Rodríguez *et al.*, 2011). Flowering was earlier and more intense in cv. Caturra than in cv. Catimor, and the peak of flowering activity was observed between the end of the month of May and mid-June.

Figure 3.8. Flowering on a coffee plant (cv. Caturra) of the study.

Figure 3.9. Count of buds found in given phenological phase per node position, at each recording occasion. Buds in the Quiescent and Swollen Bud phases were removed from the plot for better representability.

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Figure 3.10 illustrates the relationship between light availability and probability of the buds of each plant to flower or develop new vegetation. It emerges that the most sunlit plants of each variety were the ones which were capable to produce the highest number of new inflorescences and new leaves alike. The buds of the plants that were most in the shade, instead, produced only few new entities, regardless of the type of development they would undergo.

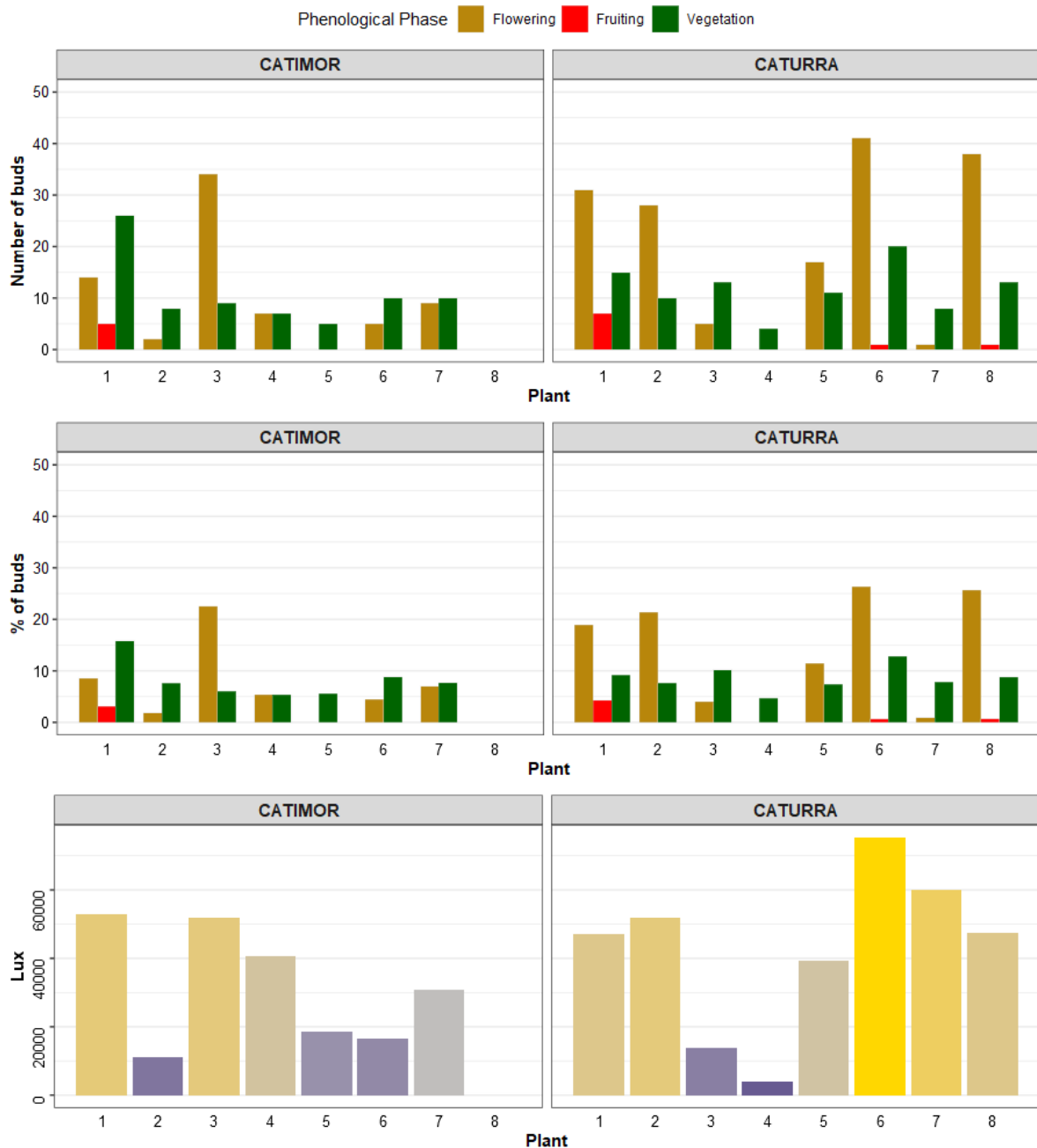**Figure 3.10**. Number (upper row) and percentage (middle row) of buds per phenological phase and amount of light (Lux) intercepted by the plants (lower row). Quiescent and Swollen Bud phenological phases are not shown for easiness of reading.

We could then deduce that increased light availability favours the development and productivity of coffee. Many authors, however, have investigated the effect of light on coffee plants' growth and reproductive cycle, from the early years of the research on this species (Kumar, 1979; Nutman, 1937; Ostendorf, 1962). The results of experiments conducted in tropical coffee-producing countries showed that photosynthesis was higher in shaded plants than in sun-grown plants, when they were kept at the same temperature conditions (Kumar and Tieszen, 1980). The cultivation of coffee in full sunlight, practised in Brazil, often leads to very high production of beans which usually causes extensive plant dieback (Fahl *et al.*, 1994). Moreover, coffee berries grown in a well-lit environment usually achieve inferior fruit and cup quality than those grown in the shade (Bosselmann *et al.*, 2009; Muschler, 2001).Therefore, more research has to be conducted on the actual implications of the Mediterranean temperature and light environment on the production and quality of coffee beans.

3.1.3   Conclusions

Cultivating coffee in the Mediterranean basin could seem like an impossible goal, or a presumption of researchers. However, we once again emphasize the capacity of adaptation of many plants of different origins to the various climatic conditions that can be found in Sicily. Coffee could be the next of the many species that found suitable conditions for their cultivation in the Mediterranean island.

The preliminary results that we obtained encourage this idea, in the sense that with the adequate cropping techniques such as greenhouse cultivation or the employ in promiscuous systems, coffee plants have shown a good growth from one year to the next, good response to the environmental conditions that stimulate development, and satisfactory flowering. The next studies to be carried out in more locations in the Mediterranean region should focus on the observation of productivity, yield and fruit quality, and subsequent cup quality of coffee grown in this new environment.

3.2   Composition of Sicily-grown coffee

The main bioactive compounds in coffee are polyphenols, chlorogenic acids, caffeine, trigonelline and diterpenes, and the variability of these chemical constituents affects the price of coffee commodities by determining their qualities. The occurrence of polyphenols in coffee beans (including the derivatives of chlorogenic acid, caffeoylquinic acid and phenolic acids) was highly related to the taste of the coffee brew. These compounds are known to have several beneficial health effects. In fact, bioactive compounds in coffee beans contribute to their antioxidative, antifibrotic, antidiabetic, neuroprotective, and anti-inflammatory activities (Carneiro *et al.*, 2021).

In common practice, the coffee cherries are dried to obtain the coffee seeds. At the same time, a by-product consisting of dried pulp and parchment called coffee husks (CH) is obtained. Dry processing generates approximately 1 kg of CH per kg of coffee beans produced (Franca and Oliveira, 2009)

This work focused on the chemical characterization of Sicilian green coffee seeds also considering the coffee husks, with a thorough description of the climatic conditions at which coffee cherries have been obtained. The study was conducted in collaboration with the Department of Biology, Physics and Chemistry of the University of Palermo, at whose laboratories the chemical analyses were conducted.

3.2.1   Material and Methods

3.2.1.1   Chemicals and Reagents

Methanol, ethanol, pyridine anhydrous, ethyl chloroformate, sodium hydroxide, hydrochloric acid, sodium carbonate, sodium bicarbonate, gallic acid, caffeine (≥99%), Folin-Ciocalteu′s phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), ethyl myristate, quercetin, rutin, kaempferol, naringenin, nicotinic acid, cyanidin 3,5-diglucoside, caffeic acid, ferulic acid, p-coumaric acid, m-coumaric acid and chlorogenic were purchased from Sigma-Aldrich (St.

Louis, MO, USA), ABTS (2,2' azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid), potassium persulphate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Fluka (Buchs, Switzerland). Chloroform, n-hexane, acetic acid, ethyl acetate, potassium hydroxide, Supelco 37- FAME mix 79248, 17 Amino acid standards mixture containing L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine 2.5 mM in 0.1 M HCl, a were from Supelco. L-Tryptophan, L-asparagine, and L-glutamine pure standards and calcium carbonate were acquired from Merck (Darmstadt, Germany). HPLC-grade water was obtained by purifying double distilled water in a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA), 0.45 µm PTFE syringe filter (Whatman). Atractyligenin sample was available at a high purity grade standard in the laboratory of one of the authors.

3.2.1.2 *Plant Material, Environmental Conditions and Sample Preparation*

The coffee cherries (*Coffea arabica* L. cv. Caturra) analysed in the study were harvested in the month of June 2022 in an orchard located in the municipality of Milazzo (ME), Sicily, Italy (38.11°N 15.14°E), 9 meters above sea level, from six randomly selected twelve-years old adult plants. The fruits were hand-picked at stage 88 – defined as "fully ripe fruit, ready for picking" – of the BBCH scale for coffee (Arcila-Pulgarín *et al.*, 2002). After harvest, 200 g of cherries from each plant were pooled together in a homogeneous sample (**Figure 3.11**). The climate of the area falls under the Mediterranean (Csa) classification, characterized by temperate climate with dry and hot summers – in the Köppen classification, with an average annual precipitation of 885 mm and a mean annual temperature of 18.56 °C (Murray C Peel *et al.*, 2007). Daily maximum, mean and minimum temperature, mean relative humidity, total rainfall, and hourly solar radiation – Global Horizontal Irradiance, GHI (W/m^2) – data for the period 2010-2022 in

the location of the study were collected from the nearby weather station managed by the Sicilian regional agri-meteorological Service (SIAS).

Sample preparation initially involved the manual separation of the coffee husks (consisting of the pericarp) from the seeds (endocarp). After separation, the samples were placed in an oven at a temperature of 40-45 °C for 72 h, ground to a fine powder in a commercial coffee grinder. Dried coffee husks and seeds were weighed and ground to powder.

Figure 3.11. Maturity degree of coffee cherries at harvest.

3.2.1.3 Radical Scavenging Properties Evaluation, DPPH and ABTS Assay

The measurement of dried green coffee seeds and coffee husks' anti-radical activity was carried out following the procedure illustrated in Di Stefano *et al.* (2022); one g of each sample (coffee seeds and coffee husks) was extracted with 4 mL of methanol for 40 min in an ultrasonic bath. The supernatant was filtered with a 0.45 μm PTFE syringe filter. 100 μL of the filtrate was mixed with 3 mL of DPPH (60 μM) and placed in the dark for 30 min. Absorbance was measured at 515 nm using a spectrophotometer (Varian Cary® 50 UV-Vis spectrophotometer) and methanol as blank. Values obtained were reported as Trolox equivalent antioxidant activity (TEAC) and expressed as mmol Trolox equivalent (TE)/100g of sample. Trolox was used as the standard and the calibration curve was prepared using solutions at different concentrations [5-400 μM] ($y = -0.0009x + 0.4037$, $R^2=0.9971$). All experiments were performed in triplicate and were reported as a mean ±SD. According to (Re and Pellegrini, 1999), ABTS was dissolved in water to a 7 mM concentration. ABTS$^{.+}$ radical cation was produced by reacting ABTS stock

solution with 2.45 mM potassium persulfate $K_2S_2O_8$ and the mixture was kept in the dark for 16h in order to make the radical reaction. At the end of time the solution will turn dark blue-green. The $ABTS^{.+}$ stock solution was diluted to an absorbance of 0.7 ± 0.02 nm measured at 734 nm. For sample preparation, 4 mL of MeOH was added to 1 g of each sample (dried seeds and coffee husks) and sonicated in an ultrasonic bath for 40 min. Solutions were collected and filtered through Whatman 0.45 μm PTFE filters. A calibration curve using Trolox in a concentration range of 2.5 μM-30 μM, was constructed. Data were performed in triplicate and reported as a mean (±SD) of triplicate measures.

3.2.1.4 *Total Phenolic Content (TPC) Analysis*

Total phenolic content (TPC) was determined using the optimized Folin–Ciocalteu method proposed by (Bonacci *et al.*, 2023). Five mL methanol/water (80:20 *v/v*) were added to 0.5 g of each sample (coffee husks and green seeds), sonicated with an ultrasonic bath for 40 min and filtered through 0.45 μm PTFE syringe filter. 125 μL of the filtrate was mixed with 625 μL of 1:5 diluted Folin–Ciocalteau reagent and 120 μL of 7% Na_2CO_3 solution. Samples were left in the dark for 1 h at room temperature to form a blue coloured complex. The intensity of the colour is proportional to phenolic compounds in the sample. The resulting colorimetric reaction is measured at 765 nm using a UV/ Vis spectrophotometer (Varian Cary 50, Agilent). A standard solution of gallic acid [25 – 250 μg/mL] was used for calibration (y=0.0025x+0.0554, R^2 = 0.9879). Results were expressed as mg gallic acid equivalents (GAE) per g of sample.

3.2.1.5 *Determination of Phenolic Compounds by UHPLC-ESI-MS in green seeds and coffee husks*

Free and bound phenolics, were analysed by UHPLC-ESI-MS, according to procedures previously described (Bonacci *et al.*, 2023).

3.2.1.5.1 Extraction of Free Phenolics (FPs).

Two grams of each sample (seeds and coffee husks) were extracted with 40 mL 80% methanol/water solution for 45 min using an ultrasonic bath. After centrifugation at 5000× g, the supernatant was collected, and evaporated with a rotary evaporator at 45 °C and the residue was redissolved in 2 mL of methanol. The solution, containing free phenolics, was filtered through a 0.45 µm PTFE syringe filter into glass vials prior to UHPLC-ESI-MS analysis.

3.2.1.5.2 Extraction of Bound Phenolics (BPs).

The extraction process was based on the procedure by Singh *et al.* (Singh *et al.*, 2013), with a few modifications. The residue after methanol/water extraction was hydrolysed with 45 mL of 4 mol/L NaOH for 4 h in the dark. Then, the mixture was acidified to pH = 2.0 with 2 mol/L HCl. After centrifugation at 3000 rpm for 15 min, the supernatant was collected and ethyl acetate (50 mL) was added, thoroughly mixed, and extracted 3 times. The ethyl acetate extracts were dried in a rotary evaporator. Finally, the dry powder was dissolved in 2 mL of methanol prior UHPLC-ESI-MS analysis.

3.2.1.5.3 Instrumentation and Chromatographic Conditions

The identification and quantification of polyphenols were performed by UHPLC-ESI-MS analysis using Q-Exactive LCq/Orbitrap MS (ThermoFisher Scientific), interfaced with UHPLC Ultimate 3000 RS (Dionex) and equipped with HESI (Heated Electrospray Ionization) source. The chromatographic separation was achieved using a Luna C18(2) (150 x 2.0 mm, 5 µm stationary phase), equipped with pre-column. The column was maintained at 30°C and the flow rate was 400 µL /min. The mobile phases solutions were H_2O with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). The binary gradient separation was as follows: 0-2 min 95% A; 2-18 min 95-5%A; 18-20 min 5% A; 20-40 min 5-95% A. The initial conditions were maintained for 3 min to equilibrate the column. One µL of each sample was injected with a run time of 40 min. The HESI source conditions were set as follows: Spray Voltage (+/-):

3000.00; Capillary Temperature (+/-): 300.00; Sheath Gas (+/-): 30.00; Aux Gas (+ / -): 15.00; S-Lens RF Level: 50.00; Ion Source: ESI. Data were collected in both positive and negative ionization modes. The scan range was set between 80-1000 m/z.

Quantification was based on the standard curves. For quantitative determination of polyphenols, nine standard calibration curves (quercetin, rutin, naringenin, cyanidin 3,5-diglucoside, caffeic acid, ferulic acid, p-coumaric acid, chlorogenic acid, kaempferol) in a range of concentration (0.1 - 10 ppm) were developed.

3.2.1.6 *Determination of Alkaloids and Atractyligenin by UHPLC-ESI-MS*

Alkaloids (trigonelline and nicotinic acid) and atractyligenin, were analysed by UHPLC-ESI-MS, according to procedures described for determination of free phenolics compound (Bonacci *et al.*, 2023). Calibration curves were determined using available standard (nicotinic acid for alkaloids and atractyligenin) in a concentration range of 0.1 - 10 ppm. Chromatographic conditions are the same as previously described for UHPLC-ESI-MS analyses of free and bound polyphenols.

3.2.1.7 *Fatty Acid Composition*

A method for determining fatty acids composition in Sicilian coffee husks and seeds was investigated using gas chromatography-mass spectrometry (GC/MS) ISQ™ 9000 Quadrupole GC-MS System (Thermo Fisher Scientific, Waltham, MA, USA) after esterification of the fatty acids to their corresponding methyl esters (Melilli *et al.*, 2020). One g of each dried sample was added with 10 mL $CHCl_3$/MeOH (2:1) solution. The mixture was sonicated for 45 min at 50 °C, cooled to room temperature, and centrifuged at 4000 rpm for 5 min at 4 °C. The supernatant was filtered through filter paper and the residual pellet was subjected to two further extractions. To remove water-soluble compounds a separation with a separating funnel was carried out with a H_2O + NaCl solution (0.88%). Obtained solution was evaporated by rotavapor. One hundred mg of oily residue was resuspended in 1 mL of hexane, after 100 μL of a solution of KOH in

MeOH (2M) was added and the mixture was vortexed for 5 minutes. 100 μL of the upper hexanic phase, containing FAMEs, 100 μL of internal standard solution (ethyl myristate 150 ppm) and 800 μL of hexane were mixed before GC/MS analysis. FAMEs analysis was carried out by using a gas chromatography-mass spectrometry (GC/MS) ISQ™ 9000 Quadrupole GC-MS System equipped with ZB-WAX® column (30 m x 0.25mm x 0.25 μm, film thickness; Phenomenex, Italy). Operating conditions were as follows: ultra-high purity helium was used as carrier gas at a flow rate of 1 mL/min; the injector temperature was set at 200 °C. The injected volume was 5 μL with a split ratio of 10:1. The initial oven temperature was maintained at 120 °C for 3 min and increased to 300 °C at a rate of 1.5 °C min^{-1}, and then Increased at a rate of 10 °C min^{-1} to 350 °C and held isothermal for 5 min. The ionization potential of the mass-selective detector was 70 eV and the scan range was 33–550 m/z. Identification of compounds was achieved by a mass spectra database search (NIST 2011 Mass Spectral Library) and confirmed by comparing retention time and MS spectra with the Supelco 37-component FAMEs Mix (C4–C24). Peak integration was carried out using Xcalibur™ software (Thermo Scientific™, Waltham, MA, USA). The amount of individual fatty acids methyl esters was expressed as g/100g of sample. Analyses were performed in triplicate.

3.2.1.8 *Determination of total protein content and amino acids content*

Total protein content of dried green coffee seeds and coffee husks was determined using Kjeldahl's (Jung *et al.*, 2003) method. A sample rate was subjected to acid-catalysed mineralization to turn the organic nitrogen into ammoniacal nitrogen and, subsequently, was distilled in an alkaline pH. The ammonia formed during this distillation was collected in a boric acid solution and determined through titrimetric dosage. The final value of ammoniacal nitrogen was multiplied by 6.25. In dried coffee husks and seed samples, the amino acid content was also investigated using the modified procedure employed acid hydrolysis of protein and derivatization of the free amino acids using ethyl chloroformate previously described by (Di

Stefano *et al.*, 2020). Five hundred mg of sample were digested with 2 mL of 6M HCl at 110°C for 24 h. After cooling, 1 mL of distilled water was added, and supernatant was filtered with 0.45 μm PTFE filters. One hundred μL of the filtrate was taken and placed in a vial and 100 μL of a 4:1 solution of EtOH-Pyridine, 20 μL of ethylchloroformiate, 100 μL of 1% ethylchloroformiate in chloroform and 40 μL of a saturated aqueous solution of NaHCO3 were also added. The lower chloroform phase, which contain the derivatized amino acids, was immediately analysed by GC/MS. Derivatized amino acids have been analysed through an ISQ[TM] 9000 Quadrupole GC-MS System with SLB ®- 5MS fused silica capillary column (30 m x 0.25 mm x 0.25 μm film thickness; Supelco). Helium was used as carrier gas at a flow rate of 1 mL/min. The column temperature program was set at 120 ∘C for 1 min, it ramped at 4 ∘C/min up to 240 ∘C, held for 3 min and increased to 280 ∘C with at a rate of 30 ∘C/min and held for 5 min under isothermal conditions. The mass spectrometer was set to positive electron impact ionization (+EI) mode. Qualitative identification was performed by comparing retention times of samples with a standard mixture of amino acids and comparing their mass spectra with NIST mass spectral library. Quantitative determination of amino acids was performed using calibration curves of standard stock solutions in a range of 0.025 - 0.5 mM. Total Amino Acid (TAA) were expressed in g/100 g of sample.

3.2.1.9 *Determination of caffeine*

Caffeine content in dried coffee husks and seeds was determined by GC/MS analysis with a method reported by (Misto *et al.*, 2021). Two g of each sample, coffee husks and seeds, were added to 0.6 g of $CaCO_3$ and 20 mL of distilled water to boiling. The solution was filtered with filter paper. Caffeine was extracted by chloroform (15 mL x 3) and evaporated by rotavapor. The solid caffeine residue was resuspended and solubilized in 25 mL of chloroform. A further 1:20 dilution of the sample was carried out after injection in GC/MS. Caffeine has been identified and quantified through an ISQ[TM] 9000 Quadrupole GC-MS System (Thermo Fisher

Scientific) in positive electron ionization mode (EI); A Fused Silica Capillary Column (30 m x 0.25mm x 0.25 μm film thickness; Supelco) column was used. Helium was used as carrier gas with a column flow rate of 1 mL/min. The temperature gradient of the GC oven program started at 160°C for 2 min, increased to 280 ◦C at a rate of 15 ◦C/min and held at 290°C for 10 min, giving a total run time of 15 min. An aliquot of 5 μL of the sample was injected into the chromatograph using the split-injection mode (1:10). The temperature of the injector was maintained at 200°C. A calibration curve with standard stock solutions in a concentration range of 10 - 150 ppm was constructed. Results were expressed as g/100 g of sample.

3.2.1.10 Determination of Carbohydrates, Water and Ashes

Ash and water contents in seeds and coffee husks were determined through the procedure described in the Association of Official Analytical Chemists, AOAC. All analyses were performed in triplicate and data were expressed as g per 100 g of sample. The carbohydrate content (TSG) was evaluated using Anthrone's method (Yemm and Willis, 1954). Carbohydrates were first hydrolysed into simple sugars using dilute HCl in hot acidic medium, glucose is dehydrated to hydroxymethyl furfural that with anthrone forms a green-colored product with an absorption maximum at 630 nm.

3.2.1.11 Mineral Content

The contents of K, Na, Ca, Mg, Fe, Cu, Mn, and Zn were determined by atomic absorption spectroscopy following wet mineralization. Briefly, the samples were digested, and approximately 100 mg of dried sample was incubated with 9 mL of 65% (w/v) HNO3 and 1 mL of 30% (w/v) H_2O_2. The temperature was set at 200 °C for 20 min. Once cooled, the digested samples were diluted to a final volume of 50 mL with distilled H_2O. All measurements were performed using an Agilent 4200 MP-AES fitted with a double-pass cyclonic spray chamber and OneNeb nebulizer. The calibration standards were prepared by diluting a 1000 mg/L multi-element standard solution (Sigma Aldrich and Scharlab S.L.) in 1% (v/v) HNO3. Instead, P was

determined using a colorimetric method (Farina *et al.*, 2017). All analyses were performed in triplicate and data were expressed as g per 100 g.

3.2.1.12 *Vitamin Content*

Riboflavin (Vit. B2), Thiamine (Vit. B1), Vit. B3, Vit. B5 and Ascorbic Acid (Vit. C) were quantified. Vit. B1, B2, B3, B5 were respectively extracted in an autoclave with a solution of 0.1 N HCl, and later, after enzymatic treatment, was determined through HPLC (Palazzolo *et al.*, 2012). Quantification was performed via HPLC equipped with a fluorimetric detector.

Ascorbic acid was extracted, with 10 mL of 1% metaphosphoric acid for 45 min at room temperature and filtered through filter paper. The filtrate (1 mL) was mixed with 9 mL of 2.6–dichlorophenolindophenol and the absorbance was measured within 30 min at 515 nm (Gentile *et al.*, 2019b). Vitamin C was quantified using a calibration curve of authentic L-ascorbic acid (0.02–0.12 mg/100 g). All analyses were performed in triplicate and data were expressed as g per 100 g.

3.2.1.13 *Statistical analysis*

Statistical analysis was conducted using R statistical software (R Core Team, 2022)**.** Student's T-test was performed on the raw data to assess the existence of significant differences in the presence of the components in the husks and seed of the coffee fruit.

3.2.2   *Results and Discussion*

3.2.2.1   *Assessment of the climatic variables in the area of observation*

The average yearly weather data for the location during the period 2010-2022 are plotted in **Figure 3.12**. During the lifespan of the plants of the study, the average temperatures ranged from 10°C to 25°C throughout the year. Minimum temperatures reached values close to 0°C repeatedly in the months between December and February, while the maximum temperatures only occasionally exceeded 40°C. The precipitations were mainly distributed in the months

between October and January, for an average cumulated rainfall of 980 ± 138 mm / year and the average relative humidity was always comprised between 60 and 75%. In the native coffee cultivation areas, temperatures range from minimum values of 10°C to maximum values of 29°C and rainfall may vary from 1700 to 2200 mm / year, thus ensuring a very high relative humidity throughout the year (Dubale and Shimber, 2000).
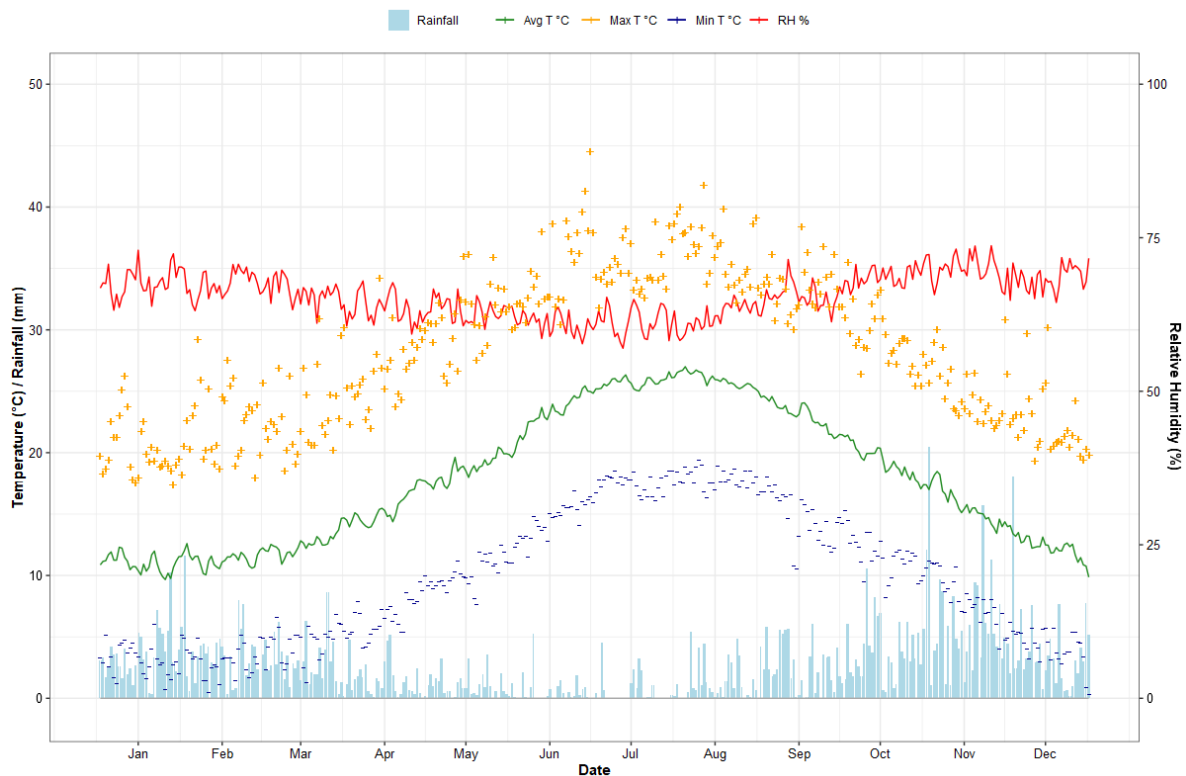
Figure 3.12. Values of daily average temperature, relative humidity and rainfall recorded each day of the year in the study location for the period 2010-2022. The "+" and "-" symbols represent the absolute daily maximum and minimum temperatures recorded for each date, respectively.

Coffee cherries need about 300 days from the moment of flowering until full maturity (Fernandes *et al.*, 2017): during the fruit development period, the maximum temperature was 41.8°C, recorded on 11/08/2021, the lowest temperature was 0.3°C, recorded on 31/12/2021, and total rainfall amounted to 995 mm. The available literature reports contrasting results on the effect of increasing temperature on the quality of coffee quality, in terms of its sensory attributes and the content of phenols, lipids and sugars (Ahmed *et al.*, 2021; Pezzopane *et al.*, 2011). Another significant climatic variable which greatly influences the coffee plant

phenology, and the final cherry and cup quality is the light exposure. The average hourly values of solar radiation (expressed as Global Horizontal Irradiance, GHI) per month of the year in the location of the study are reported in **Figure 3.13**.
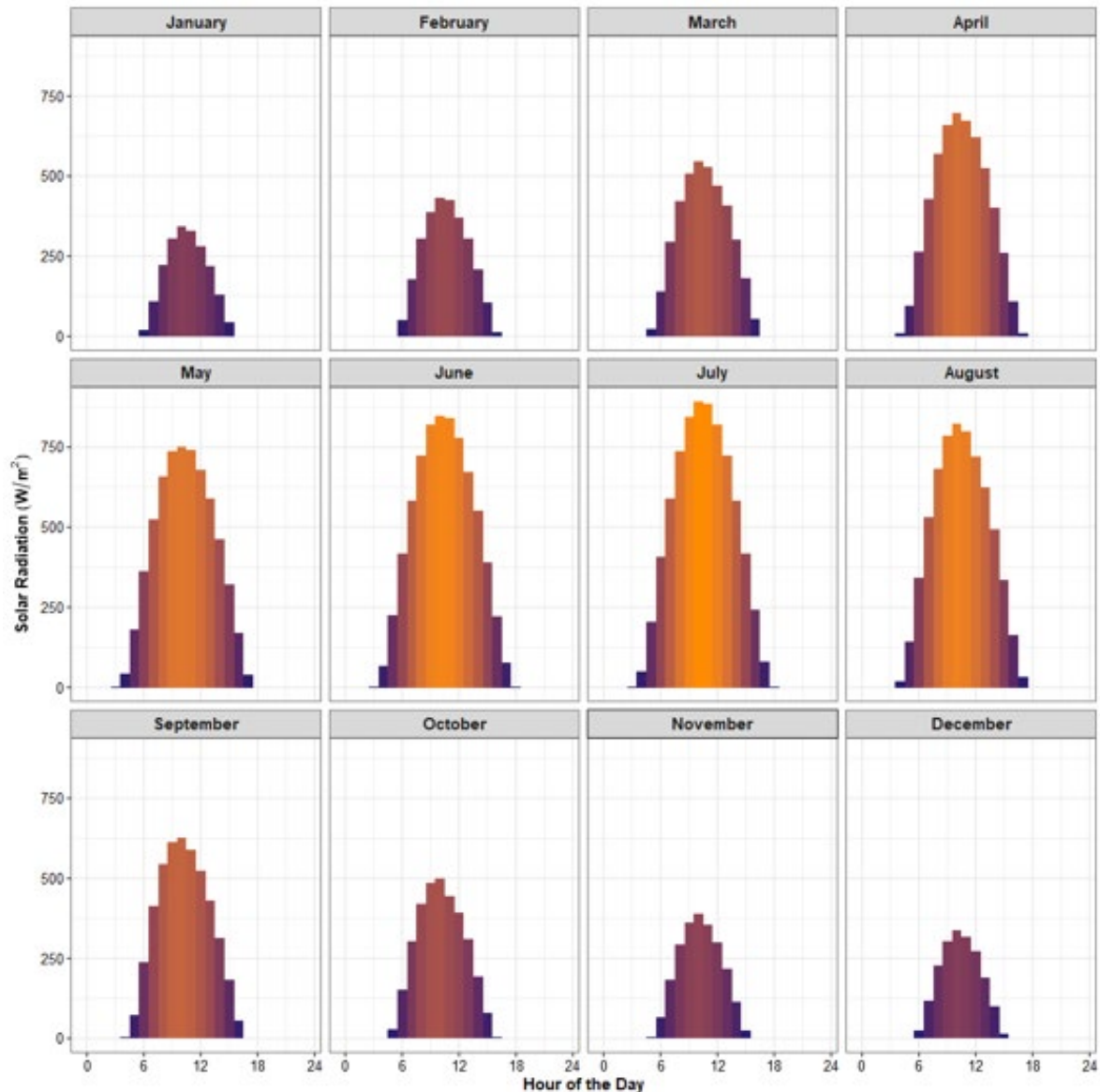
Figure 3.13. Average hourly Solar Radiation values recorded each month of the year in the location of the orchard in the period 2010-2022.

The highest solar radiation values are reached in the summer months, with peaks of 800 W/m^2/h and with the length of the day ranging from 9 to 15 hours/day (**Figure 3.13**). In general, literature reports that increased light exposure has a negative effect on the quality of the coffee cherries, regarding their sensory attributes, but also to their content in alkaloids, phenols, amino acids and minerals. Only the sugar content of the coffee cherries and beans has a positive

relation with the light exposure. However, it is even more important to consider the variation in the solar radiation values throughout the months of the year: a three-fold increase from the darkest to the most light saturated month is observed, while in the countries of the so-called "coffee belt" only a 20% variation occurs among the months of the year (Pezzopane *et al.*, 2011).

3.2.2.2 *Chemical Characterization of the green coffee seeds and coffee husks*

Anti-radical scavenging activity and the total phenolic content of dried green coffee seeds and coffee husks were evaluated, as shown in Table 1. DPPH and ABTS are two assays for the study in vitro of anti-radical activity and commonly used for the evaluation of the scavenger activity towards free radicals. DPPH, in particular, it is a method based on the decolorization of the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) in presence of an electron donor substrate. The discoloration is proportional to scavenging activity and can be monitored by spectrophotometric analysis of the absorbance at a wavelength of 515 nm using a spectrophotometer (Varian Cary® 50 UV-Vis spectrophotometer) using methanol as blank.

Through DPPH and ABTS assay, coffee seeds showed good value of scavenging activity (29.23 ± 0.27 - 26.37 ± 0.33 mmol TEAC/100g respectively) (**Table 3.1**). The total phenolic content (TPC) of green coffee seeds, obtained by Folin-Ciocalteau assay, highlighted also interesting values (67.90 ± 0.29 mg GAE/g). In addition to the seeds, same analyses were carried out for dried coffee husks. Although they are usually identified as a waste product, CH shown good nutritional characteristics and a richness in biophenols, nutritional components and scavenger activity. As also shown in Table 1, the antiradical activity and the TPC of coffee husks was lower than the seeds. Coffee husks showed in particular an antiradical scavenger activity value of 13.55±0.19 mmol TEAC/100g and of 16.80±0.37 mmol TEAC/100g for DPPH and ABTS assay respectively. As it can be seen in the same table, TPC of CH was 42.83±0.27 mg GAE/g.

Table 3.1. Antioxidant activity of green coffee seeds and coffee husks. The results are expressed as mean + S.D. (n = 3). *** symbol indicates statistically significant difference between seeds and husks for Student's t-test at P< 0.001.

	TPC *** mgGAE/g	DPPH$_{TEAC}$ *** mmol TE/100g	ABTS$_{TEAC}$ *** mmol TE/100g
Seeds	67.90 ± 0.29	29.23 ± 0.27	26.37 ± 0.33
Coffee husks	42.83±0.27	13.55±0.19	16.80±0.37

In order to classify the coffee deriving from Sicilian experimental crops, it should be compared with the chemical-nutritional characteristics of tropical coffee deriving from the equatorial countries that fall into the so-called "Coffee Belt", between the two tropics. Similar values have been observed are reported in the literature for coffee berries of *C. arabica* var. Caturra, regarding antioxidant and anti-radical activity. In particular, the measured radical scavenging activity is comparable to data obtained by analyses carried out on coffee of tropical origin: Jeszka-Skowron *et al.* (2017) obtained values similar to coffee seeds cultivated in Mediterranean climate, in a range of 21.39 ± 1.42 - 23.41 ± 1.81 mmol TEAC/100g, using coffee samples from Brazil, Rwanda, China. Results from ABTS assay can be compared with values obtained in seeds by Baeza and Acidri (Baeza *et al.*, 2016) (Acidri *et al.*, 2020): ABTS data were included in a range of 190.09 ± 10.61 - 227.13 ± 10.31 µmol TEAC / g and 220.7 ± 22.4 µmol TEAC / g, respectively.

Oliveira (Oliveira *et al.*, 2019), has found TPC values of the seeds, obtained by Brazilian coffee, comparable to Mediterranean coffee (4048.34 ± 177.05 mg GAE /100g).

Even for the CH it is possible to compare tropical green coffee with Sicilian green coffee, even if the literature in this regard is very poor.

The antioxidant and anti-radical activities of CH have been researched in various works. Arabica coffee husks (var. Caturra) showed ABTS values of 20000 µmol TEAC / 100 g (Delgado *et al.*, 2019), very similar to sicilian coffee and in coffee husks of Bourbon and Caturra varieties values were in a range of 91.49 – 158 µmol TEAC / g (Alemán *et al.*, 2019).

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As shown in **Table 3.2**, dried green coffee seeds had a total fatty acid (TFA) value of 13.85 g/100g, six times greater than that found in dried coffee husks. Main fatty acids in coffee beans were linoleic acid (6.286 g/100g), palmitic acid (4.283 g/100g), oleic acid (1.532 g/100g), stearic (1.197 g/100g). The unsaturated fatty acids (USFA) content usually, is an important guideline to evaluate its nutritional value. Compared to the TFA, green coffee seeds containing more than 50% of USFA (53.16%) and a high content of PUFA (41.64%). Total fatty acid content of dried coffee husks showed very low values (1.84 g/100g) with a % saturated fatty acids (SFA) of 71.59%, and 13.54% of PUFA, related to total. Major fatty acids identified in CH were myristic acid (0.588 g/100g), palmitic acid (0.542 g/100g), linoleic acid (0.234 g/100g), oleic acid (0.237 g/100g).

Table 3.2. Fatty acids composition of dried green coffee seeds and coffee husks (g/100g). The results are expressed as mean + S.D. (n = 3). *, ** and *** symbols indicate significant difference between coffee seed and CH for the relative fatty acid content for Student's t-test at P< 0.05, < 0.01 and < 0.001 respectively.

	Seeds	Coffee husks
Fatty acid	(g/100g)	
Decanoic acid	0.010 ± 0.00038	0.005 ± 0.00075
Undecanoic acid **	0.005 ± 0.00027	0.0036 ± 0.00005
Dodecanoic acid	0.005 ± 0.00029	0.005 ± 0.00164
Tridecanoic acid	0.009 ± 0.00016	0.004 ± 0.00061
Myristic acid *	0.052 ± 0.00274	0.588 ± 0.02087
Pentadecanoic acid	0.010 ± 0.00029	0.005 ± 0.00039
Palmitoleic acid	0.026 ± 0.00119	0.008 ± 0.00058
Palmitic acid	4.283 ± 0.02353	0.542 ± 0.00798
Heptadecenoic acid	0.017 ± 0.00035	0.009 ± 0.00129
α Linolenic acid ****	0.004 ± 0.00015	0.004 ± 0.00003
Linoleic acid ***	6.286 ± 0.01584	0.234 ± 0.00587
Oleic acid	1.532 ± 0.00937	0.237 ± 0.00778
Stearic acid **	1.197 ± 0.01484	0.095 ± 0.00161
Arachidonic acid	0.007 ± 0.00003	0.004 ± 0.00091
Eicosatrienoic acid (ω-6) **	0.019 ± 0.00056	0.006 ± 0.00009
Eicosatrienoic acid (ω-3)	0.064 ± 0.00221	0.005 ± 0.00064
Arachic acid **	0.527 ± 0.00814	0.023 ± 0.00128
Erucic acid	0.016 ± 0.00059	0.008 ± 0.00070
Heneicosanoic acid	0.017 ± 0.00015	0.005 ± 0.00069
Tricosaenoic acid	0.025 ± 0.00082	0.006 ± 0.00202
Nervonic acid *	0.005 ± 0.00024	0.020 ± 0.00036
Lignoceric acid	0.080 ± 0.00299	0.011 ± 0.00138
TFA	13.854 ± 0.00048	1.841 ± 0.00024
% SFA **	46.836 ± 0.01793	71.593 ± 0.225
% USFA **	53.163 ± 0.02789	28.406 ± 0.287
% MUFA	11.5208 ± 0.05277	14.861 ± 0.393
% PUFA ***	41.643 ± 0.02418	13.545 ± 0.199

%SFA = Σ saturated fatty acids/total fatty acid * 100; %USFA = Σ unsaturated fatty acids/total fatty acid; %MUFA = Σ mono-unsaturated fatty acids/total fatty acid; %PUFA = Σ poli-unsaturated fatty acids/total fatty acid.

Green *C. arabica* seeds of different geographical origins (China, Ethiopia, Indonesia, Kenya, Guatemala, Honduras, Colombia, Brazil) have been shown to have %PUFA similar to that of coffee seeds grown in a Mediterranean climate (43.03%-45.98%) (Zhu *et al.*, 2021). A review by Klingel *et al.* highlights the total fatty acid content of arabica coffee husks of about 0.5-3% (Klingel *et al.*, 2020a).

The total protein content was determined by Kjeldhal's method, and an amount of 11.31 g/100 g and 13.05 g/100 g was found in dried seeds and CH respectively.

The amino acids analysis in seeds and CH used a standard protein hydrolysis procedure with 6 M hydrochloric acid. The amino acids, before analysis by GC-MS require a pre-column derivatization. The use of internal and external standards has been found to be essential for the accurate quantification of each amino acid. The amino acid profile of the samples is presented in **Table 3.3**.

Table 3.3. Amino acids (AA) content in coffee seeds and coffee husks expressed as % amino acids/ Total amino acids (% w/ w); total amino acids (TAA), essential (EAA) and no essential amino acids (NEA) expressed as g/100 g. The results are expressed as mean + S.D. (n = 3). *, **, *** and **** symbols next to each AA indicate statistically significant differences between the fruit parts for Student's t-test at $P < 0.05, < 0.01, < 0.001$ and < 0.0001, respectively, for that AA.

	Seeds	Coffee Husks
Amino acids	% w/ w	
*Alanine****	4.32 ± 0.022	4.18 ± 0.014
*Glycine*****	21.69 ± 0.150	17.78 ± 0.159
*Histidine*****	5.45 ± 0.078	4.60 ± 0.042
*Valine*****	3.42 ± 0.019	1.29 ± 0.012
*Glutamine**	7.97 ± 0.031	7.89 ± 0.030
*Leucine*****	0.92 ± 0.032	0.43 ± 0.011
*Isoleucine*****	2.53 ± 0.074	1.36 ± 0.022
*Cystine****	7.04 ± 0.181	6.76 ± 0.023
Proline	1.88 ± 0.013	1.88 ± 0.022
*Asparagine*****	5.91 ± 0.155	2.45 ± 0.019
*Glutammic acid*****	3.58 ± 0.086	14.15 ± 0.122
*Aspartic acid*****	3.58 ± 0.084	9.81 ± 0.102
*Methionine*****	1.69 ± 0.012	2.54 ± 0.018
Threonine	0.11 ± 0.002	0.22 ± 0.041
*Arginine****	3.14 ± 0.078	2.37 ± 0.037
*Serine*****	4.48 ± 0.047	10.78 ± 0.071
*Phenylalanine*****	9.03 ± 0.036	6.38 ± 0.005
*Lysine*****	2.85 ± 0.068	4.77 ± 0.146
*Cysteine****	1.03 ± 0.022	1.16 ± 0.024
*Tyrosine*****	8.03 ± 0.213	0.51 ± 0.058
*Tryptophan*****	1.39 ± 0.014	1.87 ± 0.025
	g/100 g	
TAA	10.56 ± 0.012	11.12 ± 0.0006
ΣEAA	3.99 ± 0.117	3.70 ± 0.015
ΣNEA	5.75 ± 0.149	7.415 ± 0.015

In seeds predominant amino acids were expressed as % amino acids/total amino acids (%w/w). Glycine (21.69% w/w), phenylalanine (9.02% w/w), tyrosine (8.02 %w/w), glutamine (7.97 %w/w), cystine (7.04 %w/w), asparagine (5.90 %w/w), histidine (5.45 %w/w) were the main.

Even in CH, glycine was found to be the amino acid present in higher concentrations (17.78 %w/w), followed by serine (10.78 % w/w), aspartic acid (9.81 %w/w) and tyrosine (8.51 %w/w). The results are shown in Table 3. Total amino acid (TAA) was higher in CH (11.12 g/100 g), as reported in Table 3. Unfortunately, regarding the qualitative and quantitative determination of amino acids in coffee seeds and husks, the literature only reports data for *C. robusta*. To date, there are no data for the amino acid profile of green *Coffea arabica* seeds and husks.

In Ameca *et al.* work's (2018), the total protein content in seeds was investigated in a range of 10.85 – 13.24 g/100 g, in agreement with our results. A review by Klingel *et al.*, (2020b) highlights a range of total protein content of arabica coffee husks of 8-11 g/100 g.

Coffee is the main source of polyphenols in the diet of European populations, and it accounts for up to 40% of polyphenol intake, mostly in the form of chlorogenic, ferulic, and p-coumaric acids. Chlorogenic acids (CGAs), which include many different isomeric forms, are the predominant phenolic compounds in coffee beans. Chlorogenic acids are a family of molecules formed between quinic and cinnamic acids and metabolized into several molecules in the body. The most common chlorogenic acid is 5-O-caffeoylquinic acid (CQA), called simply chlorogenic acid.

The major CGA isomers found in coffee include as 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA) (Farah *et al.*, 2008)

Free phenolic compounds are known to be easily extractable with aqueous/organic solvent. Conversely, bound phenolic compounds, which are covalently bonded to the structural components of plant matrix, cellulose, pectin, polysaccharides, proteins or lipids, need alkaline hydrolysis to be released from the insoluble residues. In addition to their antioxidant activity, the related phenolic compounds have important effects on the inhibition of the growth of cancer cells, as well as in the regulation of inflammatory processes and in the regulation of the microbiota, for this it is important to know also the bound fraction.

Through UHPLC-ESI-MS-have been analysed phenolic compounds. As **Table 3.4** shows, free and bound phenolics were compared, and the major class of biophenols in green coffee seeds was chlorogenic acids. Compounds mostly present in coffee seeds in bound form were isoferulic acid (6.23 g/100g), caffeic acid (1.73 g/100g), while in free form were caffeoylquinic acid (1.63 g/100g) and chlorogenic acid (1.13 g/100g).

Table 3.4. Chlorogenic acids, phenolic acids, anthocyanins, anthocyanidins and flavonoids content in free and bound forms in green coffee seeds and coffee husks. The results are expressed as mean + S.D. (n = 3).

	Seeds		Coffee husks	
	Free	Bound	Free	Bound
		g/100 g		
Phenolic acids				
Caffeic acid	0.009 ± 0.0001	1.73967 ± 0.005	n.d.	0.25173 ± 0.007
m-Coumaric acid	0.0005 ± 0.0003	0.0014 ± 0.0001	0.00017 ± 0.0002	0.00074 ± 0.0001
p-Coumaric acid	0.0005 ± 0.00003	0.02465 ± 0.0004	0.00036 ± 0.0006	0.00874 ± 0.0003
Trihydroxycinnamic acid	n.d.	0.00214 ± 0.0003	0.00217 ± 0.0005	0.00285 ± 0.0002
Ferulic acid	n.d.	0.47372 ± 0.0008	n.d.	0.04566 ± 0.0008
Isoferulic acid	0.0553 ± 0.0002	6.23114 ± 0.002	n.d.	0.99312 ± 0.002
Quinic acid	0.0498 ± 0.0006	0.08159 ± 0.0003	0.17397 ± 0.006	0.03717 ± 0.0006
Chlorogenic acids (CGAs)				
Caffeoylquinic acid isomers*	1.63879 ± 0.003	0.01415 ± 0.0002	0.03761 ± 0.003	0.00385 ± 0.0003
Chlorogenic acid**	1.13663 ± 0.001	0.00579 ± 0.0009	0.01528 ± 0.001	0.00032 ± 0.0004
Feruoyl quinic acid isomers	0.62192 ± 0.0009	0.01028 ± 0.0004	0.04697 ± 0.0008	n.d.
Coumaroyl quinic acid isomers	0.0438 ± 0.0005	n.d.	0.01201 ± 0.0007	n.d.
Dicaffeoyl quinic acid isomers	0.40361 ± 0.005	n.d.	0.05761 ± 0.0007	n.d.
Diferuoyl quinic acid isomers	0.02067 ± 0.001	0.01 ± 0.0003	n.d.	n.d.
Coumaroyl Caffeoyl quinic acid isomer	0.01336 ± 0.0007	n.d.	n.d.	n.d.
Feruoyl Caffeoyl quinic acid isomers	0.05385 ± 0.0009	n.d.	n.d.	n.d.
Dimethoxycinnamic acid	0.00113 ± 0.0002	n.d.	n.d.	n.d.
Dimethoxy cinnamoyl quinic acid isomers	0.01055	n.d.	n.d.	n.d.
Total	4.0592 ± 0.002	8.59453 ± 0.0004	0.34615 ± 0.003	1.34418 ± 0.002
Anthocyanins e anthocyanidins				
Cyanidin	0.0066 ± 0.0002	0.0075 ± 0.0003	0.0084 ± 0.0007	0.00793 ± 0.0002
Cyanidin_isomers	0.00288 ± 0.0006	n.d.	0.00175 ± 0.0005	n.d.
Total	0.00948 ± 0.0002	0.0075 ± 0.0003	0.01015 ± 0.0005	0.00793 ± 0.0002
Flavonols				
Rutin	n.d.	n.d.	0.00507 ± 0.0006	0.00227 ± 0.0004
Quercetin	n.d.	n.d.	0.00221 ± 0.0001	0.00122 ± 0.0001

Quercetin_3-4_diglucoside	n.d.	n.d.	0.00252 ± 0.0002	0.00023 ± 0.00007
Quercetin_3-O_e_7-O_glucoside	n.d.	n.d.	0.00111 ± 0.0006	0.0026 ± 0.0001
Mangiferin	n.d.	n.d.	0.00018 ± 0.0001	0.00099 ± 0.00002
Naringenin	n.d.	n.d.	0.00044 ± 0.00002	0.00007 ± 0.00005
Kaempferol	0.00522 ± 0.0005	0.00567 ± 0.0001	0.00634 ± 0.0003	0.00472 ± 0.0004
Kaempferol_diglucoside	n.d.	n.d.	0.00049	n.d.
Total	0.00522 ± 0.0005	0.00567 ± 0.0002	0.01836 ± 0.0001	0.00601 ± 0.0001

Total chlorogenic acids and phenolic acids (free + bound) quantified by Ciaramelli (2019) were in a range of 112.73 – 132.10 µg/mg from green arabica coffee deriving from Colombia, Brazil, and Burundi.

With regards to the phenolic compounds released in the seeds, according to the study by Baeza *et al.*, levels of total chlorogenic and phenolic acids were found in the range of 59.60-69.345 mg/g for coffee of Colombian, Brazilian, Ethiopian and Kenyan origin (Baeza *et al.*, 2016)

According to Bicho's study (2012) performed on *C. arabica* of Brazilian origin, of the Catuai and Obata cultivars, the levels of total chlorogenic acids were around 4-5 g/100g. The data obtained from the latest study report total quantities of isomers of caffeoylquinic acid in the range of 3.94-6.09 g/100g, slightly higher than those of coffee grown in a Mediterranean climate (2.78 g/100g); the amount of total feruyl quinic acid isomers (0.56-1.9 g/100g) and total dicaffeoyl quinic acid isomers (0.09-0.49 g/100g) determined in green coffee beans of tropical and Mediterranean origin was instead comparable.

Cyanidins were found in trace in Sicilian green coffee seeds, slightly higher in free form with a total ACNs value of 0.0094 g/100g against 0.0075 g/100g in bound form. Even flavonoids in seeds, represented by kaempferol, in both free and bound form were 0.0052 g/100g – 0.0057 g/100g respectively.

The quali-quantitative profile of polyphenols was also evaluated in coffee husks. Chlorogenic acids were lower than seeds both in free and bound form (0.35 - 1.34 g/100 g respectively); also, the main compound found in bound form was isoferulic acid (0.99 g/ 100g) and caffeic acid (0.25 g/100g). Compared to seeds, CH showed a mild abundance of flavonoic fraction, with a total of 0.018 g/100g in free form (almost double compared to the seeds) and

0.006 g/100 g in bound form. The main flavonoid in coffee husks in free form was kaempferol (0.006 g/100g) and anthocyanidins and alkaloids values obtained were comparable to values carried out on the seeds. Trigonelline showed content values very similar to seeds.

Not many studies focused on coffee husks; Clifford & Ramirez-Martinez studied CH samples from Venezuela (Caturra and Bourbon varieties) and found values of chlorogenic acids of 1.01-1.16 g/100 g, very similar to coffee husks of Mediterranean origin. (Clifford and Ramirez-Martinez, 1991).

The presence of caffeine in dried green coffee seeds and coffee husks was also evaluated by GC/MS; dried green coffee husks was found to be lower, with values of 1.55 g/100g compared to seeds (2.26 g/100 g). Awwad *et al.*, (2021) included values of caffeine in a range of 2.03 - 2.54 g/ 100g of coffee from Brazil, Ethiopia, India, similar to those of Sicilian green coffee. Furthermore, caffeine content analysed by Braham & Bressani, (1979) resulted equal to 1.27%. As shown in **Table 3.5**, from the alkaloid group, nicotinic acid and trigonelline have been highlighted, with a major contribution in trigonelline (1.21 g/100g) and minimal in nicotinic acid (0.0014 g/100g). Also diterpenes, represented by the atractyligenin, were found (0.023 g/100 g).

Table 3.5. Nicotinic acid, trigonelline and atractiligenine content in green coffee seed and husks. The results are expressed as mean + S.D. (n = 3).

	Seeds	Coffee husks
	g/100 g	
Alkaloids		
Nicotinic_acid	0.00137 ± 0.0001	0.00264 ± 0.0002
Trigonelline	1.21372 ± 0.03	1.21852 ± 0.02
Diterpens		
Atractyligenin	0.03679 ± 0.001	n.d.

3.2.2.3   *Proximate Composition Analysis*

Proximate compositions of coffee husks have been scarcely published in the literature (Moreira *et al.*, 2018) with a rather large variability of the individual values for lipids (0.5–3%), protein (7–17%), ash (3–7%) and carbohydrates (16–85%) contents. The water content, as shown in

Table 3.6. Proximate composition, mineral and vitamin content of the analysed cherry parts. The results are expressed as mean + S.D. (n = 3). *, **, *** and **** symbols indicate statistically significant differences between the fruit parts for Student's t-test at P< 0.05, < 0.01, < 0.001 and < 0.0001, respectively., was found to be lower in green coffee seeds than in the CH, with value of 11.05 g/100g and 76.04 g/100g, respectively. Carbohydrates (23.77 g/100 g), sugars (9.56 g/100 g), fibers (16.6 g/100 g) and ashes (4.13 g/100 g) were instead higher compared to carbohydrates (3.4 g/100 g), sugars (2.29 g/100 g), fibers (8.29 g/100 g) and ashes (1.10 g/100 g) of coffee fruits husks. The main disaccharide present in the arabica seeds is sucrose (de Melo Pereira *et al.*, 2020), present in percentages between 8 and 9%, similar to the ones that we have measured in the Mediterranean coffee seeds.

Table 3.6. Proximate composition, mineral and vitamin content of the analysed cherry parts. The results are expressed as mean + S.D. (n = 3). *, **, *** and **** symbols indicate statistically significant differences between the fruit parts for Student's t-test at P< 0.05, < 0.01, < 0.001 and < 0.0001, respectively.

	Seeds	Pulp
	g/100g	
*Water****	11.05 ± 0.09	76.04 ± 1.21
*Carbohydrates*****	23.77 ± 0.49	3.40 ± 0.282
*Sugars*****	9.56 ± 0.18	2.29 ± 0.12
*Fiber***	16.61 ± 0.56	8.29 ± 0.0.11
*Ash*****	4.13 ± 0.11	1.11 ± 0.04
Minerals		
Ca	0.13 ± 6.43	0.127 ± 3.78
Fe	0.008 ± 2.00	0.005 ± 0.57
Mg	0.168 ± 7.21	0.123 ± 40.12
*K**	2.017 ± 6.08	1.860 ± 50.06
*Na**	0.078 ± 3.21	0.68 ± 2.08
P	0.170 ± 8.39	0.170 ± 96.89
*Cu**	0.010 ± 0.01	0.008 ± 0.57
Mn	0.006 ± 1.15	0.005 ± 0.58
Zn	0.013 ± 1.15	0.011 ± 1.00
Vitamins		
B1	0.0001 ± 0.01	n.d.
B2	0.0018 ± 0.03	n.d.
B3	0.012 ± 2.65	n.d.
B5	0.0001 ± 0.01	n.d.
C	0.0040 ± 0.01	n.d.

The results obtained from the analysis of minerals and vitamins in dried seeds and coffee husks are also shown in **Table 3.6**. The minerals most present in the coffee seed were K (2.01 g/100 g), P (0.170 g/100 g), Mg (0.168 g/100 g) and Ca (0.133 g/100 g). The mineral content in CH, was found to be similar to seeds. Also, here a higher content in K (1.86 g/100g), P (0.170

g /100 g), Mg (0.123 g/100 g) and Ca (0.127 g/100 g) was highlighted. The obtained values are in the same order of magnitude than those reported in the literature for *Coffea arabica* seeds grown in the coffee-producing countries, with a considerably higher content in potassium and a lower zinc content (Muñiz-Valencia *et al.*, 2014). No statistically significant difference was observed between the fruit parts with regard to the content of any mineral.

The vitamin content in seeds was also investigated, in particular those of group B (B1, B2, B3, B5 and vitamin C) (**Table 3.6**). The results showed very low values of B1 and B5 (0.0001 g/100g) and B2 (0.0018 g/100 g) and higher for B3 (0.012 g /100 g). Ascorbic acid was found to be 0.0040 g/100 g. The vitamin content in the coffee husks showed no quantifiable values. The most present vitamin in the *Coffea arabica* seeds was vitamin B3, amounting to 0.120 g/100g, a value comparable to the range observed in tropical countries (Canzi *et al.*, 2023; Sainn *et al.*, 2019).

The results obtained make it possible to compare the seeds, the portion of the drupe used for the production of coffee powder, and the husks, the portion identified as a waste product.

3.2.3   Conclusions

Our results indicate the possibility to cultivate coffee in the Mediterranean climate obtaining seeds with interesting qualitative traits to fit again into the approach of reusing waste, to enhance their quantity of bioactive molecules. Both the coffee husks and the green coffee seeds have good results in terms of bioactive components. It is possible to say that the crops of Made in Sicily coffee are comparable, from the point of view of bioactive and nutritional components, to those present in cafes of tropical origin, despite growing in very different climatic conditions. This allows to reconsider the cultivation limits for the species and opens wide research possibilities. It is interesting to note that the coffee husks, usually understood as a waste product, contain important quantities of nutritional compounds and can be reused as a source of nutraceutical components, in order to be reused in food fortification processes, in a

perspective of a circular economy. The qualitative and chemical evaluation of the coffee husks intended as a waste product, has highlighted its potential use.

The local Sicilian product will be able to represent a niche product destined for demanding groups of customers who are looking for an exclusive product to live a sensorial and cultural experience. The use of Sicilian coffee could be stimulated by the new demands of consumers who are increasingly looking for a product that has a low environmental impact, high nutraceutical qualities and is both a sensory and taste experience.

3.3 References

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4   Conclusions

Throughout the course of the Ph.D., it was possible to observe, in many cases for the first time, some particular features of the growth of some of the most important tropical species in a totally new environment and climate.

The climatic conditions found in the Mediterranean basin differ greatly from those at which mango is cultivated in subtropical regions, in particular due to a very high daily thermal variability and erratic precipitation patterns. This translates into a significantly different phenological cycle observed in the plants growing in this area, compared to the one known for the species in subtropical or tropical environments. This confirms the plasticity of adaptation of mango to various conditions, and at the same time the wide suitability of the Mediterranean climate. The new findings about the phenology of the mango trees in the Mediterranean conditions are the basis on which new cultivation techniques can be developed.

The protected cultivation systems in which the plants are grown have positive effects on preventing the spread of diseases and disorders, by the use of simple physical means such as windbreaks or application of inert materials like kaolin; at the same time, though, they alter the conditions of growth of plants and fruit. While the cultivation inside plastic greenhouses exposes the mango fruit to noticeable stress conditions, thus lengthening its development period, the best solution is probably represented by less invasive systems such as removable plastic covers or shading nets, which do not alter the plant's and fruit's physiology.

Coffee is the latest species of tropical origin to be introduced in cultivation in the Mediterranean basin: thinking that it will achieve production volumes comparable to the *coffee belt* countries represents a utopia. However, the first observations that we present are encouraging and suggest that even this species can succeed in the new environment and represent a unique, peculiar product which can increase economic opportunities for the farmers.

The coffee plants that we studied in the Mediterranean – even if in greenhouse cultivation – tolerated well the cold temperatures reached during the winter and also survived a late cold spell in the month of April, showing good vegetative development especially across years. Flowering on the same plants was consistent, indicating good production potential.

The coffee beans harvested from adult plants growing in the open air in a typical Mediterranean climate reached a chemical quality absolutely comparable to the one of the fruits obtained in the species' native or traditional cultivation areas. The evaluation of brewing, cup and sensory quality of this coffee requires a longer period of data collection and investigation. However, the tradition of coffee consumption in the Mediterranean countries represents, alone, a strong motivation for experimenting with this species and developing new products.

The beauty of this thesis, finally, lied in the possibility to travel, literally and figuratively, across all the world in the footsteps of the diffusion of these plant species, which transcend borders and cultural differences, and unite people of all origins, from the fields to the table, from the *café* to the *καφενείο* until the *sobremesa*.

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