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PhD in Plant and Animal Production Sciences

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Varietal and eco-physiological adaptation of almond in the Latium region

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SUMMARY

1 General introduction

The almond cultivation has always played a fundamental role in the economies and cultures of the Mediterranean countries (Delplancke et al., 2013). Almond tree is native of central Asia and its cultivation rapidly spread throughout the Mediterranean regions from central Asia during the Greek domination (Zeinalabedini et al., 2010, Di Guardo et al., 2021).

Nowadays Spain has the largest almond surface area with 720,000 ha cultivated in 2021 and an average yield of 0.6 t/ha, while the USA is the largest almond producer in the world with an average yield of 4.7 t/ha and a production of in-shell almond exceeded 2.3 million tons in 2021 produced over a surface of about 500,000 (FAOSTAT, 2022).

This huge yield difference is mainly due to the different growing environments, planting systems, cultivar choice, age of orchards and orchard management techniques between the two major producing countries.

While American almond growing is structured according to intensive and specialised fruit growing models mainly using the high yielding cultivar Nonpareil, almond cultivation in Spain alternates between traditional low-density planting and more recent high-density (HD) and super high density (SHD) plantings. In Spain hard-shelled cultivars are still cultivated as the local varieties Desmayo Largueta and Marcona, appreciated for their high-quality oil and tocopherol contents (Socias i Company and Felipe, 2007). In the early 1970s, some foreign late-flowering cultivars were introduced in Spain, including the Italian cultivars Tuono and Cristomorto, and the French releases Ferragnès and Ferraduel (Gradziel, 2017).

A yearly average of 85,000 tons of in-shell almonds are produced in Italy on a harvested area of about 53,000 ha and average yield/ha of 1.6 tons (Figure 1). In Italy, this nut crop is mainly cultivated in Sicily and Apulia, which are still the main Italian almond-growing areas as confirmed by their production consistency. In 2021 almond production in Sicily reached almost 54,000 tons produced on 32,000 surveyed hectares, while in Apulia production was about 27, 000 tons obtained on 20,000 ha of orchards (Istat, 2022).

In the south of Italy, a large almond germplasm diversity can be found. Since its introduction from Asia (Di Guardo et al., 2021), its cultivation has spread rapidly into that area, and thanks to the selection of local populations carried out over time, a wide genetic diversity has rapidly developed in different cultivation areas (Distefano et al., 2013) favoring the selection of various cultivars still used today. However, according to the yield performance, technological traits of the kernels and the early blooming of these cultivars, their spread in cultivation affected only some of them.

Furthermore, many studies on Italian almond germplasm have been recently carried out (Rigoldi et al., 2015; Distefano et al., 2013; De Giorgio et al., 2007; Monastra et al., 1982). The aims of these studies included phenotyping and genetic characterization of accessions to determine the relationships between them and in order to select agronomic traits to be used for future breeding programs.

Table 1 shows the main accessions present in Sicily, Apulia and Sardinia, almost of them currently cultivated. In Sicily, an almond collection field, called “Museo vivente del mandorlo”, was established in the province of Agrigento in 1997, where around 300 local accessions are preserved (Distefano et al., 2013), while in Apulia over 200 almond accessions are currently retained at the Experimental Agronomic Institute of Bari (De Giorgio et al., 2007).

Table 1. List of the main almond cultivars collected at the “Museo vivente del mandorlo” (Sicily) (Distefano, 2013), Agronomic Experimental Institute of Bari (Apulia) (De Giorgio, 2001) and at the AGRIS agency (Sardinia) (Rigoldi et al., 2015).

Origin	**Genotype**			
Sicily	Acquaviva	Chiarchiara	Mannara du chianu	Pizzutella
	Amara	Chiatta	Mastraciccia	Principato
	Angelica	Cumma	Mennula du vattiu	Pullara (di Fauma)
	Barunissa	Cuore	Mezzalira	Rapparina
	Belvedere	Cupani piccola	Miricanedda	Regina
	Bianculidda di Pezzino	Cuttì	Mullisa grande	Reginella
	Bona	Daduna	Mullisa piccola	Romana
	Bottara	Don Filippo	Nambaredda	Sancisuca
	Bronte 1	Don Peppino	Nerone	Sarbaggia di Sciascia
	Cacciatura	Don Pitrino	Nivera manza	Sciarra
	Cacinova	Don Vincenzo	Nivera selvaggia	Scummissa
	Calamonaci	Enna 2	Nuciddara (Ispica)	Selvatica favata
	Callara	Fascionello	Nuciddara (Naro)	Tabacchina
	Canicattinisa	Fastuchina	Nuciddara (di Fauma)	Tricula
	Castrianisa	Filippazzo	Piatta mollisa	Tuono siciliana
	Catrubba	Fellamasa Casteltermini	Pirsichina	Vaiana (mennula di)
	Cavalera	Giardinella	Pizzuta d’Avola	Villana
	Cavaliere	Lisciannarisa	Pizzuta grande	Zagarrì
Apulia	A Grappolo	Della Madonna	Mincone	Pulita
	Albanese	D’Aloia	Mollese di Canneto	Putignano
	Antonio De Vito	Falsa Barese	Monaca	Rachele
	Banchiere	Falsa Catuccia	Montrone	Rachele Tenera
	Barese	Ferrante	Naturale di M. Vella	Rachelina
	Barlettana	Ficarazza	Nocella	Rana Gentile
	Cacciola	Fico D’India	Occhio D’Argento	Reale
	Caporusso	Filippo Ceo	Occhio Rosso di Trani	Riviezzo
	Caputo	Fragiulietta	Pappamucco	Santeramo
	Catalini	Fragiulio	Pastanella	Santoro
	Catuccia	Franciscudda	Pavone	Scorza Verde
	Catucedda	Galgano	Pendulo	Senz’arte
	Centopezze	Genco	Pepparuddo	Trianella
	Ciavea	Giunco di Cozze	Pettolecchia	Tribuzio
	Cicerchia Amara	Irene Lanzolla	Piangente	Tuono
	Cinquanta Vignali	Lorenza Tribuzio	Pignatidde	Viscarda
	Cosimo di Bari	Mancina	Piscalze	Vuoi o non Vuoi
	Cristomorto	Marchione	Pizzutella	Zanzanidde
Sardinia	Antioco Pala	De Mrasciai	Lutzeddu	Rebeccu 3
	Antoni Piras	Efisi Sinzoba	Malissa Tunda	Riu Loi
	Arrubia	Emilio 91	Niedda I	Schina de Porcu
	Basibi	Farci	Niedda II	Stampasaccusu
	Bianca	Farrau	Nuxedda	Sunda G.
	Bocchino	Fiori	Olla	Sunda N.
	Ciatta Inglese	Folla 'e Pressiu	Orri	Troito A
	Ciatta Malissa	Franciscu	Pitichedda	Troito B
	Corrochina	Ghironi	Provvista	Vargiu
	Cossu	Ibba	Rebeccu 1	Vavani Perra
	De Efisi Sinzoba	Is Stumbus	Rebeccu 2	

The nut market is currently experiencing a period of steady growth both in terms of consumption and production (INC, 2019). As a result, interest in almond cultivation has recently increased, especially in suitable Mediterranean countries, involving both growers and confectionary industry.

According to this trend, almond cultivation is also expanding in Italy, both in traditional and newly introduced areas such as in Latium region. Furthermore, there is a progressive replacement of old orchards in favor of specialized SH and SHD plantings managed with high mechanization levels and following the guidelines given by the Spanish almond sector, which contribute to an increase in average per-hectare productions (Maldera et al., 2021).

On the Latium coast, one of the main areas recently affected by the spread of Italian almonds, while in 2017 the almond orchards were only 40 ha, five years later the surveyed orchards had reached of about 450 ha and 150 of which are yielding since 2020. New plantations are specialized and designed at high-density, often drip irrigated, introducing intermediate-late and late flowering cultivars as Tuono, Lauranne®Avijor, Penta®, Soleta, Guara and Makako®.

The above-mentioned cultivars in this introducing area are mainly grafted on interspecific hybrid GF-677 and Garnem (Rubio-Cabetas et al., 2017), although there is no shortage of attempts to use dwarfing rootstocks such as the Rootpac® series, Rootpac®20 (*P. cerasifera* x *P. besseyi*) overall, according to their low vigor and early bearing influence, especially when used in SHD planting systems (Vahdati et al., 2021).

The new crop introduction in a suitable environment needs an accurate preliminary assessment to guide the cultivars choice according to the market requests, of the planting layouts, and of the more suitable orchard management techniques to be applied. For instance, the new trend in introducing HD and SHD orchard cultivation models emphasizes the risks of environmental pollution and reduction of ecosystems and their associated biodiversity.

Conversely, extensive agroecosystems as the oldest Italian almond orchards, in some cases still managed as intercropping, even if they contribute to preserving agricultural biodiversity, often release unsatisfactory productions. According to these agroecosystems criticalities in Italy new models of sustainable orchard intensification

(SI) are being developed devoted to increase yields without determining high impact on the environment (Buckwell et al., 2014). This new approach is interesting also almond and should be promoted particularly in areas where the crop is newly introduced, such as in central Italy.

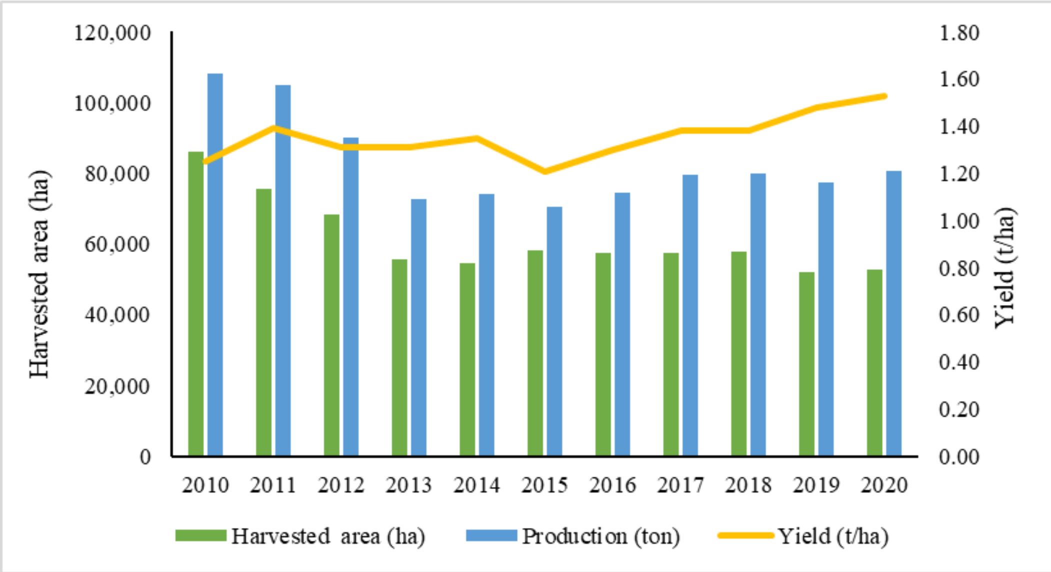

Figure 1. Evolution of almond orchards in Italy from 2010 to 2020: harvested area, in-shell almond production and average yield per hectare.

1.1 Aims and scopes of the research and thesis structure

The almond tree is a historical crop in southern Italy, where it has been cultivated since ancient times. Innovations in cultivation techniques, late-flowering varieties released by breeding, the growing almond market demand and climate changes have contributed to extend almond cultivation in non-traditional areas. According to this trend, in Latium the development of new almond chain is taking place, although knowledge about its adaptation in these new growing areas is still poor.

Based on this evidence, the aims of this thesis were i) to assess the agronomical behavior of almond cultivars in the new introducing area, ii) to develop suitable protocols for innovative nutrition strategies through specific cultivar-site foliar diagnostics and iii) to provide technical and scientific support to the stakeholders. The activities conducted during the PhD course have been organized into three main research tasks, described in chapters as follow:

Chapter I - **"Evaluation of phenological and agronomical traits of different almond grafting combinations under testing in central Italy".** Two different trials have been carried out: the first trial was focused on phenological, and agronomic influences induced by the clonal rootstock GF677 on the grafted cultivars Tuono, Supernova and Genco, compared to those induced by the peach seedling rootstock, in order to identify the best grafting combination to develop "high density" plantings in the new growing area. The second trial tested the phenological and agronomical influences induced by three different clonal rootstocks (GF677, Rootpac® 20 and Rootpac® R), on the Spanish cultivar Guara to identify suitable dwarfing rootstock devoted to "super high density" orchards in the same environment.

An article on the subject was published in a peer-reviewed journal (https://doi.org/10.3390/agriculture11121252).

Chapter II - **“Development of a new protocol for a sustainable foliar nutrition in almond orchards”.** In this trial, traditional soil fertilization has been compared with total foliar nutrition in almond orchards. A three-year investigation has been conducted, with the aim to evaluate the effect of different nutrition management approaches on four-year-old commercial almond orchard cultivated with cultivar Tuono. Four applications of two different foliar fertilizers containing macro- and micro-nutrients have been administered, and the effects of foliar nutrition were evaluated in the second year of the trial by means of foliar diagnostics, computation of DOP index for each nutrient, measurement of eco-physiological traits such as leaf chlorophyll and flavanols levels and the nitrogen balance index, as well as agronomic traits. The scope was to develop a sustainable protocol for nutrition in almond orchards, limiting the environmental pollution related to the soil nutrition applications.

An article on the subject was published in a peer-reviewed book series (https://doi.org/10.17660/ActaHortic.2022.1333.40).

Chapter III - **“Cultivar-specific assessments of almond nutritional status through foliar analysis”**. The trial aimed to assess the nutritional status of the almond cultivars recently introduced in central Italy through leaf analyses and diagnostics, supported by non-destructive measurements of leaf biometrical and eco-physiological traits such as content of total chlorophyll, flavonols, anthocyanins and nitrogen balance index, using a hand-held meter (Dualex®). During the growing seasons 2019-2020 leaf samples of almond cultivars were collected at 90 and 120 days after full bloom (DAFB) and analyzed for traits mentioned above. Furthermore, the cultivars were tested for their phenological behavior through the research-years. The final purpose of the trial was to release specific reference values of leaf macro- and micronutrients of almond cultivars according to their phenological stages, to be used as monitoring of the nutritional status and health of the plants.

An article on the subject was published in a peer-reviewed journal (https://doi.org/10.3390/horticulturae8090822)

2 CHAPTER I

Evaluation of phenological and agronomical traits of different almond grafting combinations under testing in central Italy

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Abstract: In the new introducing almond areas, it is necessary to test the more promising almond cultivar and rootstock combinations able to guarantee the best agronomic performances according to the specific pedoclimatic conditions. With this aim, two almond trials have been established in an experimental farm located in the Latium region (Italy). The first trial (A) focused on the phenological, and agronomical influences induced by the clonal rootstock GF677 on the grafted cultivars Tuono, Supernova and Genco, in comparison to those induced by peach seedling rootstocks, in order to identify the best grafting combination for developing "high density" plantings in this new growing area. The second trial (B) tested the phenological and agronomical influences induced by three different clonal rootstocks (GF677, Rootpac® 20 and Rootpac® R), on the Spanish cultivar Guara to identify suitable dwarfing rootstocks for "super high density" plantings in the same environment. Flowering and ripening calendars of the trial A highlighted as the medium-late flowering cultivars Genco, Supernova and Tuono could be subject to moderate risk of cold damages. The clonal rootstock GF677 seems to anticipate flowering and vegetative bud break by a few days in Tuono when compared to the same cultivar grafted on peach seedling rootstocks. Furthermore, the yield per plant was always higher in plants grafted on GF677. The observations carried out in trial B highlighted as the flowering of cultivar Guara were affected by the rootstock, with Rootpac® 20, which postponed its full bloom of about one week when compared to other rootstocks,

whereas GF677 imposed more vigor to the cultivar than Rootpac® 20 and Rootpac® R.

Keywords: *Prunus dulcis* (Mill.) D.A.Webb; clonal rootstocks; blooming; fruit-set; yield efficiency.

1. Introduction

The almond tree (*Prunus dulcis* (Mill.) D.A. Webb) is the most widely cultivated and economically important nut species in the world (de Los Cobos et al., 2021). About 30% of human nut consumption concerns almonds, followed by walnuts (20%), cashews, and pistachios (Nut & Dried Fruit, 2019). According to FAO data (Faostat, 2021) the cultivated area is about two million hectares, mainly located in Spain, the USA, and Australia, whereas the almond production is mainly achieved in California (USA), where two million tons of in-shell almonds were harvested in 2019. Europe is the main consumer of almonds, followed by North America and Asia (INC, 2020). In the Mediterranean basin, temperate nuts have always been an essential food source, thanks to their nutritional value and proven potential to reduce human degenerative diseases (Salas-Salvadó et al., 2011), their high adaptability in this environment, and to their storage over long periods (Sottile et al., 2020). In addition, almond cultivation over the years in this region has contributed to the selection of the most widespread cultivars, such as Tuono, grown by almond growers to date (de Los Cobos et al., 2021). The traditional production of almonds in Italy was mainly based on growing local varieties often characterized by hard shell nuts. The resulting low yield of shelled almonds and the lack of production uniformity in terms of nut and kernel traits contributed to the shrinkage of domestic market demand from the second half of the 1900s, also accompanied by an increase in almond cultivation in Spain and California (Anania et al., 1999). For some years, almond cultivation has been attracting renewed interest from the growers so much that, in Italy, a planting increase of new almond orchards has recently been recorded, designed with high levels of specialization (ISTAT, 2021). In the growing areas of the Mediterranean basin including Italy and Spain, almond tree was considered to be a species suitable of being cultivated in poor soils characterized by low water availability (Álvarez et al., 2020); for this reason, the existing older almond orchards are mainly located in marginal areas, often inter-cropped with other crops, and usually give low yielding (López-López et al., 2018).

Almond cultivation involves the use of rootstock, and the oldest orchards were made by grafting the main cultivars on seedling-derived rootstocks, considered more suitable for poor soils (Rubio-Cabetas, 2016; Bielsa et al.,2016), as the seedling was able to explore the soil and capture water and nutrients, not only in the topsoil, but also in the deeper layers (Vahdati et al.,2021). Thus, the agronomic approach to the use of grafted plants involved almond cultivation as well as other nut species such as walnut Rezaee et al., 2008), chestnut and, more recently, hazelnut. This first almond rootstock generation was mainly selected from the seed of bitter almonds or peaches harvested from plants considered to be resistant to root knot nematodes (*Meloidogyne* spp.). However, these rootstocks showed several limitations, such as the susceptibility to iron chlorosis and to root knot nematodes (Dirlewanger et al., 2004). The most successful clonal rootstock for almond is still represented by GF677 and more recently by Garnem, two inter-specific hybrids of *P. dulcis* × *P. persica* (Rubio-Cabetas et al., 2017). Between the two clonal rootstocks, the first one, selected in the 1950s by the INRA (Bordeaux- France), is a vigorous rootstock able to reduce the plant juvenility and promote a high and constant yearly cropping. Furthermore, when compared to seedling rootstocks, it better tolerates the waterlogging, and it is more resistant to iron chlorosis. Its greatest disadvantage is the unsuitability for almond replanting (Rubio-Cabetas, 2016). The red leaf rootstock Garnem, released by CITA (Zaragoza-Spain) in 2000 (Felipe, 2000), is characterized by similar vigor to GF677 (Zarrouk et al., 2005), as well as for the iron chlorosis tolerance, and showed a higher drought tolerance. This clonal rootstock also shows high resistance to the main root knot nematodes species affecting *Prunus spp*., and it is suitable for almond replanting. However, it shows low tolerance to root hypoxia caused by waterlogging (Rubio-Cabetas, 2016; Pinochet, 1997; Esmenjaud et al., 1997). Due to the increasing interest in almond cultivation, both in traditional and new growing areas, often following the guidelines of "high density" (HD) and "super high density" (SHD) orchard design and management, it is necessary to test and release new almond rootstocks able to keep plants of small size, which are easier to manage mechanically. Currently, the SHD almond orchards are very popular in Spain, and they have started attracting the interest of Italian growers, not only in the traditional Italian almond regions such as Apulia and Sicily, but also in new introducing areas, as in Latium (central Italy) (Maldera et al., 2021). The most used clonal rootstocks for SHD almond orchards are the Rootpac® series, derived from the hybridization of *P. besseyi* × *P. cerasifera*

(Pinochet, 2010; Gasicet al., 2020) and released by the Spanish Agromillora group (Barcelona, Spain) in 2011. Within the Rootpac® series, the Rootpac® 20, also named Densipac, is the most spread for grafting almond in SHD orchards since it is characterized by low vigor and prompt compact growth habits to the grafted plants (Maldera et al., 2021). Furthermore, it seems to be tolerant to calcareous soils and asphyxia, and moderately resistant to *Meloidogyne* spp. (Rubio-Cabetas et al., 2017). Based on this evidence, which concerns the development of new almond chains both in traditional and new growing areas, the aim of this study was to test the phenological and agronomical behavior of different almond cultivar and rootstock combinations in the coastal area of Central Italy (Latium region), where the almond cultivation is increasing very quickly (Istat, 2021).

2. Materials and Methods

2.1. Plant Material and Trials Site

Two trials have been established and carried out over two experimental almond orchards planted in late 2017 (trial A) and late 2018 (trial B), respectively, in the experimental farm of ARSIAL (Regional Agency for Innovation and Development of Agriculture in Latium), located in the municipality of Tarquinia (Latium region - Italy. Latitude 42°16′20″ N; longitude 11°42′26″ E; altitude 32 m a.s.l.). The first trial (A) focused on the phenological and agronomical influences induced by the clonal rootstock GF677 on the grafted cultivars Tuono, Supernova and Genco, in comparison to those induced by peach seedling rootstocks on the same cultivars, with the aim to identify the best combination of rootstock GF677 and late flowering almond cultivars. This agronomic knowledge has great interest in exploring the eligibility of these cultivars to achieve HD plantings in this new growing area. The second trial (B) tested the phenological and agronomical influences induced by three different clonal rootstocks (GF677, Rootpac® 20 and Rootpac® R, respectively), on the Spanish cultivar Guara, to guide the development of new SHD plantings in the same environment. Although Guara and Supernova are considered two different commercial varieties and are marketed as such, recently some studies have highlighted that they may both be synonyms of the cultivar Tuono (Marchese et al., 2008; Di Stefano et al., 2013; Dicenta et al., 2015). Each grafting combination of the two trials was represented by 15 grafted plants spaced at 6 m × 5 m and trained in a vase shape and managed by applying an integrated pest and disease control. The planting layout of trial B, even if aimed at future SHD orchards establishment, was chosen specifically wide to check the effect of the dwarfing rootstocks in the absence of punctual competition between adjacent plants in the row, thus exploring their intrinsic physiological-agronomic influence on the selected cultivar. The orchard was drip irrigated, and the amount of water supplied was about 1500 m^3 ha^{-1} $year^{-1}$. The soil had a clay-loam texture, a pH of 7.6, and a total organic matter content of 1.9%. The average climatic parameters recorded during the growing seasons of the trials (years 2018–2021) are reported in Table 1.1. On average, the annual rainfall in the site was about 840 mm. July was the critical month with very low rainfall, the highest average value of maximum temperature, and a high daily evaporative demand. Furthermore, the more critical climatic events occurred in late February 2018, where night temperatures of 28th fell below −5 °C without causing damages on young plants still unproductive and characterized by the presence of very few swelling or breaking fertile buds, and in early spring 2020, when during the night of 24 March, the minimum temperature fell below −2 °C, compromising almost the entire fruit set, and during the nights of 8 and 9 April of the growing season 2021, when the minimum temperature fell below −1 °C, without causing frost damage to the developing fruits.

Table 1.1. Minimum (t), maximum (T) and mean monthly temperature (mean T) and sum monthly precipitation (rainfall) at the experimental farm ARSIAL-Tarquinia (Viterbo province-Italy) in the period February to September, of the four years of investigation (2018–2021).

Month	**Year**	**t** (°C)	**T** (°C)	**Mean T** (°C)	**Rainfall** (mm)
February	2018	5.3	12.3	7.1	157.1
	2019	4	15.5	9.8	53.4
	2020	8.4	12.9	10.1	31.4
	2021	7.2	11.5	9.8	76.1
March	2018	6.8	14.1	10.3	230.0
	2019	5.9	17.8	11.9	4.2
	2020	8.8	12.9	10.5	65.7
	2021	8.6	11.2	9.5	163.3
April	2018	10.9	20.1	15.9	80.1
	2019	7.5	18.9	13.5	72.0
	2020	10.4	16.0	13.1	72.3
	2021	11.4	15.1	12.8	156.1
May	2018	16.3	21.9	20.1	83.1
	2019	12.2	19.8	15.1	70.2
	2020	17.5	19.2	18.3	19.2
	2021	15.4	17.9	16.1	45.3
June	2018	20.8	22.4	21.6	37.1
	2019	16.6	30.1	23.7	5.5
	2020	18.9	22.9	21.1	25.1
	2021	20.9	22.7	21.8	67.2
July	2018	23.4	27.1	25.3	31.0
	2019	18.8	30.7	25.4	19.8
	2020	23.5	26.5	24.4	25.9
	2021	22.3	25.8	24.1	29.4
August	2018	23.3	27.8	25.1	59.9
	2019	19.2	31.1	25.5	0.0
	2020	24.1	28.3	25.5	49.3
	2021	23.9	27.2	25.3	14.2
September	2018	19.1	24.4	21.7	56.1
	2019	16.9	28.1	22.3	103.3
	2020	17.4	26.1	21.2	112.5
	2021	19.7	22.9	21.5	53.5

2.2. Phenological observations

Phenological traits of each grafting combination were recorded weekly from February until September, starting in 2018 for trial A, and in 2019 for trial B. The survey of the phenological stages has been carried out according to the BBCH scale adapted for almond (Sakar et al., 2019), and with the support of field-captured images. These phenological remarks allowed the development of specific cultivar-site blooming phenograms and ripening calendars.

2.3. Leaf Chlorophyll, Flavonols and Anthocyanins Content and Nitrogen Balance Index

Five plants for each grafting combination were chosen in both trials, and twenty leaves per plant (two measurements per leaf) randomly selected from non-fruiting twigs were subjected to measuring eco-physiological parameters such as total chlorophyll (Chl), flavonols (Flav) and anthocyanins (Anth), using a hand-held meter (FORCE-A, Dualex®, Orsay, France). The instrument also automatically determines the nitrogen balance index (NBI), using the ratio between Chl and Flav (Wijewardana et al., 2018; Silvestri et al., 2019; Socias I Company et al., 2004). The measurements, carried out for three consecutive years starting in the growing seasons 2019, were taken 90 days after full bloom (DAFB).

2.4. Fruit Set, Production, Yield Efficiency and Nut Traits

The incidence of fruit set was determined during the last year of investigation for both trials, since in the early growing seasons, the nuts production was inconsistent due to the plant juvenility, whereas during the growing season of 2020, the frost damages occurred in late April and compromised the first cropping. Four fruit-bearing branches per plant were selected, one for each cardinal point, and the number of flowers per branch was counted during the full bloom. The fruit set incidence was determined at the end of April according to Socias i Company et al. (2004), when the plants were at stage 81 (fruit at full size) of the BBCH scale (Sakar et al.,2019). Yield per plant was determined in the growing season 2021 by harvesting all nuts from the 15 plants for each grafting combination. Furthermore, at the end of each growing season, the trunk cross sectional area (TCSA) of each plant was measured at 30 cm above the ground (above the grafting point), and the yield efficiency (YE), expressed as the ratio between yield and TCSA, was calculated for the year 2021. During the last year of the trials, nut and kernel traits were also recorded, testing a sample of 200 nuts per grafting combination (Felipe, 2000).

2.5. Statistical Analysis

The data collected over the period of the trials have been subjected to a two-way ANOVA to validate the "rootstock" effect. Differences have been accepted as statistically significant when $p < 0.05$, and mean separation was done using Fisher's test. Percentages were subjected to angular transformation according to the formula (x + 0.5) 1/2 before data analysis. All data were processed in XLSTAT, a statistical software for Microsoft Excel.

3. Results of trial A

3.1. Blooming and Ripening Phenograms

Figure 1.1 presents the blooming and ripening phenograms of trial A. The earliest flowers emergence was observed in early February 2018 on a few fertile buds of the young plants of Supernova grafted on seedling rootstocks, where the full bloom occurred in late February. Its full bloom was earlier by about 10 days when compared to other grafting combinations in the same year. Nevertheless, only slight differences were noticed for the studied cultivars regarding the influence of GF677 on flowering time, except for cultivar Tuono, that in 2019 and 2020, showed its full bloom stage about one week before for plants grafted on seedling rootstocks. In general, the full bloom of the three cultivars studied was concentrated in the first half of March. The clonal rootstock GF677 had more influence on almond ripening that was anticipated of about one week for cultivars Tuono and Supernova grafted on GF677. This evidence was more marked in the growing season 2021, when the plants were aimed towards physiological maturity. The cultivar Genco had later fruit maturity without differences induced by rootstock and showing the start of ripening in the first half of August and a full maturity (hull spill 100%) occurred in the second half of September, about one week later than other cultivars (Figure 1.1).

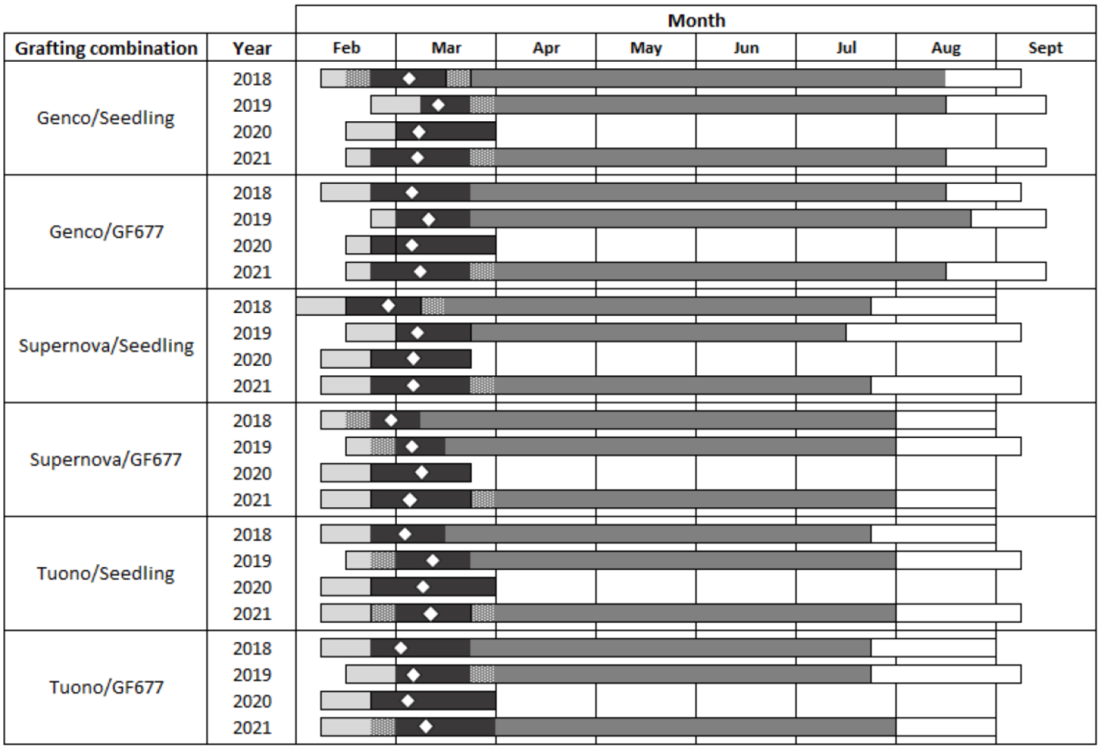

Figure 1.1. Phenograms of blooming and ripening recorded over four years of investigation (2018–2021) for cultivars Genco, Supernova and Tuono grafted on clonal rootstock GF677 and seedling rootstocks, respectively (light grey histogram = beginning flowers emergence; dark grey histogram = blooming; white dots = overlapping of successive phenological stages; mid-dark grey histogram = fruit development; white histogram = fruit maturity: 1% hull split).

3.2. Leaf Chlorophyll, Flavonols and Anthocyanins Content and Nitrogen Balance Index

Leaf chlorophyll, flavonols and anthocyanins content and nitrogen balance index (NBI) of cultivar Genco detected at 90 DABF and expressed as yearly mean values and as three years' investigation mean values, from 2019 to 2021 (Table 1.2), did not show significant differences between plants grafted on GF677 and those grafted on seedling rootstocks. Similarly, no differences were observed for the cultivar Supernova, with the exception for anthocyanins that showed three years mean values of 0.11 μg cm^{-2} in plants grafted on seedling rootstocks versus mean values of 0.09 μg cm^{-2} in plants grafted on GF677. Conversely, all leaf eco-physiological parameters monitored for cultivar Tuono showed slight rootstock-related differences, which were most relevant for the chlorophyll content and NBI and characterized by three-year mean values of 29.55 μg cm^{-2} and 13.89 in plants grafted on GF677, versus mean values of 28.36 μg cm^{-2} and 12.77 recorded in those grafted on seedling rootstocks.

Table 1.2. Content of total chlorophyll (µg cm^{-2}), flavonol (µg cm^{-2}) anthocyanin (µg cm^{-2}), and nitrogen balance index (NBI) in leaves collected from plants of trial A. Data are reported as mean ± standard deviation per year, and as three-year mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between treatment and year (Fisher's test $p < 0.05$).

	Rootstock	Year	Chl	Flav	Anth	NBI
Genco	**GF677**	**2019**	26.08 ± 3.62 b	2.23 ± 0.20 ab	0.08 ± 0.02 b	11.76 ± 1.71 bc
		2020	26.86 ± 4.76 b	2.29 ± 0.10 a	0.09 ± 0.03 b	11.91 ± 2.30 bc
		2021	27.38 ± 3.29 b	2.23 ± 0.10 ab	0.08 ± 0.02 b	12.44 ± 1.66 b
	Seedling	**2019**	21.70 ± 4.69 c	2.29 ± 0.10 a	0.12 ± 0.04 a	9.46 ± 2.40 d
		2020	33.01 ± 6.05 a	2.21 ± 0.10 b	0.07 ± 0.03 b	15.00 ± 2.81 a
		2021	25.21 ± 3.81 b	2.28 ± 0.10 a	0.11 ± 0.03 a	11.09 ± 1.94 c
	Average GF677		*26.78 ± 3.97*	*2.25 ± 0.10*	*0.08 ± 0.03*	*12.04 ± 1.92*
	Average Seedling		*26.64 ± 6.83*	*2.26 ± 0.10*	*0.10 ± 0.04*	*11.85 ± 3.33*

	Rootstock	Year	Chl	Flav	Anth	NBI
Supernova	**GF677**	**2019**	26.96 ± 5.45 c	2.25 ± 0.11 ab	0.08 ± 0.03 cd	11.97 ± 2.57 c
		2020	29.12 ± 5.32 b	2.21 ± 0.15 b	0.09 ± 0.04 bc	13.23 ± 2.63 b
		2021	26.58 ± 3.23 c	2.24 ± 0.12 ab	0.10 ± 0.03 b	11.85 ± 1.68 c
	Seedling	**2019**	19.82 ± 4.65 d	2.29 ± 0.12 a	0.15 ± 0.05 a	8.42 ± 2.09 d
		2020	34.82 ± 5.95 a	2.20 ± 0.11 b	0.07 ± 0.03 d	15.89 ± 2.87 a
		2021	26.86 ± 4.05 c	2.23 ± 0.10 b	0.10 ± 0.03 b	12.01 ± 1.89 c
	Average GF677		*27.55 ± 4.89*	*2.23 ± 0.13*	*0.09 ± 0.03 b*	*12.35 ± 2.41*
	Average Seedling		*27.16 ± 7.87*	*2.24 ± 0.11*	*0.11 ± 0.05 a*	*12.11 ± 2.83*

	Rootstock	Year	Chl	Flav	Anth	NBI
Tuono	**GF677**	**2019**	28.37 ± 3.71 b	2.15 ± 0.14	0.06 ± 0.02 c	13.22 ± 1.96 b
		2020	32.68 ± 5.47 a	2.11 ± 0.19	0.07 ± 0.03 bc	13.55 ± 2.67 a
		2021	27.56 ± 4.53 b	2.16 ± 0.15	0.09 ± 0.03 a	12.92 ± 2.43 b
	Seedling	**2019**	23.63 ±4.10 c	2.25 ± 0.12	0.09 ± 0.03 a	10.31 ± 2.01 c
		2020	33.06 ± 7.45 a	2.17 ± 0.13	0.08 ± 0.04 ab	15.24 ± 3.35 a
		2021	28.41 ± 3.07 b	2.22 ± 0.11	0.08 ± 0.02 ab	12.75 ± 1.68 b
	Average GF677		*29.55 ± 5.14 a*	*2.14 ± 0.16 b*	*0.07 ± 0.03 b*	*13.89 ± 2.64 a*
	Average Seedling		*28.36 ± 6.48 b*	*2.21 ± 0.13 a*	*0.08 ± 0.03 a*	*12.77 ± 3.17 b*

Analyzing the annual mean values of the eco-physiological parameters, in 2019, chlorophyll and NBI were significantly higher in grafting combinations on GF677 for all cultivars investigated, while in 2020, the values of these traits were higher in plants grafted on seedling rootstocks. In the third year of the trial, the parameters investigated have been more uniform, showing only slight differences between the grafting combinations, for all three cultivars.

3.3. Fruit Set Incidence

The incidence of fruit set, determined following the literature guidelines (Sakar et al., 2019; Socias I Company et al., 2004), was estimated in late April 2021 for trial A, as reported in Table 1.3.

Table 1.3. Incidence of fruit set in cultivars Genco, Supernova and Tuono grafted on clonal rootstock GF677 and seedling rootstocks (trial A), calculated at the end of April 2021. Data are reported as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between rootstocks (Fisher's test $p < 0.05$).

Cultivar	Rootstocks	Number of flowers	Number of fruits at full size	Fruit set (%)
Genco	**Seedling**	484	179	36.01 ± 7.96 a
	GF677	577	169	29.21 ± 7.78 b
Supernova	**Seedling**	585	228	38.37 ± 8.42
	GF677	579	195	34.06 ± 7.75
Tuono	**Seedling**	577	190	32.72 ± 5.02
	GF677	552	175	30.71 ± 7.35

The fruit set recorded in trial A did not show significant differences when subjected to statistical analysis, except for cultivar Genco, which was influenced by the rootstock effect. In general, the fruit set was higher for cultivars grafted on seedling rootstocks, with recorded values in the growing season 2021 of 36.01, 38.37 and 32.72% for Genco, Supernova and Tuono, respectively. Conversely, the same cultivars grafted on clonal rootstock GF677 showed a fruit set incidence about 7% lower in Genco, 4% in Supernova, and 2% in Tuono, when compared to the same cultivars grafted onto seedlings (Table 1.3).

3.4. *Yield Per Plant, Trunk Cross-Sectional Area, Yield Efficiency and Nut Traits*

The yield, yield efficiency (YE), and kernel/nut ratio recorded in the trial A during the growing season 2021, are shown in Table 1.4. Statistical differences for plant yield were observed for cultivars Genco and Supernova. In general, the clonal rootstock GF677 positively influenced the yield per plant expressed as in-shell almonds during the first year of remarkable production, showing mean values of 2.10, 1.78 and 0.47 kg per plant in Tuono, Genco and Supernova, when compared to the mean values of 1.82, 1.04 and 0.23 kg per plant recorded for the same cultivars grafted on peach seedling rootstocks.

Table 1.4. Yield per plant, yield efficiency (YE), and kernel/nut ratio recorded for cultivars Genco, Supernova and Tuono in the trial A during the growing season 2021. Data are reported as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between rootstocks (Fisher's test $p < 0.05$).

Cultivar	Rootstock	Yield per plant (kg)	YE (kg cm^{-2})	% kernel
Genco	**GF677**	1.78 ± 0.30 a	0.020 ± 0.010	31.28 ± 2.07
	Seedling	1.04 ± 0.49 b	0.040 ± 0.010	29.62 ± 4.34
Supernova	**GF677**	0.47 ± 0.12 a	0.006 ± 0.003	30.74 ± 3.35
	Seedling	0.23 ± 0.17 b	0.010 ± 0.003	30.85 ± 2.64
Tuono	**GF677**	2.10 ± 0.46	0.040 ± 0.010	30.64 ± 2.83 a
	Seedling	1.82 ± 0.16	0.040 ± 0.002	28.14 ± 4.11 b

The cultivar Tuono also showed the highest YE in both grafting combinations, with mean values of 0.04, and like those of Genco grafted onto seedling rootstocks. Conversely, Supernova showed the lowest YE in plants grafted on GF677 (0.006), and the lowest TCSA mean values (37.44 cm^2) in plants grafted on peach seedlings rootstocks. Slight influences also emerged for the nut traits, especially for cultivars Genco and Tuono, which showed a kernel/nut ratio higher by about two percent points when grafted on GF677 (31.2 and 26.6%, respectively), in comparison to those observed in plants grafted on peach seedlings (29.6 and 28.1%), respectively. No rootstock influence was found for the cultivar Supernova, which showed an average kernel/nut ratio of almost 31% for both grafting combinations. The rootstock GF677 positively influenced the vigor of plants in cultivar Supernova (see mean values of TCSA in Figure 1.2 for the growing season 2021), whereas a contrary behavior was noticed for cultivar Tuono, where the more vigor plants were those grafted onto seedling rootstocks.

As confirmed by the Figure 1.2, which reports the mean values of TCSA from 2018 (year of planting) to 2021 for the plant material of the trial A, the cultivar Genco was characterized by similar vigor in both rootstock types, as well as increased vigor induced by GF677 on Supernova, and by peach seedling rootstocks in Tuono, which were confirmed over the whole period of investigation.

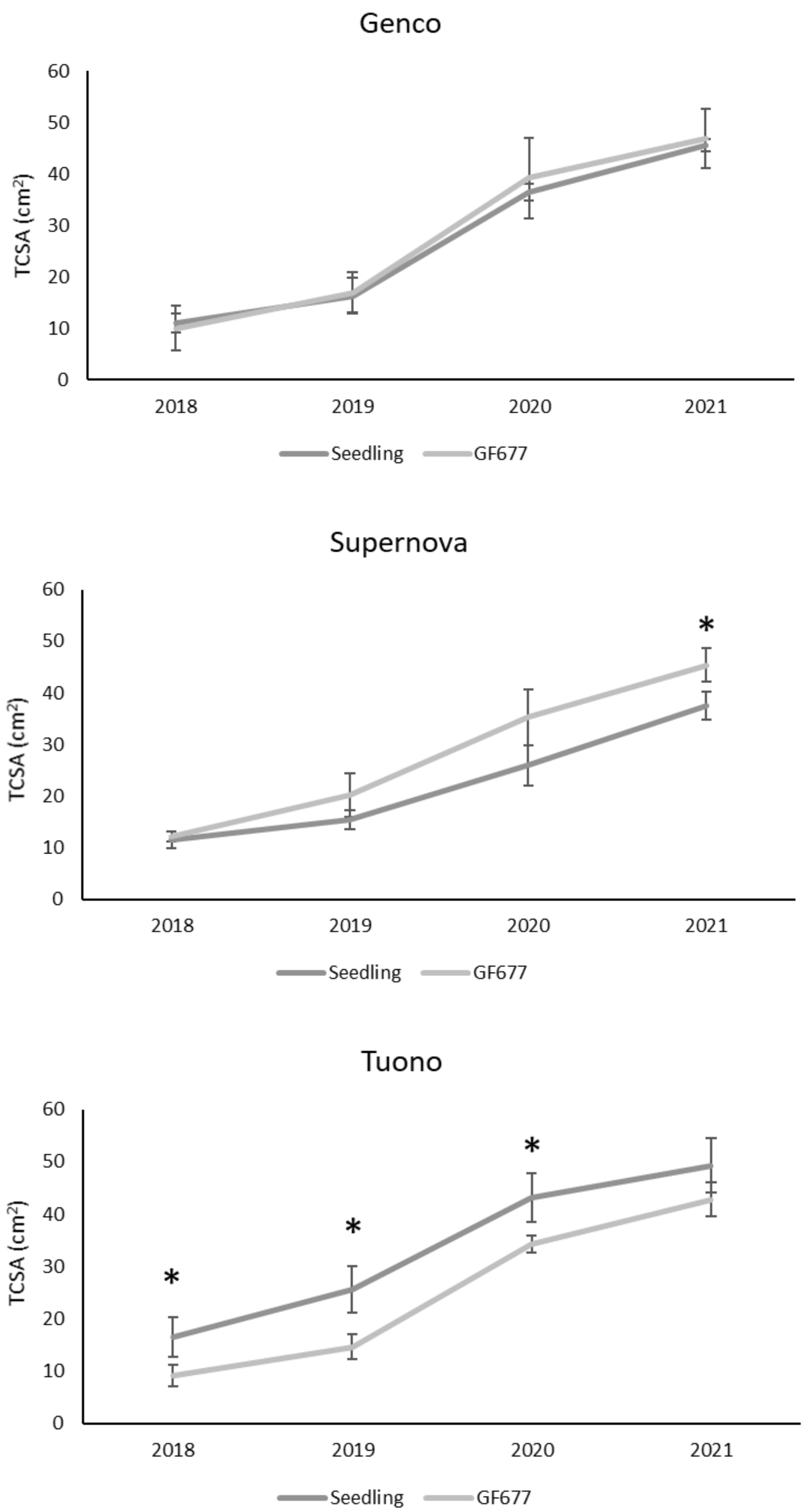

Figure 1.2. Comparison of annual mean values of trunk cross-sectional area (TCSA) computed at 30 cm above the ground and recorded at the end of growing season over the period 2018–2021 for cultivars Genco, Supernova and Tuono, grafted on GF677, and on peach seedling rootstocks Lowercase letters on the graph mean values indicate significant differences between rootstock per year (Fisher's test $p < 0.05$).

4. Results of trial B

4.1 Blooming and Ripening Phenograms

Figure 1.3 presents the blooming and ripening phenograms of trial B. The trial focused on the phenological and agronomical influence of clonal rootstocks on cultivar Guara, the blooming phenograms indicated as the rootstock Rootpac® 20 led a flowering postponement of about a week in comparison to those recorded in the other grafting combinations being tested. Thus, while the full bloom of Guara grafted on GF677 and Rootpac® R occurred within the first half of March, the same cultivar grafted on Rootpac® 20 showed its full bloom at the beginning of the second half of March. Conversely, no rootstock-induced differences were noted in terms of almond maturity for the cultivar Guara over the period of the trial.

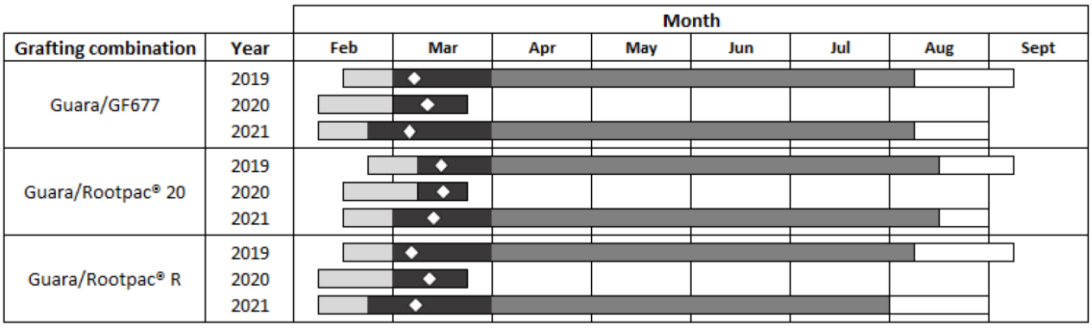

Figure 1.3. Phenograms of blooming and ripening recorded over four years of investigation (2018– 2021) for cultivars Guara grafted on clonal rootstock GF677, Rootpac® 20 and Rootpac® R, respectively (light grey histogram = beginning flowers emergence; dark grey histogram = blooming; white dots = overlapping of successive phenological stages; mid-dark grey histogram = fruit development; white histogram = fruit maturity: 1% hull split).

4.2 *Leaf Chlorophyll, Flavonols and Anthocyanins Content and Nitrogen Balance Index*

All parameters were significantly influenced by rootstock, as reported in Table 1.5. The highest mean values of total Chl were observed in leaves of cultivar Guara grafted onto Rootpac® 20, with 29.33 µg cm^{-2}, followed by those of plants grafted on GF677 and Rootpac® 20, with values of 28.90 and 28.18 µg cm^{-2}, respectively.

Flavonols in leaves of Guara reached average values of 2.2 µg cm^{-2}, showing only slight differences between rootstocks. Plants grafted on Rootpac® R showed the highest mean values of anthocyanin (0.11 µg cm^{-2}), while the highest NBI was recorded in leaves of Guara grafted on Rootpac® 20, with mean values of 13.81, followed by plants grafted on GF677 and Rootpac® R, which showed mean values of 13.30 and 12.89, respectively. Additionally, also in trial B, the annual mean values of the eco-physiological parameters investigated showed higher values in 2019 in Guara grafted onto GF677, while in 2020, the plants which showed higher values for the parameters investigated were those grafted onto the Rootpac® R and Rootpac® 20. Furthermore, also in this trial in 2021, the parameters investigated have been more uniform, showing similarly mean values for all grafting combinations.

Table 1.5. Content of total chlorophyll (μg cm^{-2}), flavonol (μg cm^{-2}) anthocyanin (μg cm^{-2}), and nitrogen balance index (NBI) in leaves of cultivar Guara collected from plants of trial B. Data are reported as mean ± standard deviation per year and as three-year mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between treatment and year (Fisher's test $p < 0.05$).

Rootstock	Year	Chl	Flav	Anth	NBI
GF 677	**2019**	29.13 ± 4.36 bc	2.14 ± 0.14 cde	0.07 ± 0.03 d	13.70 ± 2.56 b
	2020	28.68 ± 4.35 c	2.26 ± 0.14 a	0.09 ± 0.08 c	12.74 ± 2.05 b
	2021	28.81 ± 4.60 bc	2.18 ± 0.12 abc	0.08 ± 0.05 cd	13.12 ± 2.04 b
Rootpac® 20	**2019**	27.83 ± 3.46 c	2.25 ± 0.11 abc	0.10 ± 0.02 bc	12.62 ± 1.99 b
	2020	30.8 ± 3.80 ab	2.12 ± 0.12 e	0.08 ± 0.05 cd	14.84 ± 2.15 a
	2021	28.83 ± 3.95 abc	2.13 ± 0.12 bcd	0.09 ± 0.04 c	13.73 ± 2.28 ab
Rootpac® R	**2019**	25.05 ± 4.84 d	2.22 ± 0.13 ab	0.15 ± 0.03 a	10.75 ± 2.38 c
	2020	31.31 ± 5.91 a	2.14 ± 0.16 de	0.08 ± 0.03 cd	14.85 ± 3.52 a
	2021	27.85 ± 5.96 c	2.17 ± 0.19 bcd	0.10 ± 0.04 b	13.08 ± 3.66 b
Average GF677		*28.9 ± 4.26 b*	*2.21 ± 0.14 a*	*0.08 ± 0.03 b*	*13.3 ± 2.35 a*
Average Rootpac® 20		*29.33 ± 5.06 a*	*2.17 ± 0.15 b*	*0.09 ± 0.04 b*	*13.81 ± 2.90 a*
Average Rootpac® R		*28.18 ± 5.42 b*	*2.19 ± 0.15 ab*	*0.11 ± 0.06 a*	*12.89 ± 3.14 b*

4.3. Fruit Set Incidence

The incidence of fruit set was estimated in late April 2021. Table 1.6 shows the fruit set of the cultivar Guara in trial B. Whereas no statistically significant difference was highlighted considering the rootstock effect, the higher fruit set was recorded for plants grafted onto GF677 that showed mean values of 31.2%, while the lowest mean values (26.3%) were recorded for plants grafted onto Rootpac® 20.

Table 1.6. Percentage of fruit set of plants in trial B (cultivar Guara), calculated at the end of April 2021. Data are reported as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences among rootstocks (Fisher's test $p < 0.05$).

Cultivar	Rootstocks	Number of flowers	Number of fruits at full size	Fruit set (%)
Guara	**GF677**	584	182	31.25 ± 8.91
	Rootpac® 20	550	143	26.31 ± 6.72
	Rootpac® R	579	172	29.71 ± 7.12

4.4. Yield Per Plant, Trunk Cross-Sectional Area, Yield Efficiency, and Nut Traits

Table 1.7 reports plant yield, YE and kernel/nut ratio recorded in 2021 in trial B. Referring to the yield per plant, the cultivar Guara grafted on GF677 performed slightly better than the other grafting combinations, with an average of 1.5 kg per plant of in-shell nuts harvested in the growing season 2021, compared to an average yield per plant of 1.2 and 1.0 kg in the plants grafted on Rootpac® R and Rootpac® 20, respectively.

Table 1.7. Yield per plant, yield efficiency (YE), and kernel/nut ratio recorded for cultivar Guara in the trial B during the growing season 2021. Data are reported as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between rootstocks (Fisher's test $p < 0.05$).

Cultivar	Rootstocks	Yield per plant (kg)	YE (kg/cm^2)	Kernel/nut ratio (%)
Guara	**GF677**	1.49 ± 0.62	0.04 ± 0.02	30.53 ± 2.64
	Rootpac® 20	1.00 ± 0.20	0.03 ± 0.01	29.30 ± 3.35
	Rootpac® R	1.21 ± 0.16	0.04 ± 0.01	29.07 ± 2.95

Due to the different vigor imposed by the clonal rootstocks tested, the differences in YE are not relevant, at least at the third leaf on field of the plants and ranged between 0.3 and 0.4. Similarly to the YE, the kernel/nut ratio of Guara was also not affected by the rootstock effect, showing mean values of 29–30% in all grafting combinations, and in accordance with the literature (Felipe and Socias I Company, 1987). Unlike the other parameters analyzed, the mean values of TCSA determined at the end of growing season 2021 were significantly different between rootstock combinations, showing the higher values for Guara grafted on GF677 (35.05 cm^2), intermediate values in Rootpac® 20 (31.86 cm^2), and lower ones in Rootpac® R (25.97 cm^2). This trend is also confirmed on a three-year basis, as highlighted by the TCSA measurements begun in the year of the trial's implantation (growing season 2019) and shown in Figure 1.4.

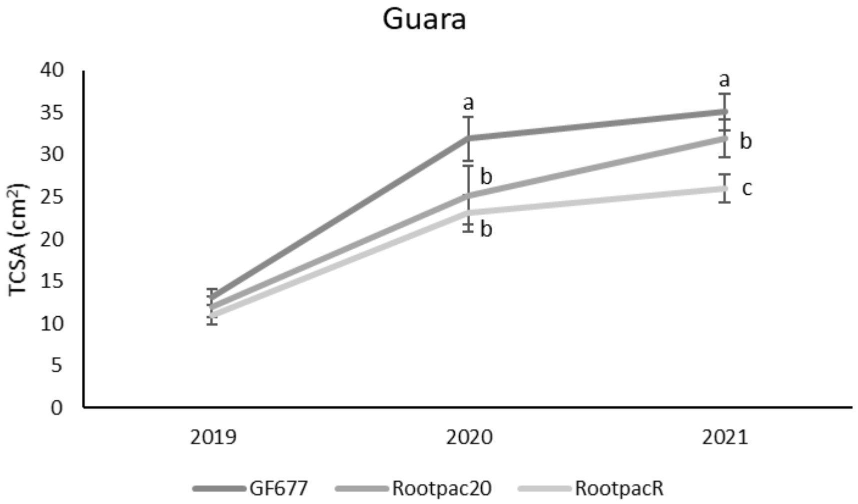

Figure 1.4. Comparison of annual mean values of trunk cross-sectional area (TCSA) computed at 30 cm above the ground and recorded at the end of growing season over the period 2019–2021 for cultivars Guara grafted on clonal rootstocks GF677, Rootpac® 20 and Rootpac® (trial B). Lowercase letters on the graph mean values indicate significant differences between rootstock per year (Fisher's test $p < 0.05$).

5. Discussion and Conclusions

Flowering and ripening calendars of the trial A highlighted as the medium-late flowering cultivars Genco, Supernova and Tuono could be subject to moderate risk of cold damages in the new introducing environment, as verified in early spring 2020, when the frost coincided with the end of plant flowering. Furthermore, it has been observed that the kind of rootstock does not have a significant influence on flowering time (Lordan et al., 2019), which is consistently influenced by the scion genotype (Monet and Bassi, 2017), whereas other trials carried out using different almond cultivars showed the rootstock influence on flowering (Romero et al., 2018). The clonal rootstock GF677 seems to anticipate flowering and vegetative bud break by a few days in Tuono when compared to the same cultivar grafted on peach seedling rootstocks, according to the literature (Monastra et al., 1982), and it should therefore be taken into account when choosing scion/rootstock combination for new plantings. On the contrary, in trials carried out in Spain, it was observed that interspecific hybrid rootstocks including GF677 tended to delay flowering time (Vahdati et al., 2021). In addition, the GF677 rootstock also had a slightly delaying effect on the ripening time of the fruits, particularly for Genco, as also confirmed by Vahdati et al. 2021. This finding is relevant, since a late drying of the hull and fruit could lead to contamination as a consequence of possible late summer rains (Danyluk et al., 2008), that are quite frequent in the environment considered; meanwhile, an earlier harvesting could determine difficulties during shucking and shelling almond nuts, with a consequent loss of product quality (Tombesi et al., 2010). From the phenological observations carried out in trial B, it was found that the flowering of cultivar Guara was affected by the kind of rootstock with the Rootpac® 20, which postponed its full bloom of about one week when compared to other rootstocks. Conversely, no effects on ripening time were observed, whereas in the literature, it has been reported that plum rootstocks, less vigorous than peach–almond hybrids, anticipate fruit ripening (Duncan et al., 2019). The leaf eco-physiological parameters monitored during the two trials, especially total chlorophyll content and NBI, have confirmed their functionality in determining the physiological status of the plants, since they are related to the secondary metabolism of the plants, and then they can be interpreted as a response to eventual nutritional deficiencies, water stress, or physiological disorders, also related to the correct functionality of the grafting point of grafted plants (Overbeck et al., 2018). The differences recorded at 90 DABF over the three years of the trials, mainly as ratio between Flav/Anth and Chl/NBI, and similarly to those observed by several authors (Scogings, 2018; Carvalho et al., 2011; Deng et al., 2019), confirm the hypothesis that meanwhile the secondary compounds in the leaves decreases, the leaf nitrogen, that it is directly related to Chl level, tends to increase (Padilla et al., 2014). The fruit set incidence determined in the last year of investigation was generally higher in plants grafted onto seedlings in the trial A, whereas in trial B, the rootstock GF677 expressed the best performance on Guara for this trait, when compared with the other clonal rootstocks. In general, in both trials, the fruit set incidences recorded were in line with the literature (Socias I Company et al., 2004). It is known that the annual yield in almond is highly influenced by the adverse weather events, especially during the post-setting/early fruit growth phases. In our trials, the yield per plant recorded during the growing season 2021 was always higher in plants grafted on GF677, as well as for the kernel/nut ratio, confirming the literature findings (Corina et al., 2015; Palasciano and Logoluso, 1998). Furthermore, referring to trial B, it is also possible to assert that, in the new environment of almond introduction, the rootstockGF677 imposed more vigor to the cultivar Guara than the tested clonal rootstocks Rootpac® 20 and Rootpac® R, as confirmed by Miarnau et al. (2010) for Spanish environments. Furthermore, it has been observed by Romero et al. (2018) that less vigorous rootstocks offer lower quality nuts than more vigorous ones. However, in recent studies (Maldera et al., 2021) it has been concluded that SHD almond orchards show higher YEs than those of conventional systems. The results obtained in the early cropping years of the almond scion/rootstock combinations of the two trials, to also be acquired in the next growing seasons, represent the most suitable field tool to guide future choices in the implementation of new HD almond orchards in central Italy, as this is currently taking place in the coastline of the Latium region. Furthermore, the outputs from trial B will be crucial to orientate, in this environment, the implementation of SHD orchards, since the dwarfing rootstocks tested in the absence of punctual competition between adjacent plants in the row, will allow a complete evaluation of their intrinsic physiological-agronomic behavior.

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3 CHAPTER II

Development of a new protocol for a sustainable foliar nutrition in almond orchards

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Abstract

The almond market is experiencing a period of steady growth in terms of both production and consumption. In Italy, the interest in almond cultivation is increasing, as confirmed by the presence of new almond orchards in areas not fully suited to its cultivation and where the knowledge of plant behaviour related to soil and climate conditions is still poor. Optimizing fertilization is one of the most important factors to guarantee high yields and nut quality, as well as to reduce environmental pollution. The trial was established in 2019 in a commercial orchard located in Viterbo province (Italy), with the aim to evaluate the effect of total foliar nutrition applications on almond trees compared to plants managed applying traditional soil fertilization as a control. Plants were subjected to four foliar nutrition applications with two different foliar fertilizers containing macro- and micro-nutrients. The effects of foliar nutrition were evaluated in the second year of trial (growing season 2020) by applying foliar diagnostics, the deviation from optimum percentage (DOP) for each nutrient, and eco-physiological traits such as leaf chlorophyll and flavonol levels and the nitrogen balance index. Furthermore, the yield efficiency was also calculated. No differences were observed for leaf nitrogen, potassium, and calcium concentrations between treatments, while the average concentrations of phosphorus and magnesium were higher in plants subjected to foliar nutrition than in the controls. In general, the

concentrations of micronutrients in the leaves, as well as the nut yield, were higher in plants subjected to foliar nutrition. The experiment is still in progress and will contribute to develop a sustainable protocol for nutrition in almond orchards, limiting the environmental impact caused by the addition of fertilizers to the soil.

Keywords: *Prunus dulcis* (Mill.) D.A. Webb, mineral nutrition, leaf diagnostics, DOP index, yield efficiency

1. Introduction

The world production of nuts has maintained a continuous growth over the last decade, reaching 4.6 million t in 2019 (FAOSTAT, 2020). Many scientific reports place nuts among the so-called healthy foods (Richardson et al., 2009; Dikariyanto et al., 2021), and therefore there is currently a strong market demand for nuts, including almonds. In Italy, about 60,000 t of in-shell almonds were imported in 2019 (INC, 2020), and consequently this crop has attracted the interest of growers. Consequently, almond orchards, which are traditionally located in southern Italian areas, have recently spread to new and not fully suitable areas, where the knowledge on the relationships between the almond eco-physiology and soil and climate conditions is still poor.
Mineral nutrition is one of the most significant practices in almond cultivation (Morais et al., 2020) and its proper management could ensure high nut quality and yield. Currently, foliar nutrition is routine in fruit crops thanks to its benefits, which include an easy and fast leaf nutrient uptake, allows multi-nutrient input and consequently a diminished contamination associated to fertilization. Furthermore, foliar nutrition can quickly correct nutritional deficiencies (Fernández et al., 2008), increase fruit-set (Solar and SŠtampar, 2001), and even increase fruit quality and yield (Ağlar and Saraçoğlu, 2018; Cristofori et al., 2018).

Based on such evidence, this study aims to develop a sustainable foliar nutrition protocol to be applied in recently established commercial almond orchards and develop appropriate leaf diagnostic references for the cultivar Tuono, which is grown in central Italy.

2. Materials and methods

2.1 Plant material and nutrition treatments

The trial was established in 2019 on a four-year-old commercial almond orchard of the cultivar Tuono, grafted on GF677 rootstock, and located in central Italy (Viterbo province; 42°19’19” N, 11°40’02” E). Trees in the orchard were spaced 5×3 m, trained in a vase shape and managed applying an integrated pest and disease control. The orchard was drip irrigated and the amount of water supplied was about 2,000 m^3 ha^{-1} $year^{-1}$.

The soil had a clay-loam texture, a pH of 6.5 and a total organic matter content (TOC) of 2.1%. The plants of the trial were managed applying a three-years total foliar nutrition (TFN) protocol (2019-21 growing seasons), which included four applications of macro- and micro-nutrients during the growing season (Table 2.1), and using plants managed applying a traditional soil fertilization protocol as control (Table 2.2).

Table 2.1. Total foliar nutrition (TFN) applications in the almond trial carried out over the growing seasons 2019-2021.

Treatment	**Fertilizer product** (NPK)	**Amount of fertilizer** (kg ha^{-1})	**Period**
1	Kappa M (21-12-18)	5.0	Mid-May
2	Frutcol NF (5-8-15)	2.5	Early June
3	Frutcol NF (5-8-15)	2.5	Early July
4	Frutcol NF (5-8-15)	3.3	Early August

Table 2.2. Traditional soil fertilization applications in the almond trial (control; C) carried out over the growing seasons 2019-2021.

Treatment	**Fertilizer product** (NPK)	**Amount fertilizer** (kg ha^{-1})	**Period**
1	Nitrophoska (12-12-17)	400	Early March
2	Ammonium nitrate (34)	200	Early April
3	Potassium nitrate (13-46)	300	Late June
4	Ammonium nitrate (34)	100	Late September

2.2 Leaf analyses and DOP index calculation

Twelve plants per treatment were selected to be subjected to all measurements, that started during the second year of the trial (2020 growing season). Forty leaves per plant were randomly collected on non-bearing shoots in the central portion of the plant crown, considering all different leaf orientations (Montañés and Sanz, 1994; Milošević and Milošević, 2016). Leaves were sampled at 90 and 120 days after full bloom (DAFB). The leaf nutrient concentrations (P, K, Ca, Mg, Fe, Mn, B, Cu, Zn) was determined using ICP-OES spectrometry (8000 DV, PerkinElmer, Shelton, CT, USA), following the protocol described by (Stazi et al., 2016). For inorganic carbon (C) and nitrogen (N), an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used. Three replicates per treatment and sampling date were analyzed to determine the leaf concentration of nutrients. Each replication was composed by sub-samples of ten leaves.

The leaf dry weight (DW) was determined using a programmed drier at 103±2°C for 24 h (when the samples reached constant weight). Three replicates per treatment and sampling date (ten leaves per sample) were analyzed.

The leaf concentration of each macro- and micro-nutrient was compared with the optimal one, calculating the deviation from optimum percentage (DOP) index (Montañés et al., 1993; Zarrouk et al., 2005; Xu et al., 2015; Milošević and Milošević, 2016) as:

$$DOP = (C \times 100/Cref) - 100$$

where C is the leaf concentration determined for a given nutrient and Cref is the optimal leaf concentration for the same nutrient as proposed by Mills and Jones (1996) (the optimal concentrations are shown in Table 2.3). Nutrient concentrations were expressed as % of leaf dry matter in the case of macro-nutrients and as $\mu g\ g^{-1}$ DW in the case of micro-nutrients.

Table 2.3. Range of optimal values of macro- (in % of DW) and micro-nutrients (in mg kg^{-1} DW) for almond leaves sampled in summer as proposed by Mills and Jones (1996). The average value of the intervals was used to calculate the DOP index.

Macronutrient (%DW)		**Micronutrient** (mg kg^{-1} DW)	
N	2.35 (2.20-2.50)	Fe	140 (30-250)
P	0.20 (0.10-0.30)	Mn	60 (20-100)
K	1.20 (1.00-1.40)	B	45 (30-60)
Ca	2.50 (2.00-3.00)	Cu	12 (4-20)
Mg	0.50 (0.25-0.75)	Zn	46 (18-75)

The same leaves used for mineral analysis were previously analyzed to determine the specific leaf area (SLA; expressed in cm^2 g^{-1} DW) (Reich et al., 1997; Wilson et al., 1999; Burns et al., 2021) and the leaf dry matter content (LDMC; expressed in mg g^{-1} FW) (Viégas et al., 2005; Vile et al., 2005). The leaf area was measured using the software Image J (Collins, 2007) and both of leaf parameters were determined during the growing seasons 2020-21.

2.3 Leaf chlorophyll, flavonols and anthocyanins content and nitrogen balance index

The same leaves, before to be collected by the plant, were used to measure eco-physiological parameters such as total chlorophyll (Chl), flavonols (Flav) and anthocyanins (Anth) during the growing seasons 2020-21 using a hand-held meter (FORCE-A, Dualex®, Orsay, France). The instrument also automatically determines the nitrogen balance index (NBI), using the ratio between Chl and Flav (Wijewardana et al., 2018; Silvestri et al., 2019). The NBI index has a low sensitivity to plant phenology and reflects nitrogen availability better than when the two parameters (Chl and Flav) are used individually (Cerovic et al., 2015).

2.4 Yield efficiency and nut and kernel traits

Yield was determined by harvesting all nuts from the twelve plants selected for each treatment. At the end of the growing seasons 2020-21 the trunk cross sectional area (TCSA) of each plant was measured at 30 cm above the ground, and the yield efficiency (YE), expressed as the ratio between yield and TCSA, was calculated for the year 2020 only, since the following year's production was compromised by a few frosts occurred in early April. Nut and kernel traits were recorded testing a sample of 200 nuts per treatment, as proposed by Felipe (2000).

2.5 Statistical analysis

Statistical analyses were carried out using InfoStat Professional v.1.1 program. All data were subjected to analysis of variance (two-way ANOVA). The percentage values were subjected to angular transformation according to the formula (x+0.5) ^ 1/2 before data analysis. Fisher's test was carried out to identify statistical significance between means among treatments, sampling dates and their interactions.

3. Results and discussion

3.1 Leaf characteristics

Table 2.4 shown the mean values of specific leaf area (SLA) and leaf dry matter content (LDMC) in plants treated with total foliar nutrition (TFN) in comparison to those managed with traditional fertilization (C) at 90 and 120 DAFB detected in the growing season 2020 and 2021. No significant differences in SLA values were found at 90 DAFB between the TFN and control. Data set ranges from 97 m^2 g^{-1} DW in control in 2021 to 116 m^2 g^{-1} DW in TFN in the same year. No significant differences were also recorded for LDMC at 90 DAFB both in 2020 and 2021, and its average values ranged from 382 mg g^{-1} FW in C to 368 mg g^{-1} FW in TFN. Conversely, significant differences due to the year effect were observed at 120 DAFB for SLA, where the highest mean value was observed in 2021 (107 m^2 g^{-1} DW) compared to that of the previous year (85 m^2 g^{-1} DW). Leaf dry matter content (LDMC) at 120 DAFB showed significative differences for the interaction treatment × year. The highest LDMC mean values were observed in leaves sampled in control plants in 2020 (415 mg g^{-1} FW), whereas those collected in TFN plants in the same year showed average values of 360 mg g^{-1} FW. Significant differences related to the treatment effect were also observed, with average values ranging from 396 mg g^{-1} FW in C to 371 mg g^{-1} FW in TFN.

Table 2.4. Specific leaf area (SLA) and leaf dry matter content (LDMC) in plants treated with total foliar nutrition (TFN) and traditional fertilization (C) protocols sampled at 90 and 120 DAFB. Data shown were obtained for the 2020 and 2021 growing seasons and are means ± SD (n=30).

90 DAFB			
Treatment	**Year**	**SLA** (cm^2 g^{-1} DW)	**LDMC** (mg g^{-1} FW)
C	2020	103.42 ± 14.64	366.89 ± 9.02
	2021	97.04 ± 17.74	382.71 ± 16.06
Two-year average		*100.23* ± *15.81*	374.80 ± *14.92*
TFN	2020	101.44 ± 14.01	379.56 ± 7.75
	2021	116.88 ± 20.50	368.91 ± 21.55
Two-year average		109.16 ± *18.58*	374.24 ± *16.40*
Average treatments	2020	102.43 ± 13.65	373.23 ± 10.39
	2021	106.96 ± 21.01	375.81 ± 19.50
LSD (p<0.01)			
Treatment		*n.s.*	*n.s.*
Year		*n.s.*	*n.s.*
*Treatment * Year*		*n.s.*	*n.s.*

120 DAFB			
Treatment	**Year**	**SLA** (cm^2 g^{-1} DW)	**LDMC** (mg g^{-1} FW)
C	2020	82.25 ± 8.42	415.00 ± 22.58 a
	2021	104.29 ± 16.16	377.71 ± 8.51 b
Two-year average		93.27 ± *16.83*	396.35 ± *25.38 a*
TFN	2020	88.57 ± 18.71	360.44 ± 28.34 b
	2021	110.04 ± 12.10	383.32 ± 10.22 ab
Two-year average		99.31 ± *18.74*	371.88 ± *23.57 b*
Average treatments	2020	85.41 ± 14.22 b	387.72 ± 37.53
	2021	107.17 ± 13.94 a	380.51 ± 9.43
LSD (p<0.01)			
Treatment		*n.s.*	**
Year		**	*n.s.*
*Treatment * Year*		*n.s.*	**

In each column, different letters indicate significant differences between means (Fisher's test p<0.05). n.s. = not significant; **significant at p<0.05.

3.2 Leaf chlorophyll, flavonol and anthocyanin levels and nitrogen balance index

The leaf chlorophyll levels measured at 90 DAFB showed significant differences among ‘treatment’, ‘year’ and interaction ‘treatment x year’ (Table 2.5). In both treatments, values ranged between 42 μg cm^{-2} for TFN in 2020 and 32 μg cm^{-2} for control in 2021.

The highest average value was observed in TFN plant with 39 μg cm^{-2} against 36 μg cm^{-2} observed in control. Highest Chl values were observed in 2020 (41.9 μg cm^{-2}).

Flavonols content was significantly different due to the effects of ‘year’ and interaction ‘treatment x year’, whereas no significant differences were observed between treatments. Its mean values ranged between 2.22 μg cm^{-2} in 2021 and 2.11 μg cm^{-2} in 2020, both observed in control plants.

As recorded for Flav, also the content of Anth was higher in the leaves analyzed in 2021 than in those monitored in 2020, without showing significant differences between treatments.

Similarly to Chl, the NBI showed significant differences among ‘treatment’, ‘year’ and interaction ‘treatment x year’. The highest values were observed in 2020 in both treatments (average values above 19.5), while the lowest ones were recorded in 2021 in the control plants with values of 14.55.

The leaf eco-physiological parameters measured at 120 DAFB were significantly affected by the year (Table 2.5). The mean Chl contents were higher in 2020 (41.28 μg cm^{-2}) than in 2021 (35.77 μg cm^{-2}), as well as the mean Flav contents that were of 2.14 μg cm^{-2} in 2020 and 1.96 μg cm^{-2} in 2021. Conversely, the mean contents of Anth at 120 DABF, although influenced by the effect of the year, showed similar values with no differences due to treatment.

The highest mean value of NBI at 120 DABF was observed in 2020 (19.3), while the plants treated with TFN showed values of the index slightly higher than those of control plants.

Table 2.5. Leaf total chlorophyll (Chl), flavonol (Flav) and anthocyanin (Anth) contents and nitrogen balance index (NBI) in plants treated with foliar (TFN) and traditional fertilization (C) protocols. Data shown were obtained for the 2020 and 2021 season and are means ± SD (*n*=30).

90 DAFB					
Treatment	**Year**	**Chl** (µg cm^{-2})	**Flav** (µg cm^{-2})	**Anth** (µg cm^{-2})	**NBI**
C	2020	41.47 ± 2.95 a	2.11 ± 0.09 c	0.03 ± 0.02 c	19.69 ± 1.73 a
	2021	32.22 ± 2.32 c	2.22 ± 0.13 a	0.06 ± 0.02 a	14.55 ± 1.57 c
Two-year average		*36.85 ± 5.34 b*	*2.17 ± 0.12*	*0.04 ± 0.02*	*17.12 ± 3.06 b*
TFN	2020	42.47 ± 2.98 a	2.16 ± 0.08 b	0.04 ± 0.02 b	19.53 ± 2.09 a
	2021	35.81 ± 4.35 b	2.13 ± 0.11 bc	0.04 ± 0.01 b	16.84 ± 2.25 b
Two-year average		*39.14 ± 5.01 a*	*2.15 ± 0.09*	*0.04 ± 0.02*	*18.18 ± 2.55 a*
Average treatments	2020	41.98 ± 2.99 a	2.14 ± 0.09 b	0.03 ± 0.09 b	19.61 ± 1.91 a
	2021	34.01 ± 3.91 b	2.18 ± 0.12 a	0.05 ± 0.12 a	15.69 ± 2.25 b
LSD (p<0.01)					
Treatment		**	*n.s.*	*n.s.*	**
Year		**	**	**	**
*Treatment *Year*		**	**	**	**

120 DAFB					
Treatment	**Year**	**Chl** (µg cm^{-2})	**Flav** (µg cm^{-2})	**Anth** (µg cm^{-2})	**NBI**
C	2020	41.43 ± 2.41	2.14 ± 0.13	0.03 ± 0.02	19.25 ± 2.26
	2021	35.63 ± 3.27	1.98 ± 0.22	0.02 ± 0.02	18.02 ± 2.63
Two-year average		*38.53 ± 4.08*	*2.06 ± 0.20*	*0.03 ± 0.02*	*18.63 ± 2.52*
TFN	2020	41.14 ± 2.83	2.14 ± 0.12	0.03 ± 0.02	19.34 ± 1.79
	2021	35.91 ± 3.09	1.93 ± 0.25	0.02 ± 0.02	18.74 ± 3.10
Two-year average		*38.52 ± 3.95*	*2.04 ± 0.22*	*0.03 ± 0.02*	*19.04 ± 2.54*
Average treatments	2020	41.28 ± 2.62 a	2.14 ± 0.12 a	0.03 ± 0.12 a	19.29 ± 2.03 a
	2021	35.77 ± 3.17 b	1.96 ± 0.23 b	0.02 ± 0.23 b	18.38 ± 2.88 b
LSD (p<0.01)					
Treatment		*n.s.*	*n.s.*	*n.s.*	*n.s.*
Year		**	**	**	**
*Treatment *Year*		*n.s.*	*n.s.*	*n.s.*	*n.s.*

3.3 Leaf analysis and DOP index

The leaf macronutrient concentrations (N, C, P, K, Ca, Mg) are shown in Table 2.6. Differences between sampling dates were observed for the N concentrations, with the highest average values being observed in mid-July (4.45% DW). The interaction treatment × sampling date was also significant (only at $p<0.05$), with higher values in mid-July in the control. Regarding C, significant differences were found for treatments and the treatment × sampling date interaction (in both cases only at $p<0.05$). Significant differences for the P concentrations were observed only between treatments, with the highest values being observed in the plants subjected to TFN (0.11% DW vs. 0.07% in the control). The K and Ca concentrations were significantly different only between sampling dates. Significant differences for the Mg concentrations were observed only between treatments, with the highest average values being observed in the plants subjected to TFN (0.28% DW vs. 0.24% in the control).

Table 2.6. Leaf macro-element concentrations (in % of DW) in plants treated with foliar (TFN) and traditional fertilization (C) protocols. Data shown were obtained for the 2020 season and are means ± SD (*n*=3).

Treatment	**Sampling date**	**N** (% DW)	**C** (% DW)	**P** (% DW)	**K** (% DW)	**Ca** (% DW)	**Mg** (% DW)
C	**Mid-June**	3.18 ± 0.09 b	41.99 ± 0.13 a	0.08 ± 0.01	1.08 ± 0.05	1.05 ± 0.04	0.25 ± 0.01
TFN		3.70 ± 0.68 ab	41.96 ± 0.17 a	0.10 ±0.00	1.52 ± 0.62	1.22 ± 0.47	0.28 ± 0.00
C	**Mid-July**	4.61 ± 0.18 a	42.39 ± 0.40 a	0.06 ± 0.02	0.46 ± 0.02	2.78 ± 0.25	0.23 ± 0.06
TFN		4.28 ± 0.53 ab	37.49 ± 3.49 b	0.11 ± 0.04	0.62 ± 0.13	3.00 ± 1.15	0.29 ± 0.04
Averages	**Mid-June**	3.44 ± 0.54 b	41.97 ± 0.14	0.09 ± 0.01	1.30 ± 0.48 a	1.13 ± 0.33 b	0.26 ± 0.02
	Mid-July	4.45 ± 0.42 a	39.94 ± 3.98	0.08 ± 0.04	0.54 ± 0.12 b	2.89 ± 0.80 a	0.26 ± 0.06
C	**Av. Samp. date**	3.89 ± 0.76	42.19 ± 1.36 a	0.07 ± 0.02 b	0.77 ± 0.32	1.91 ± 0.92	0.24 ± 0.04 b
TFN		4.00 ± 0.66	39.72 ± 3.83 b	0.11 ± 0.03 a	1.77 ± 0.63	2.11 ± 1.25	0.28 ± 0.03 a
LSD (p<0.01)							
Treatment		*n.s.*	*	**	*n.s.*	*n.s.*	**
Sampling date		**	*n.s.*	*n.s.*	**	**	*n.s.*
*Treatment *Samp. date*		*	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

A large variability was observed for the microelements Fe, Mn, B, Cu and Zn (Table 2.7). The leaf Fe concentration was influenced significantly by the treatment, and the interaction treatment × sampling date was also significant (only at $p<0.05$). The highest values were observed in the plants of the TFN treatment sampled in July (284 mg kg^{-1} DW). The leaf Mn concentration was influenced by the treatment and sampling date, and the interaction treatment × sampling date was also significant (only at $p<0.05$). The highest leaf Mn concentrations were observed in plants subjected to TFN in samples collected in mid-July (105 mg kg^{-1} DW). Also, the leaf B concentration was significantly influenced by the treatment. Differences were also observed between sampling dates for Cu concentrations, with higher values recorded in mid-June. The concentration of Zn was affected significantly by the treatment and the interaction treatment × sampling date was also significant (in both cases only at $p<0.05$).

Table 2.7. Leaf micro-element concentrations (in mg kg^{-1} DW) in almond plants treated with foliar (TFN) and traditional fertilization (C) protocols. Data shown were obtained for the 2020 season and are means ± SD (*n*=3).

Treatment	Sampling date	Fe (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	B (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)
C	**Mid-June**	138.52 ± 38.31 b	39.09 ± 2.02 c	8.53 ± 3.04	24.77 ± 15.41	34.63 ± 8.84 ab
TFN		88.81 ± 11.61 b	63.01 ± 6.17 b	10.80 ± 1.20	21.55 ± 10.33	37.99 ± 3.98 ab
C	**Mid-July**	81.71 ± 21.21 b	57.24 ± 17.34 bc	4.91 ± 1.22	6.27 ± 2.21	26.60 ± 6.48 b
TFN		284.04 ± 62.01 a	105.32 ± 13.78 a	14.14 ± 7.54	15.08 ± 6.30	53.32 ± 20.52 a
Averages	**Mid-June**	113.67 ± 37.45	51.04 ± 13.24 b	9.67 ± 2.50	23.16 ± 12.62 a	36.31 ± 6.67
	Mid-July	182.87 ± 104.52	81.28 ± 29.22 a	9.52 ± 6.05	10.67 ± 6.43 b	39.96 ± 20.60
C	**Av. Samp. date**	110.12 ± 41.85 b	48.16 ± 15.11 b	6.72 ± 2.91 b	15.52 ± 14.27	30.61 ± 8.50 b
TFN		186.42 ± 91.46 a	84.17 ± 24.33 a	12.47 ± 5.43 a	18.31 ± 8.83	45.55 ± 16.79 a
			LSD (p<0.01)			
Treatment		*	**	**	*n.s.*	*
Sampling date		*n.s.*	**	*n.s.*	**	*n.s.*
*Treatment *Samp. date*		**	*	*n.s.*	*n.s.*	*

The DOP index values calculated for the two treatments and sampling dates are shown in Figure 2.1. The N content in both treatments was higher than reference values (Cref), and the highest values were recorded for leaves collected in July. The elements P and Mg showed lower DOP index in comparison to Cref for all sampling, highlighting the lowest values for both nutrients in samples collected in July from control plants.

Similarly, to previous nutrients, also K showed lower DOP index for all sampling with the exception to leaves collected in June from TFN plants, that had values of +27 Cref.

The Ca showed considerable dynamism going from negative DOP values in June for both treatments to positive DOP index in July.

The Fe and Mn contents had higher DOP index in plants subjected to foliar treatments, highlighting the most significant increase in July (+ 103 and +76 Cref for Fe and Mn, respectively).

The B was lower than its Cref for all sampled leaves, whereas values comparable to the related Cref were recorded for Zn that ranged between +16 for plants treated with

TFN in July to -42 in control plants. Finally, the Cu showed DOP values higher than Cref for nearly all samples, whereas the leaves collected in july from control plants highlighted negative DOP value (-48 Cref).

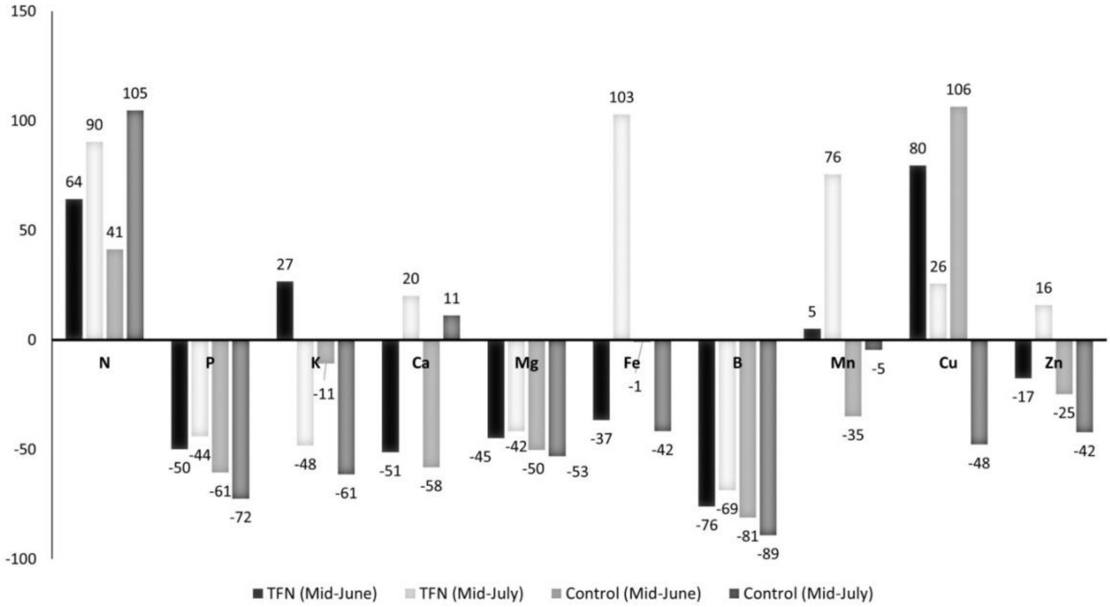

Figure 2.1. Deviation from optimum percentage (DOP) for the different macro- and micronutrients using the reference values proposed for almond.

3.4 Yield efficiency and nut and kernel traits

The yield, YE and nut and kernel traits for both treatments are shown in Table 2.8. Statistical differences were observed for plant yield. The TFN treatment performed better than the control, with an average of 1.6 kg plant^{-1} of in-shell nuts harvested, compared to an average of 1.2 kg plant^{-1} in the control. Significant differences were also observed in kernel weight, that was higher in TFN treatment than in the control (1.62 and 1.49 g, respectively). The nut traits and nut kernel^{-1} ratio were not influenced by the fertilizer treatments, and the values were like those previously reported in the literature for the same cultivar (Felipe, 2000).

Figure 2.2 shown the average TCSA calculated at the end of growing seasons 2020 and 2021. Significant differences emerged between treatments in 2020 (only at $p<0.05$), with higher values in the plants subjected to TFN than in the controls (71.1 and 56.7 cm^2, respectively), whereas no significant differences were observed in the following growing season 2021.

Table 2.8. Yield efficiency (YE) and nut and kernel traits in almond plants treated with foliar (TFN) and traditional fertilization (C) protocols. Data shown were obtained for the 2020 season and are means ± SD (*n*=12).

Treatment	**Yield** (kg plant^{-1})	**YE** (kg cm^{-2})	**Nut weight** (g)	**Kernel weight** (g)	**Nut kernel^{-1}** (%)
C	1.20 ± 0.22 b	0.01 ± 0.00	4.03 ± 0.21	1.49 ± 0.07 b	36.98 ± 1.17
TFN	1.61 ± 0.57 a	0.02 ± 0.00	4.27 ± 0.16	1.62 ± 0.05 a	37.95 ± 1.04
Effects	**	n.s.	n.s.	**	n.s.

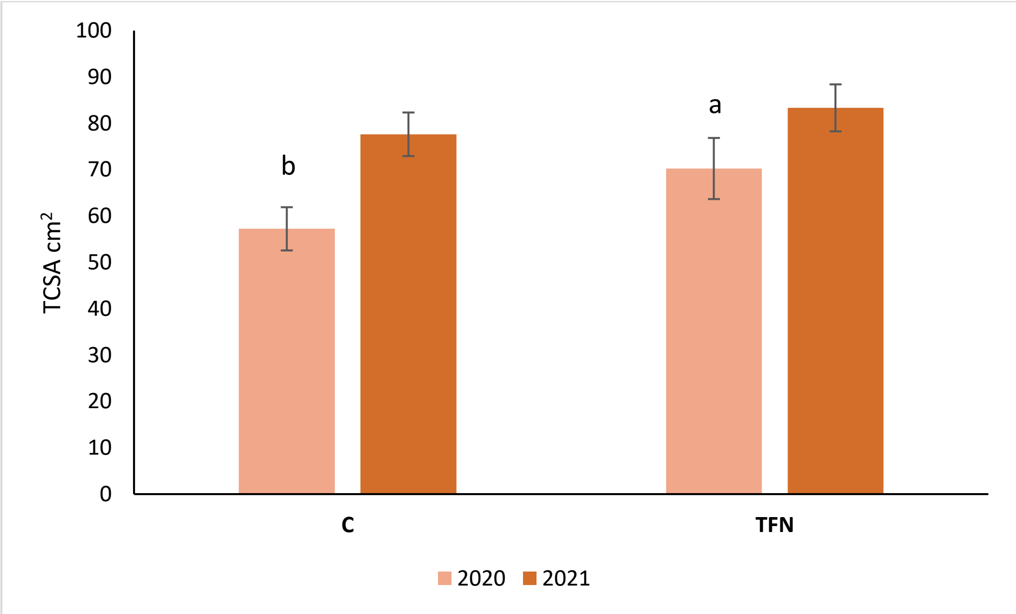

Figure 2.2. TCSA at 30 cm above the ground in almond with TFN and traditional fertilization (C) protocols.

4. Conclusions

The results obtained in the two years of field measurements and lab analyses, did not reveal substantial differences that justify the unsuitability of such a plant nutrition approach.

The leaf diagnostic data showed that P and Mg have a higher leaf concentration in plants subjected to TFN, whereas no noteworthy differences were recorded for N, K, and Ca, indicating that foliar nutrition does not worsen the plant's nutritional balance while strongly affecting the leaf concentrations of micro-elements.

According to the results of the first two years of field measurements it has been confirmed that plants subjected to TFN did not show any nutritional deficiency stress, and this is also confirmed by leaf eco-physiological parameters. Nevertheless, the effects to foliar nutrition treatments on yield and nut and kernel traits would need further investigation to consolidate the sustainability of this new almond nutrition strategy.

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4 CHAPTER III

Cultivar-specific assessments of almond nutritional status through foliar analysis

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Abstract

The Mediterranean almond growing is increasing, as confirmed in Italy where new almond orchards in areas not previously interested by this nut crop are recently established. In these new introducing areas, as in the coastal of Latium region, the knowledge of eco-physiological behavior of the cultivars related to specific pedoclimate conditions and orchard management is still poor. Optimizing fertilization strategies according to cultivar needs is one of the main key factors to guarantee high yields and nut quality, as well as to reduce environmental pollution. With this aim, an almond field collection has been established in 2017 on a local experimental farm and intermediate and late-bloom almond cultivars Genco, Guara, Lauranne®Avijor, Penta®, Supernova, Soleta, Tuono and Vialfas, grafted on clonal rootstock GF677, were introduced. During the growing seasons 2019-2020 leaf samples of almond plants were collected at 90 and 120 days after full bloom (DAFB) and analyzed for biometrical and eco-physiological traits and leaf nutrients content through foliar diagnostics. The analysis of the data acquired during the trial revealed differences in foliar nutrient content due to both cultivar and phenological stage of the plant. The correlation of the analyzed foliar traits also highlighted the reciprocal influences between some leaf nutrients, including those between certain nutrients and eco-physiological parameters of the leaf, such as the nitrogen balance index (NBI), which can be easily investigated using proper field instruments and used for a preliminary screening of the plant's nutritional status. Indeed, according to Pearson correlation matrix almond leaf nitrogen content showed a medium-high positive correlation with

NBI either at 90 or 120 DAFB. Furthermore, the sum of deviation of optimum percentage index ($\sum$DOP) expressed as mean of cultivars at 90 DAFB was quite stable during the two research-year, whereas at 120 DAFB it was significantly higher in 2020.

Findings of this study helps to develop novel environmental-friendly almond nutrition and fertigation strategies supported by proper cultivar-site specific foliar diagnostics which also consider punctual cultivar nutritional requirements during the growing season.

Keywords: *Prunus dulcis*, specific leaf area, eco-physiological parameters, leaf diagnostics, orchard management

1. Introduction

The nut market is currently experiencing a period of steady growth both in terms of production and consumption (INC, 2020). Among the world's most widely cultivated nut species, almond (*Prunus dulcis* (Mill.) D. A. Webb.) ranks first, with a yearly average in-shell nut production of about 4.1 million tons harvested from a cultivated area of about 2 million hectares. California (U.S.) is the largest almond producer with about 2.3 million tons of in-shell nuts per year, whereas in Europe the main producer is Spain, where about 400,000 tons in-shell nuts per year are produced (FAOSTAT, 2021).

Italian almond harvested area is currently listed in 53,000 hectares and the in-shell almond production was about 85,000 tons in 2020 (ISTAT, 2021). During the first half of the 20th century, Italy was the world leader in almond production with an average annual production of 230,000 tons harvested from about 300,000 hectares of almond orchards (Sottile et al., 2014). The relatively recent development of Californian and Spanish almond cultivation led Italy into a deep crisis of own almond sector, determining the loss of production leadership in the 70s. The Italian almond contraction was also induced by the lack in research and finalized breeding programs, as well as by low field applications of technological innovation (Losciale, 2018). More recently, the interest in almond cultivation is growing up again in Italy, as confirmed by the new almond plantings established both in traditional and new growing areas. These new plantations are usually established planting both traditional cultivars as

Tuono and Genco or more recently released late-flowering cultivars as Belona, Soleta, Penta®, Vialfas, Makako, Lauranne®Avijor (Laghezza et al., 2018). Most of these new varieties were recently obtained and patented thanks to the Spanish breeding programs carried out by both public and private institutions.

The objectives of the main almond breeding programs worldwide are differently focused. In California (U.S.), where soft-shelled almond cultivars are preferred (Pérez de los Cobos et al., 2021), the breeding program started in the 1920s under the aegis of United States Department of Agriculture - Almond Board of California (USDA) and was focused in improving nut quality, self-compatibility and in realizing the suitable pollinators of the main cultivar Nonpareil (Gradziel et al., 2007). In the Mediterranean area, almond breeding started in France in 1960 at National Institute of Agronomic Research (INRA - Bordeaux), in 1970 in Spain at Center of Agrifood Research and Technology (CITA - Aragon), Institute of Agrifood Research and Technology (IRTA - Catalonia) and Spanish National Research Council (CEBAS CSIC - Murcia), as well as in Italy at Council for Agricultural Research and Analysis of Agricultural Economics (CREA - Rome). These European breeding programs have similar objectives which consist in releasing late flowering and new self-compatible cultivars, increase almond kernel quality and releasing disease-resistant varieties (Batlle et al., 2017).

The French almond breeding was very active during the 1970s-80s when the varieties Ferragnes, Ferraduel (Grasselly and Crossa-Raynaud, 1980) and in 1989 Lauranne (Grasselly et al., 1992) were released. More recently the Spanish almond breeding contributed significantly to the innovation of the European varietal platform since CITA released in 2006 the varieties Belona and Soleta (Socias i Company and Felipe, 2007), Mardia in 2007 (Socias I Company et al., 2008) and Vialfas in 2015 (Socias i Company et al., 2015). Additionally, in the same period IRTA released Constantí, Marinada and Vairo (Vargas et al., 2008). Furthermore, thanks to the almond breeding carried out at CEBAS-CSIC the cultivars Antoñeta and Marta were released in 1990 (Egea et al., 2000), while the extra-late flowering cultivars Penta® and Tardona were released in 2009 (Dicenta et al., 2009).

Apart from Ferragnes and Ferraduel, the above-mentioned cultivars are self-compatible thanks to the presence of the dominant allele S_f (López et al., 2006) transmitted in the breeding pedigrees using the Italian self-compatible cultivars Tuono and Genco as parentals (Socias I Company, 2017), as well as the Italian cultivar

Cristomorto was used to transmit the late-flowering trait in the released cultivars (Socias I Company, 2017).

Recently, it has been noticed as the genetic contribution given by cultivars Tuono and Cristomorto was about 60% in the INRA breeding program and 47% in the Spanish programs, leading to alert for the increase in inbreeding depression in the new released cultivars (Alonso Segura and Socias I Company, 2007; Pérez de los Cobos et al., 2021).

The traditional Italian almond cultivation has been established using local varieties often characterized by early blooming which compromised its spread in new growing areas.

The recent increase of almond market demand is driving the expansion of almond cultivation from southern to central Italy, often planting Spanish cultivars replacing the Italian ones. Due to the new trend in Italian almond sector and the climate changes affecting the Mediterranean basin, almond cultivation is at risk of being introduced in areas not suited to the local climates, also associated with poor knowledge in plant adaptation for phenology, physiology, and orchard resilience (Mirabdulbaghi, 2015, Losciale, 2018).

A key factor that may mitigate the above criticalities, in addition to the study of varietal adaptation for phenology, is to develop sustainable nutrition models to promote agronomic performance and the proper physiological behavior of cultivars. Furthermore, a right almond orchard nutrition should aim to minimize the environmental pollution (Muhammad et al., 2017). To achieve these goals, cultivar-site specific foliar diagnostic protocols to monitor the plant nutrition status during the growing season are required.

Some studies carried out on different *Prunus* species (Sanz et al., 1992; Montañés and Sanz, 1994; Betrán et al., 1997; Lucena, 1997; Zarrouk et al., 2005; Ben Yahmed et al., 2020) including almond (Saa et al., 2013) demonstrated as the best time for monitoring the nutritional status of the plant through foliar diagnostics is at 60 and 120 days after full bloom (DAFB) thanks the whole development of the leaves and the adequacy of the phenological stages of the plant.

A proper leaf sampling and diagnosis provide a powerful tool for the growers to determine the nutritional status of the orchard (Milošević and Milošević, 2016). The content of leaf nutrients can be compared with their critical values (CVs) determined by Ulrich (1952) and expressed as concentration of the nutrients into leaf tissues. These

CVs are considered the minimum leaf nutrient content necessary to achieve at least 90% of potential plant yield (Saa et al., 2014; Muhammad et al., 2017).

Hence, comparison of leaf nutrient content with their CVs and their Sufficiency Ranges (SR) is the most applied method for leaf nutritional diagnosis and both approaches operate through the measurement of total nutrients content in leaf tissues to be compared with reference values (Sumner, 1978; Lucena, 1997; Benito et al., 2015).

Considering that the leaf nutrient reference values for almond in the Mediterranean environment are very poor, the present study aims to their determination with a novel approach site-cultivar specific testing the main almond varieties recently introduced in Central Italy. This approach will be useful to develop novel environmental-friendly almond nutrition strategies supported by proper foliar diagnostics.

2. Material and methods

2.1 Plant material and pedoclimatic condition

The trial was carried out in the experimental farm of ARSIAL (Regional Agency for Innovation and Development of Agriculture in Latium) located in the municipality of Tarquinia (Latium region - Italy. Latitude 42°16'20'' N; longitude 11°42'26'' E; altitude 32 m a.s.l.), where a collection orchard of almond cultivars was planted in late 2017.

The following eight cultivars were introduced in the trial: Genco, Guara, Lauranne®Avijor, Penta®, Supernova, Soleta, Tuono and Vialfas. Although the cultivars Supernova and Guara are considered two different commercial varieties and are individually marketed, literature highlighted as they may be both synonyms of the cultivar Tuono (Marchese et al., 2008; Distefano et al., 2013; Dicenta et al., 2015).

The studied cultivars, each represented by three replicates (three plants per cultivar), were grafted on rootstock GF677 (*P. persica* x *P. dulcis*), spaced at 6m x 5m and trained as free vase. The experimental almond orchard was managed with a natural green cover crop according to the rules of the almond Integrated Production System and it had yearly application of the following quantities of soil fertilizers: 120 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_2O. The plants were individually drip irrigated and the amount of water supplied was about 1,500-2,000 m^3 ha^{-1} per growing season.

The physical and chemical soil characteristics of the experimental almond orchard were reported in Table 3.1. The clay-loam soil had almost 2% of organic matter mainly located in the topsoil profile and showed sub-alkaline soil reaction (pH = 7.6).

Table 3.1. Main physical and chemical soil characteristics in the experimental almond orchard located at Tarquinia municipality (Latium region – Italy) at the ARSIAL experimental farm.

Trait	Unit	Value
Texture		
Sand	%	45.4
Lime	%	25.6
Clay	%	30.0
pH	-	7.6
Total limestone	g kg^{-1}	8.0
Organic matter	%	1.9
Cation exchange capacity	meq	29.1
Calcium (Ca^{2+})	meq	23.7
Magnesium (Mg^{2+})	meq	4.1
Potassium (K^{+})	meq	0.5
Sodium (Na^{+})	meq	0.8
Basic saturation	%	100.0
Mg/K Ratio	-	7.5

The average climatic parameters recorded during the three growing seasons of the trial (years 2019-2021) are reported in Figure 3.1 as processed data from Table 1.1 since the experimental station was the same of trials discussed in Chapter I.

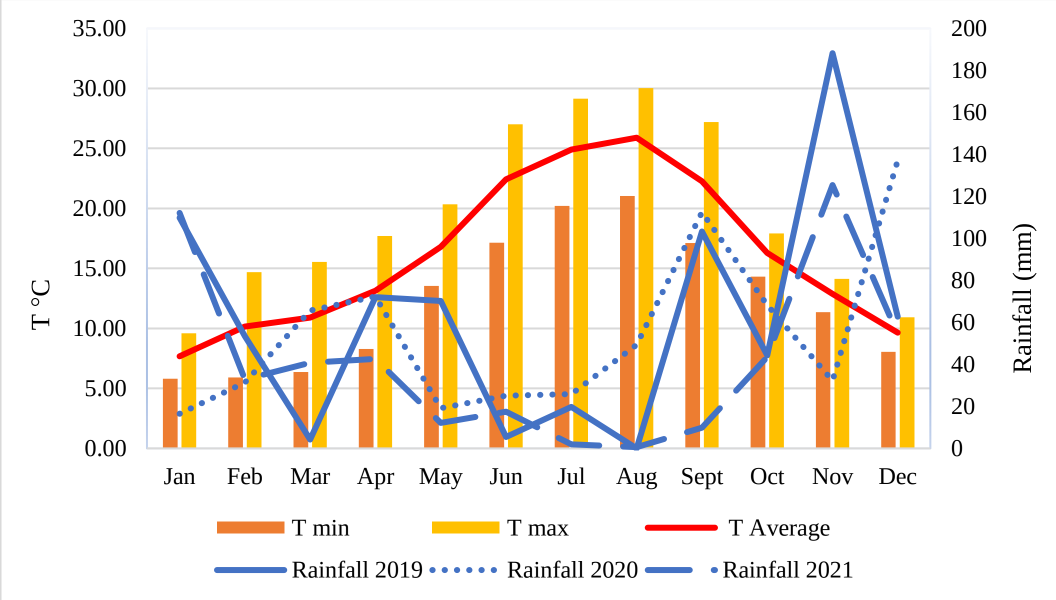

Figure 3.1. Yearly rainfall per month and mean values of monthly air temperature at the research area over the years 2019-2021. Data source: ISPRA (http://www.scia.isprambiente.it).

2.2 Phenological observations

In order to develop the almond flowering and ripening calendars, the phenological traits of each cultivar were recorded from 2019 through weekly measurements taken during the research years according to the BBCH scale adapted for almond (Sakar et al., 2019).

2.3 Yield Per Plant, Trunk Cross-Sectional Area, Yield Efficiency, and Nut Traits

Trunk cross sectional area (TCSA) of each plant was measured at 30 cm above the ground (above the grafting point) after harvesting time and the yield per plant was determined in the growing season 2021 by hand harvesting nuts from the three replicates per cultivar. The yield efficiency (YE), expressed as the ratio between yield and TCSA, was also calculated in 2021 only for cultivars that escaped the frost damages occurred during the second week of April and thus succeeded in yielding

2.4 Leaf sampling, leaf analysis and DOP index

Leaf samples of each cultivar under test were collected at 90 and 120 days after full bloom (DAFB), applying the following protocol. Sixty mature leaves per cultivar were collected from the middle portion of non-bearing shoots, taking care to sample not damaged leaves oriented in all cardinal points as recommended by Montañés and Sanz

(1994) and more recently by Milošević and Milošević (2016). The leaf samples were placed into single plastic bags and stored inside a polystyrene box with cooling gel, until to be processed in the laboratory.

The determination of macro- and micronutrients in the leaves has been carried out following the protocol described by Stazi et al. (2016). The samples were dried at +37 °C for 72 h and shredded. Approximately 250 mg of each dry sample was inserted directly into a microwave-closed vessel (tetrafluoromethoxy vessels). Two milliliters of 30% (m/m) H_2O_2, 0.5 ml of 37% HCl and 7.5 ml of HNO_3 solution were added to each vessel. The program of heating was carried out in one step, where the temperature was increased linearly from +25 to +180 °C in 37 min and then maintained at +180 °C for 15 min. After the digestion and cooling, the samples were diluted to a final volume of 25.0 ml with Milli-Q water. Analyses of leaf nutrients per cultivar were performed in triplicate. Each sample replication was composed by sub-samples of ten mature leaves.

For the elements determination were used the following emission lines: phosphorus (P) 213.620 nm, zinc (Zn) 213.857 nm, boron (B) 249.677 nm, manganese (Mn) 257.610 nm, iron (Fe) 259.939 nm, magnesium (Mg) 279.077 nm, calcium (Ca) 317.933 nm; copper (Cu) 324.752 nm, potassium (K) 766.490 nm.

An ICP-OES spectrometry with an axially viewed configuration (8000 DV, PerkinElmer, Shelton, CT, USA) was used. For detections were chosen the frequency showing the lowest interference, and high analytical signal and background ratio, for every element. For inorganic carbon (C) and nitrogen (N), an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used.

After almond leaf macro- and microelements determination their contents were compared with the optimal ones, calculating the deviation from optimum percentage (DOP) index (Moñtanés et al., 1993; Zarrouk et al., 2005; Xu et al., 2015; Milošević and Milošević, 2016) as follow:

DOP = (C x 100/Cref) -100

where *C* is the leaf concentration determined for a given nutrient and *Cref* is the optimal leaf concentration for the same nutrient as proposed by Mills et al. (1996).

Through the DOP index it is possible to determine the nutritional status of the plant according to ΣDOP index of the investigated leaf nutrients. The ΣDOP index is calculated by adding the absolute values of the DOP index determined for every single nutrient. If the detected nutrients are in balance with the reference values, the ΣDOP index will have low values (Montañés Garcìa et al., 1991; Milošević and Milošević, 2016). For its determination the mean of single nutrient reference values proposed by Mills et al. (1996) were used.

A portion of leaf samples collected at 90 and 120 DAFB (twenty leaves per cultivar analyzed in duplicate: ten leaves per sample) were analyzed to determine the Specific Leaf Area (SLA) (Wilson et al., 1999; Burns et al., 2021) using the software Image J (Collins, 2007). This leaf trait is obtained by the ratio between the leaf blade area to its relative dry weight, expressed in cm^2 g^{-1} (Reich et al., 1997).

Furthermore, the same leaves previously weighted in the lab to determine their fresh weight, were dried by heating at +103 ± 2°C to constant weight in order to determine the Leaf Dry Matter Content (LDMC) which is given by the ratio between the dry weight and the relative fresh weight of the leaf blade and expressed in mg g^{-1} (Viégas et al., 2005; Vile et al., 2005).

2.5 Leaf content of total chlorophyll, flavonols, anthocyanins and Nitrogen Balance Index

Leaves analyzed for nutrient contents were previously subjected to in-field measuring eco-physiological parameters (two measurements per leaf) such as total chlorophyll (Chl), flavonols (Flav) and anthocyanins (Anth), using a hand-held meter (FORCE-A, Dualex®, Orsay, France). The instrument also automatically determines the Nitrogen Balance Index (NBI), expressed as ratio between Chl and Flav (Wijewardana et al., 2018).

The instrument quantifies the chlorophyll content by exciting the leaf tissue with radiation at two different wavelengths (red and near infrared). Furthermore, it quantifies the leaf flavonoids amount as a logarithmic ratio between the infrared fluorescence of chlorophyll excited by a red wavelength and a UV wavelength (Silvestri et al., 2019).

It is well known as the flavonoids concentrated in the epidermal cells absorb UV light by lowering the fluorescence of chlorophyll in the infrared range (Vidovic, 2014).

The NBI, expressed as ratio between the total chlorophyll content and flavonoids, is less sensitive to plant phenology and reflects the leaf nitrogen availability better than the two parameters evaluated individually (Cerovic et al., 2015; Overbeck et al., 2018).

The leaf eco-physiological parameters analyzed using DUALEX PLUS® were correlated with the values of detected foliar nutrients in order to obtain a correlation matrix able to explain possible relationships between analyzed leaf parameters. The NBI, expressed as ratio between the total chlorophyll content and flavonoids, is less sensitive to plant phenology and reflects the leaf nitrogen availability better than the two parameters evaluated individually (Cerovic et al., 2015; Overbeck et al., 2018). The leaf eco-physiological parameters analyzed using DUALEX PLUS® were correlated with the values of detected foliar nutrients in order to obtain a correlation matrix able to explain possible relationships between analyzed leaf parameters.

2.6 Statistical analysis

Statistical analyses were carried out using InfoStat Professional v.1.1 program. For the analysis of variance (two-way ANOVA), the parameters "cultivar", "year" and their interaction were used, analyzing the sampling dates individually (90 DAFB and 120 DAFB). Differences were accepted as statistically significant when $p < 0.05$. Fischer's test was carried out to identify significance among the cultivars and the years of samples. Furthermore, correlations among data collected through leaf analyses and measurements at 90 and 120 DAFB, respectively, were determined by Pearson coefficient testing at $p < 0.05$. Principal component analysis (PCA) was applied to the self-scaled data matrix constituting the values of foliar eco-physiological parameters and foliar nutrient content analysed in the two phenological phases of the growing seasons considered for foliar analysis (90 and 120 DAFB).

3. Results and discussions

3.1 Phenological observations and almond phenograms

Figure 3.2 shows the main stages of almond blooming, fruit development and ripening over the three years of the investigation.

The beginning of bud swelling, which signals the end of winter rest, was mainly observed from the second half of February. Genco was the first cultivar that showed this stage, occurred in the second week of February in the growing seasons 2020 and 2021 and similarly to Guara and Soleta in 2021 and in Tuono in 2020.

The Vialfas and Penta® cultivars were the latest to show this phase, with bud swelling observed in the last week of February.

Within about four weeks, the emergence of the floral organs was completed, whereas the beginning of blooming (about 10% of open flowers) was recorded from the end of February in Supernova and Genco both in 2019 and 2021, and from early March in the year 2020 for the same cultivars. In 2020, also Lauranne®Avijor, Guara and Soleta showed the beginning of flowering in the first week of March, whereas in the other years their blooming started in the second half of March, similarly to Tuono. The full bloom (more than 50% of open flowers, represented in the phenogram by the yellow dot) in cultivars Supernova and Genco was observed between the second and third weeks of March over the three research years. In Guara and Soleta this phenological phase was brought forward by approximately one week only in the year 2020, while during the other research years the full bloom was concentrated between the second and third week of March.

Vialfas and Penta®, recognized as extra-late flowering cultivars, showed their beginning of flowering from the second half of March, while their full bloom was recorded between late March and early April.

The blooming window was of about 3-4 weeks, until its last stage identified by the petals and other flower whorls desiccation. Soon after occurred the fruit set and the start of the fruit development stage, coinciding with the beginning of ovary growth when the corolla rudiments can still be inserted at the base of floral receptacle. In 2019, the fruit set of the cultivars Genco, Lauranne®Avijor, Soleta and Tuono, occurred at the beginning of April, whereas for the other ones it happened between the second and third week of the month, with Vialfas and Penta® being the latest.

In the springs of 2020 and 2021, late frosts at the end of March and the beginning of April occurred in the experimental station with minimum night temperatures of -2 °C and -1 °C, respectively, when the plants were between the fruit set and early fruit development stages, compromising the yield of almost all cultivars. The only cultivar that was able to escape to these late frosts in both years was Penta®, where the fruit development phase was recorded from the third week of April (Figure 3.2).

In the third research year the cultivars Lauranne®Avijor, Guara and Soleta showed the fruit development stage from the second half of April. This stage continued until the final size of the fruit was reached, when the hull started to yellow, and dehiscence begun. At the beginning of August 2019, the cultivars Vialfas, Tuono, Guara and Soleta (also in 2021 for these last two cultivars) showed the beginning of hull dehiscence, as well as Lauranne®Avijor and Penta® that showed this stage at the second week of the month, while Genco in the only year of harvest, showed the beginning of dehiscence after the second half of August, confirming its late ripen that was in mid-September.

Figure 3.3 shows the main stages of the vegetative organs of the studied cultivars. The first phase, corresponding to the enlargement of the leaf buds and subsequent vegetative bud break followed by the green tip phase (young leaves are just visible) was recorded starting in the second half of February in the Italian cultivars (Genco, Supernova, Tuono) and in Guara, while in the other Spanish cultivars (Vialfas, Penta® and Soleta) and in Lauranne®Avijor the vegetative bud break was observed starting in March and lasted for about 2-3 weeks.

Leaf development was observed in almost all cultivars between the second and third week of March and lasted for approximately 3-4 weeks, until the leaves had fully unfolded and reached full size.

The cultivar Penta® leaf development was observed in the last week of March in the years 2019 and 2020, while in the last year leaf spreading started in the second week of March. The shoot development was quite homogeneous for all studied cultivars and started between the end of March and the beginning of April. The shoot development lasted for about 8-10 weeks, until late June in the late cultivars for this trait (Soleta, Tuono and partially Genco).

Finally, starting from the second half of September until early October, started the dormancy phase that was expressed as leaf yellowing and subsequent leaf fall, with the cultivars Genco and Penta® found to be the most delayed for this trait.

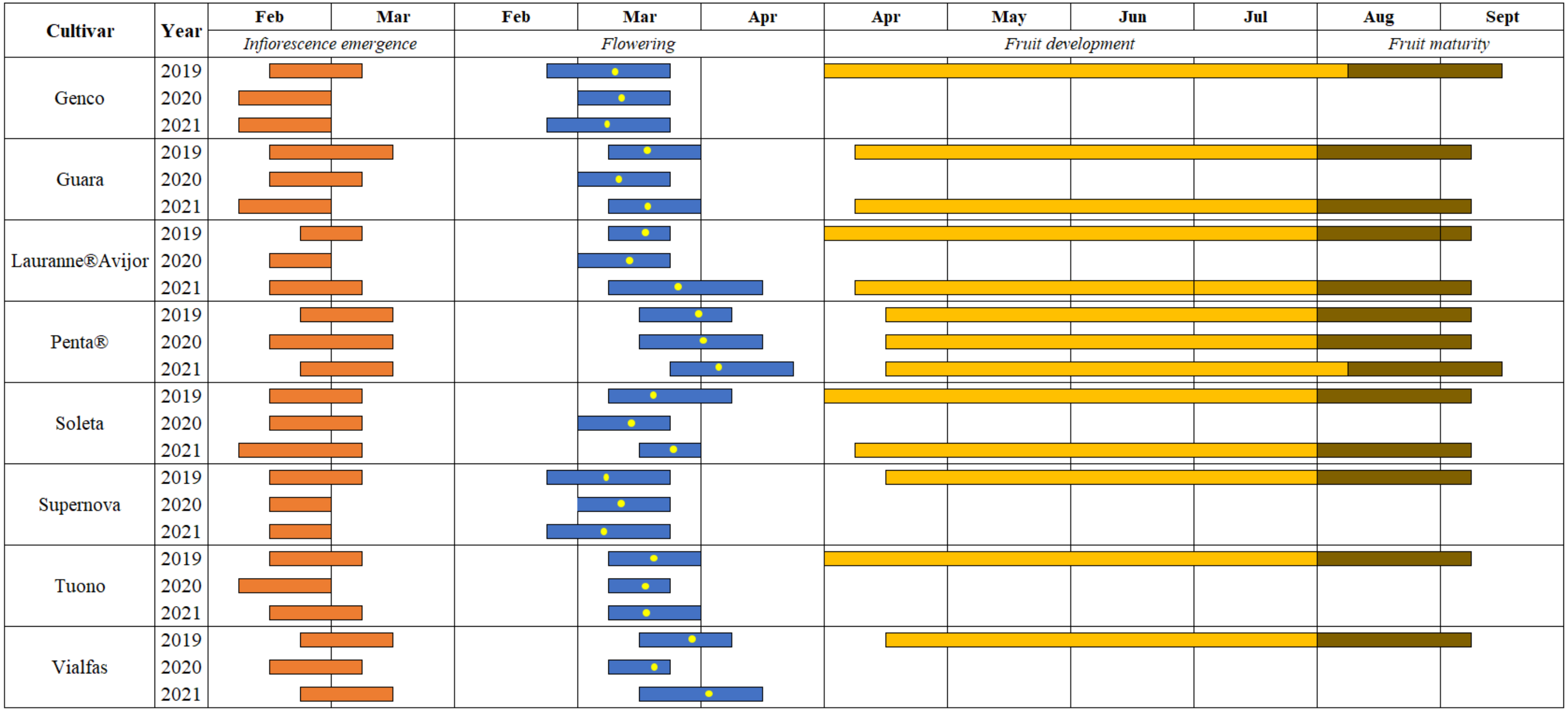

Figure 3.2. Phenogram of the main development stages of the flower organs of the cultivars tested in the collection field. The weekly measurements were carried out from February to September over the three years of investigation (2019-21).

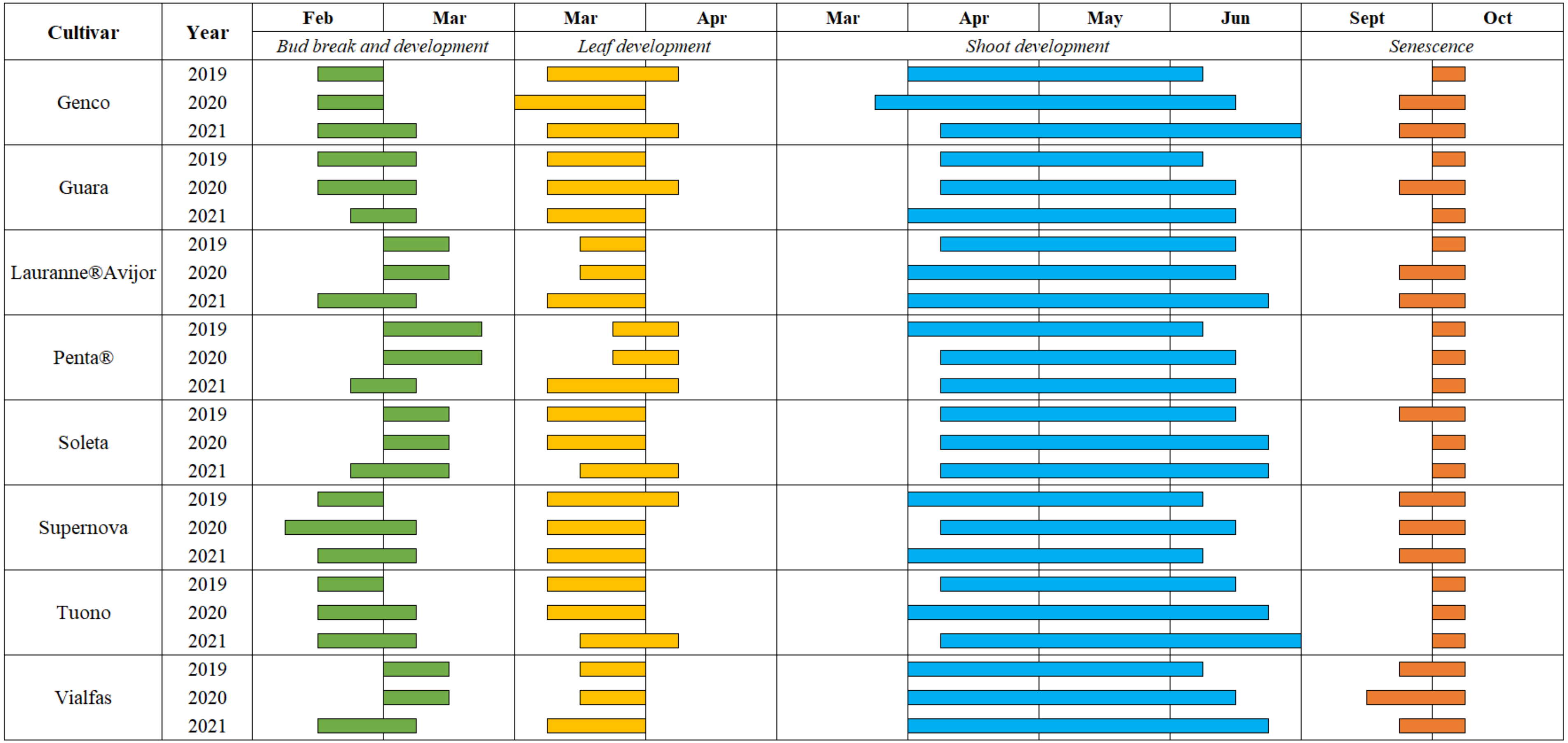

Figure 3.3. Phenogram of the main development stages of the vegetative organs of the cultivars tested in the collection field. The weekly measurements were carried out from February to September over the three years of investigation (2019-21).

3.2 Yield per plant, trunk cross-sectional area, yield efficiency, and nut traits

Figure 3.4 shows the TCSA calculated at 30 cm above ground and just above the grafting point for the almond cultivars studied over the three research years.

The cultivar characterized by the highest vigor was Soleta that showed values of TCSA higher than the other ones, especially in the last two years of investigation (27.05 and 35.90 cm^2, respectively). Genco, Supernova and Tuono showed an intermediate vigor (28.02, 29.30 and 28.42 cm^2 in 2021, respectively), whereas Penta® was the most vigorous cultivar at the end of first growing season (15.22 cm^2) and then placed among the intermediate vigor cultivars in the last year of the trial (27.00 cm^2 in 2021). Contrarily, the less vigorous cultivar over the three years of investigation resulted Vialfas with values of 5.50, 9.33 and 14.98 cm^2, in 2019, 2020 and 2021 respectively. Table 3.2 shows the average yield per plant, the yield efficiency and kernel/nut ratio of the cultivars Guara, Lauranne®Avijor, Penta® and Soleta, the only ones that have overcome frost damage and therefore fruiting in 2021.

While no significant differences were shown for yield efficiency (YE), the production of in-shell almond per plant was significantly affected by the cultivar effect. The highest yield was observed in Soleta, with mean values of 0.84 kg per plant, while Guara, which was partially damaged by frost, showed the lowest yield per plant during 2021 (mean values of almost 0.20 kg per plant). The percent kernel showed no significant differences between cultivars, with mean values ranged from 30.5% (Guara) to 27.3% (Soleta).

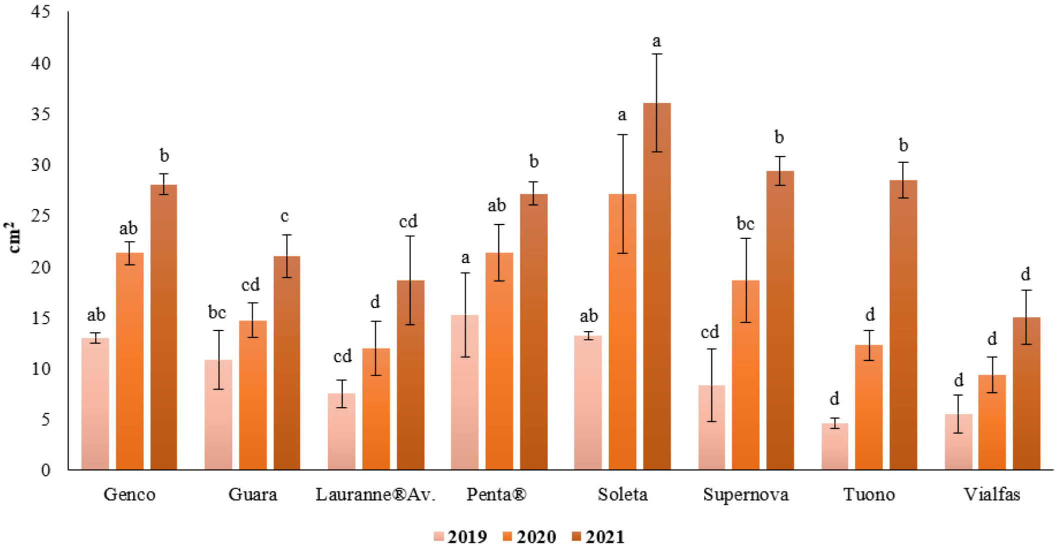

Figure 3.4. Comparison of annual mean values of trunk cross-sectional area (TCSA) calculated at 30 cm above the ground and recorded at the end of the three growing seasons in the cultivars under test. Lowercase letters indicate significant differences between cultivars per year (Fisher's test p < 0.05).

Table 3.2. Yield per plant, yield efficiency (YE), and kernel/nut ratio recorded during the growing season 2021. Data are reported as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between cultivars (Fisher's test $p < 0.05$).

Cultivar	**Yield per Plant** (kg)	**YE** (kg cm^{-2})	**Kernel/nut ratio** (%)
Guara	0.17 ± 0.07 b	0.008 ± 0.004	30.52 ± 2.09
Lauranne®Avijor	0.51 ± 0.16 ab	0.028 ± 0.016	30.10 ± 1.23
Penta®	0.66 ± 0.19 ab	0.024 ± 0.006	27.89 ± 3.10
Soleta	0.84 ± 0.30 a	0.024 ± 0.011	27.31 ± 2.87
LSD (p<0.05)	**	*n.s.*	*n.s.*

3.3 Almond leaf traits

Table 3.3 shows mean values of Specific Leaf Area (SLA, expressed as cm^2 g^{-1}) and Leaf Dry Matter Content (LDMC, expressed as mg g^{-1}) determined on leaf samples of the almond cultivars tested, at 90 and 120 DAFB, respectively.

SLA plays an important role in determining plants productivity since its values may reflect changes to the structure and nutritional content of leaves. It is well known as the SLA is mostly driven by the concentration of starch and sugars due to increased rates of photosynthesis, increasing the C:N ratio and reducing the nutritional value of the leaf (Madani et al., 2017).

LDMC is closely related to the average density of leaf tissues (Shipley and Vu, 2002; Vile et al., 2005) and tends to increase as SLA decreases (Poorter and Evans, 1998). LDMC is also strongly correlated with resource availability and with relative growth rate (Fortunel et al., 2009) and it has been recommended as a highly reliable correlate of soil fertility at least in agroecosystems not subject to severe water limitation (Vendramini et al., 2002).

The SLA values at 90 DABF showed significant differences related to the year, whereas values were not influenced by cultivar effect, as well as the interaction cultivar × year was not significant. SLA at 90 DABF ranged between 10.11 cm^2 g^{-1} in cv Tuono in 2019 to 7.16 cm^2 g^{-1} in cv Lauranne®Avijor in 2020. Furthermore, SLA expressed as mean value of cultivars studied was higher in the first year of investigation, showing mean values of 9.20 and 8.05 cm^2 g^{-1} in 2019 and 2020, respectively.

The trait measured at 120 DAFB showed significant differences related to the cultivar and year effects, whereas their interaction was not significant (Table 3.3). At this phenological stage (120 DABF) the highest mean value of SLA was observed in Tuono in 2019 (8.72 cm^2 g^{-1}) while the lowest one was observed in Supernova in 2020 (5.81 cm^2 g^{-1}).

Even at 120 DABF the trait was higher in 2019, when expressed as mean value of cultivars. Overall, the mean values of SLA were slightly lower at 120 DABF in comparison to those observed at 90 DABF for all cultivars, except for Lauranne®Avijor both in 2019 and 2020.

Leaves with low SLA values tend to be more efficient at high light concentrations and are more tolerant to water and nutrient deficiency due mainly to their thicker cuticle and slower leaf turnover (Fonseca et al., 2000; Wright et al., 2002).

Leaf Dry Matter Content (LDMC) showed significative differences for the effect of year and interaction cultivar × year, whereas no differences were emerged for cultivar effect (Table 3). The higher mean values were observed for all cultivars in 2020 at both 90 and 120 DABF. After 90 days of full bloom, the highest LDMC mean values were recorded for cultivars Soleta, Genco and Guara (484.3, 483.9 and 482.1 mg g^{-1}, respectively), whereas the lowest LDMC were observed for Guara in 2019 with mean values of 349.7 mg g^{-1}.

At 120 DABF the LDMC mean values ranged between 429.9 mg g^{-1} recorded in Soleta in 2020 to 363.7 mg g^{-1} measured in leaves of cv Tuono sampled in 2019.

The mean value of LDMC in 2019, expressed as average of cultivars (Table 3.3), was slightly lower at 90 DABF (362.8 mg g^{-1}) where compared to 120 DABF (378.4 mg g^{-1}), whereas in 2020 the reverse trend was observed, with slightly higher average values at 90 DABF (470.8 mg g^{-1}) than those recorded at 120 DABF (418.2 mg g^{-1}).

Table 3.3. Specific leaf area (SLA) and leaf dry matter content (LDMC) in almond plants at 90 and 120 DAFB over the years 2019-2020. Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar	Year	90 DAFB		120 DAFB	
		SLA (cm^2 g^{-1})	**LDMC (mg g^{-1})**	**SLA (cm^2 g^{-1})**	**LDMC (mg g^{-1})**
Genco	**2019**	9.08 ± 1.01	367.53 ± 7.29 cd	7.32 ± 0.95	391.94 ± 3.90 e
	2020	7.58 ± 0.58	483.91 ± 6.55 a	6.53 ± 0.72	420.81 ± 6.80 abc
Mean value		*8.33 ± 1.11*	*425.72 ± 62.54*	*6.92 ± 0.89 bc*	*406.38 ± 16.26*
Guara	**2019**	9.05 ± 2.09	349.77 ± 3.59 d	7.01 ± 1.02	381.17 ± 10.72 fg
	2020	7.62 ± 2.14	482.14 ± 13.05 a	6.34 ± 0.58	416.46 ± 1.49 bcd
Mean value		*8.33 ± 2.10*	*415.95 ± 71.31*	*6.67 ± 0.84 c*	*398.32 ± 20.15*
Lauranne® Avijor	**2019**	8.73 ± 0.84	363.96 ± 4.44 cd	9.79 ± 1.16	368.57 ± 12.30 hi
	2020	7.16 ± 1.39	475.49 ± 4.19 a	8.06 ± 1.23	417.52 ± 16.47 bcd
Mean value		*7.94 ± 1.35*	*419.73 ± 59.75*	*8.93 ± 1.44 a*	*393.15 ± 29.52*
Penta®	**2019**	9.83 ± 2.24	360.61 ± 6.10 cd	8.39 ± 0.96	376.84 ± 9.46 fg
	2020	9.57 ± 217	432.26 ± 17.25 b	7.59 ± 1.23	408.95 ± 6.57 d
Mean value		*9.71 ± 2.05*	*396.43 ± 40.12*	*7.99 ± 1.11 ab*	*392.89 ± 18.75*
Soleta	**2019**	7.98 ± 0.66	371.20 ± 5.85 c	6.65 ± 1.11	374.51 ± 0.40 gh
	2020	8.24 ± 2.94	484.35 ± 7.49 a	6.99 ± 0.93	429.91 ± 2.78 a
Mean value		*8.11 ± 1.98*	*427.77 ± 60.80*	*6.82 ± 0.96 bc*	*402.21 ± 29.67*
Supernova	**2019**	9.56 ± 2.88	364.16 ± 15.94 cd	7.98 ± 0.78	383.92 ± 0.55 efg
	2020	9.05 ± 3.89	466.54 ± 32.87 a	5.81 ± 1.50	411.27 ± 1.79 cd
Mean value		*9.31 ± 3.18*	*415.35 ± 59.72*	*6.9 ± 1.61 bc*	*397.6 ± 14.67*
Tuono	**2019**	10.11 ± 3.07	359.93 ± 16.89 cd	8.72 ± 2.26	363.76 ± 1.54 i
	2020	7.51 ± 0.96	466.80 ± 10.14 a	6.35 ± 0.70	425.47 ± 6.20 ab
Mean value		*8.81 ± 2.53*	*413.37 ± 58.56*	*7.54 ± 2.01 bc*	*394.62 ± 33.25*
Vialfas	**2019**	9.26 ± 0.39	365.76 ± 8.32 cd	6.98 ± 0.59	386.6 ± 0.77 ef
	2020	7.69 ± 1.99	475.55 ± 8.24 a	7.36 ± 1.42	415.03 ± 3.59 cd
Mean value		*8.48 ± 1.57*	*420.66 ± 59.18*	*7.17 ± 1.03 bc*	*400.82 ± 15.39*
Average cultivars 2019		9.20 ± 1.80 a	362.86 ± 10.54 b	7.86 ± 1.47 a	378.41 ± 10.76 b
Average cultivars 2020		8.05 ± 2.13 b	470.88 ± 21.02 a	6.88 ± 1.20 b	418.20 ± 9.25 a

3.4 Content of total chlorophyll, flavonols, anthocyanins and Nitrogen Balance Index in the almond leaves

The leaf content of total chlorophyll does not only help to quantify the photosynthesis rate of the plant (Cerovic et al., 2015), but it may be also used to assess the adaptability of a certain cultivar in a new growing environment (Li et al., 2018).

Eco-physiological parameters of almond leaves measured at 90 DABF are reported in Table 3.4a. Total Chl content at 90 DAFB was influenced by cultivar, year of investigation and by their interaction. The Chl mean values ranged between 32.87 µg cm^{-2} recorded in Lauranne®Avijor in 2020 to 18.87 µg cm^{-2} measured in Soleta in 2019. High value of total Chl at 90 DAFB, generally higher than 30 µg cm^{-2} were observed in 2020 also for Soleta Supernova, Tuono and Vialfas. Furthermore, the mean values of total Chl, expressed as mean of cultivars, was significantly higher in 2020 (mean value of 30.48 µg cm^{-2}) in comparison to those recorded in 2019 (mean value of 23.97 µg cm^{-2}).

Conversely, leaf contents of Flav and Anth at 90 DAFB were more stable than those of Chl, as shown by their mean values expressed as mean of cultivars (Table 3.4a). Indeed, Flav had mean values of 2.35 and 2.31 µg cm^{-2} in 2019 and 2020 respectively, as well as the Anth content, that showed mean values of 0.10 (2019) and 0.08 µg cm^{-2} (2020).

The NBI in the almond leaves at 90 DAFB ranged between mean values of 14.62 in Lauranne®Avijor in 2020 to mean values of 8.00 in Soleta in 2019. Even cultivars Soleta, Supernova, Tuono and Vialfas showed in 2020 high values of NBI, above 13, whereas Guara and Tuono showed in 2019 low NBI mean values, below 10. It was also confirmed by the NBI expressed as mean of cultivars, that was significantly higher in 2020 than 2019, as shown in Table 3.4a.

Table 3.4a. Leaf total chlorophyll (Chl), flavonol (Flav) anthocyanin (Anth) and nitrogen balance index (NBI) in almond leaves collected at 90 DAFB over the years 2019-2020. Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar (90 DAFB)	Year	Chl (µg cm^{-2})	Flav (µg cm^{-2})	Anth (µg cm^{-2})	NBI
Genco	**2019**	25.07 ± 4.47 gh	2.37 ± 0.06 abc	0.10 ± 0.03 cde	10.61 ± 1.96 hi
	2020	28.08 ± 3.02 f	2.35 ± 0.10 bcd	0.09 ± 0.02 def	11.97 ± 1.29 g
Mean value		*26.57 ± 4.09 cde*	*2.36 ± 0.08 a*	*0.09 ± 0.03 c*	*11.29 ± 1.79 cd*
Guara	**2019**	22.43 ± 3.29 j	2.36 ± 0.07 a-d	0.12 ± 0.03 b	9.51 ± 1.43 k
	2020	29.46 ± 4.27 de	2.30 ± 0.08 fg	0.09 ± 0.02 def	12.87 ± 1.76 de
Mean value		*25.95 ± 5.19 de*	*2.33 ± 0.08 abc*	*0.10 ± 0.03 b*	*11.19 ± 2.32 cd*
Lauranne® Avijor	**2019**	23.66 ± 2.46 ij	2.34 ± 0.06 cde	0.10 ± 0.02 cd	10.13 ± 1.17 ij
	2020	32.87 ± 3.39 a	2.28 ± 0.11 gh	0.07 ± 0.02 i	14.62 ± 1.56 a
Mean value		*28.26 ± 5.49 bc*	*2.31 ± 0.10 c*	*0.09 ± 0.02 cd*	*12.37 ± 2.64 ab*
Penta®	**2019**	24.32 ± 3.44 hi	2.29 ± 0.07 fg	0.10 ± 0.03 cde	10.59 ± 1.53 hi
	2020	28.23 ± 2.52 ef	2.32 ± 0.06 ef	0.08 ± 0.02 gh	12.21 ± 1.18 fg
Mean value		*26.27 ± 3.58 de*	*2.31 ± 0.07 c*	*0.09 ± 0.03 cd*	*11.40 ± 1.58 cd*
Soleta	**2019**	18.87 ± 2.84 k	2.36 ± 0.06 a-d	0.15 ± 0.03 a	8.00 ± 1.26 l
	2020	30.62 ± 4.29 cd	2.33 ± 0.06 de	0.08 ± 0.03 fg	13.12 ± 2.00 cde
Mean value		*24.74 ± 6.92 e*	*2.35 ± 0.06 ab*	*0.12 ± 0.05a*	*10.56 ± 3.06 d*
Supernova	**2019**	25.70 ± 3.62 g	2.38 ± 0.08 ab	0.09 ± 0.03 ef	10.86 ± 1.74 h
	2020	32.08 ± 4.69 ab	2.29 ± 0.09 fgh	0.08 ± 0.03 gh	14.14 ± 2.03 ab
Mean value		*28.89 ± 5.26 ab*	*2.33 ± 0.10 abc*	*0.09 ± 0.03 cd*	*12.50 ± 2.50 a*
Tuono	**2019**	22.93 ± 3.89 j	2.38 ± 0.06 a	0.10 ± 0.03 cde	9.62 ± 1.61 jk
	2020	30.93 ± 4.25 bc	2.26 ± 0.11 h	0.08 ± 0.02 gh	13.68 ± 1.66 bc
Mean value		*26.94 ± 5.71 cd*	*2.32 ± 0.11 bc*	*0.09 ± 0.02 cd*	*11.65 ± 2.60 bc*
Vialfas	**2019**	28.75 ± 4.45 ef	2.30 ± 0.10 fg	0.07 ± 0.02 hi	12.59 ± 2.04 ef
	2020	31.58 ± 3.88 abc	2.36 ± 0.09 a-d	0.09 ± 0.03 ef	13.30 ± 1.99 cd
Mean value		*30.16 ± 4.39 a*	*2.33 ± 0.10 abc*	*0.08 ± 0.03 d*	*12.94 ± 2.04 a*
Average cultivars 2019		23.97 ± 4.47 b	2.35 ± 0.11 a	0.10 ± 0.03 a	10.24 ± 2.02 b
Average cultivars 2020		30.48 ± 4.16 a	2.31 ± 0.10 b	0.08 ± 0.02 b	13.24 ± 1.90 a

The same leaf traits measured at 120 DABF are reported in Table 3.4b. Even in mid-July total Chl content in the leaves was influenced by cultivar, year of investigation and by their interaction. The Chl mean values ranged between 39.31 µg cm^{-2} recorded in Vialfas in 2020 to 19.83 µg cm^{-2} measured in Soleta in 2019. Values of total leaf Chl, higher than 30 µg cm^{-2}, were observed also for Lauranne®Avijor for both research years and for Guara, Penta®, Soleta and Tuono in 2020. Furthermore, the mean values of total Chl, expressed as mean of cultivars, was significantly higher in 2020 (mean value of 32.58 µg cm^{-2}) in comparison to those recorded in 2019 (mean value of 25.21 µg cm^{-2}).

Leaf contents of Flav were slightly lower than those observed in mid-June (90 DAFB) as shown by their values expressed as mean of cultivars (mean values of 2.21 and 2.27 µg cm^{-2} in the growing seasons 2019 and 2020, respectively). Furthermore, the values recorded for each cultivar over the two-year investigation were very similar between them (Table 3.4b).

The leaf Anth contents ranged between 0.12 µg cm^{-2} measured in Supernova in 2019 to 0.04 µg cm^{-2} in Penta® in 2020, meanwhile Anth expressed as mean of cultivars had mean values of 0.08 and 0.07 µg cm^{-2} in 2019 and 2020, respectively.

The NBI at 120 DAFB ranged between mean values of 17.16 in Vialfas in 2020 to values of 9.18 in Soleta in 2019. This leaf trait, expressed as mean of cultivars, was slightly higher in mid-July when compared to 90 DAFB. During 2019, the NBI at 120 DAFB had mean values of 11.49, higher than over one point in comparison to 90 DAFB (mean value of 10.24), as well as in 2020 with values of 14.58 in mid-July versus values of 13.24 recorded in mid-June.

Table 3.4b. Leaf total chlorophyll (Chl), flavonol (Flav) anthocyanin (Anth) and nitrogen balance index (NBI) in almond leaves collected at 120 DAFB over the years 2019-2020. Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar (120 DAFB)	**Year**	**Chl (µg cm^{-2})**	**Flav (µg cm^{-2})**	**Anth (µg cm^{-2})**	**NBI**
Genco	**2019**	24.63 ± 2.48 k	2.30 ± 0.11 bc	0.08 ± 0.02 ef	10.74 ± 1.16 f
	2020	28.27 ± 6.11 hi	2.30 ± 0.16 abc	0.09 ± 0.03 cd	12.94 ± 2.60 d
Mean value		*26.45 ± 4.99 c*	*2.30 ± 0.14 a*	*0.08 ± 0.03 c*	*11.84 ± 2.29 cd*
Guara	**2019**	22.20 ± 3.46 l	2.22 ± 0.15 d	0.11 ± 0.02 b	9.92 ± 1.37 fg
	2020	32.51 ± 7.19 cd	2.31 ± 0.17 ab	0.08 ± 0.04 def	14.43 ± 3.48 c
Mean value		*27.35 ± 7.64 c*	*2.27 ± 0.16 ab*	*0.09 ± 0.04 bc*	*12.17 ± 3.47 c*
Lauranne® Avijor	**2019**	30.62 ± 5.38 efg	2.03 ± 0.18 g	0.06 ± 0.02 g	15.05 ± 2.84 c
	2020	31.96 ± 6.81 cde	2.23 ± 0.18 d	0.07 ± 0.03 f	14.45 ± 3.15 c
Mean value		*31.29 ± 6.15 b*	*2.13 ± 0.21 e*	*0.06 ± 0.03 de*	*14.75 ± 3.00 ab*
Penta ®	**2019**	26.19 ± 4.47 jk	2.21 ± 0.09 de	0.07 ± 0.03 f	11.86 ± 2.11 e
	2020	34.55 ± 4.52 b	2.15 ± 0.19 f	0.04 ± 0.03 h	16.14 ± 2.26 b
Mean value		*30.37 ± 6.13 b*	*2.18 ± 0.15 de*	*0.06 ± 0.03 de*	*14.00 ± 3.06 b*
Soleta	**2019**	19.83 ± 4.81 m	2.16 ± 0.10 f	0.13 ± 0.04 a	9.18 ± 2.23 g
	2020	31.21 ± 7.36 def	2.31 ± 0.14 ab	0.09 ± 0.03 cde	13.45 ± 2.89 d
Mean value		*25.52 ± 8.43 c*	*2.24 ± 0.15 bc*	*0.11 ± 0.04 a*	*11.32 ± 3.35 cd*
Supernova	**2019**	21.57 ± 2.82 lm	2.30 ± 0.05 bc	0.12 ± 0.03 ab	9.39 ± 1.30 g
	2020	28.85 ± 5.42 ghi	2.31 ± 0.15 ab	0.09 ± 0.03 c	12.80 ± 3.03 d
Mean value		*25.21 ± 5.65 c*	*2.30 ± 0.11 a*	*0.10 ± 0.03 ab*	*11.09 ± 2.89 d*
Tuono	**2019**	27.12 ± 3.38 ij	2.17 ± 0.11 ef	0.07 ± 0.02 f	12.67 ± 1.71 de
	2020	33.40 ± 5.22 bc	2.22 ± 0 .14 de	0.07 ± 0.03 f	15.23 ± 2.59 c
Mean value		*30.26 ± 5.40 b*	*2.19 ± 0.13 cd*	*0.07 ± 0.03 d*	*13.95 ± 2.53 b*
Vialfas	**2019**	26.59 ± 4.05 fgh	2.26 ± 0.14 cd	0.06 ± 0.03 g	13.11 ± 1.58 d
	2020	39.31 ± 5.11 a	2.35 ± 0.09 a	0.05 ± 0.03 gh	17.16 ± 2.03 a
Mean value		*34.74 ± 6.93 a*	*2.30 ± 0.13 a*	*0.05 ± 0.03 e*	*15.54 ± 2.72 a*
Average cultivars 2019		25.21 ± 5.35 b	2.21 ± 0.15 b	0.08 ± 0.04 a	11.49 ± 2.67 b
Average cultivars 2020		32.58 ± 6.91 a	2.27 ± 0.17 a	0.07 ± 0.04 b	14.58 ± 3.13 a

3.5 Specific almond cultivar leaf nutrient concentration and ∑DOP index

In order to achieve almond cultivar-specific diagnostic tools focused to manage balanced fertilization of almond orchards highlighting own nutritional requirements of the main cultivars, the leaf diagnosis was applied to the studied cultivars over the two-year investigation, since it identifies the nutritional status of a plant via chemical analysis of its leaf tissues.

Leaf analyses carried out at 90 DAFB showed significant differences related to the effect of cultivar, year of investigation and their interaction, with the exception for K which showed no significance on statistical analysis, Mg that was not influenced by the effect of cultivar and year of investigation (Table 3.5a) and Cu that was not influenced by cultivar effect (Table 3.5b).

Similarly, leaf analyses carried out at 120 DAFB showed as the nutrients N, C, K, Mg and Zn were not influenced by the cultivar, while Ca, Mg, Mn, and B were not influenced by year of investigation (Tables 3.6a and 3.6b).

At 90 DAFB (Table 3.5a) cultivar Vialfas showed the higher N content (3.38% DW) in 2020, whereas the lowest mean value of N was detected in Soleta in 2019 (1.59% DW). Nitrogen contents in almond leaves were higher in 2020 for all cultivars analyzed, as confirmed by the values expressed as mean of cultivars, which were 1.97% DW in 2019 and 2.60% DW in 2020, respectively. The only cultivar Tuono did not confirmed the trend, showing same mean values of N both in 2019 and 2020 (2.4% DW) highlighting high stability for this nutrient in the leaves at 90 DAFB.

Inorganic carbon (C) was very stable in the leaves collected at 90 DAFB ranging from 44.99% to 41.06% DW recorded in Soleta in the years 2019 and 2020, respectively. This trait was only slightly influenced by the year and by the interaction cultivar x year, without cultivar-related influences.

The values of P at 90 DAFB recorded in the cultivars tested were slightly low in comparison to the average reference values reported in the literature (Mills et al., 1996).

Its content ranged between 0.11% in Vialfas and Lauranne®Avijor in 2020 to 0.05% in Genco in 2019. The nutrient was not affected by the cultivar effect and its mean values, expressed as mean of cultivars, were slightly higher in 2019 (mean value of 0.09% DW) compared to those observed in 2020 (mean value of 0.07% DW).

Although the content of K in the cultivars studied did not show statistical significance and the nutrient was highly stable in the leaves collected at 90 DAFB for all cultivars and for both years of investigation, its mean value recorded in 2019 was slightly higher in 2019 (0.97%) than in 2020 (0.92%)

At the same leaf sampling period and in the environment of the trial, low concentrations of Ca were extensively observed in the cultivars tested when compared with reference values for this nutrient (Mills et al., 1996), and highest values were recoded for Genco and Supernova (1.6% DW) in 2019 for both, whereas the lowest ones occurred for cultivars Penta® and Vialfas in 2020 (0.7% DW). On average, higher men values of Ca were recorded in 2019 compared to those of 2020.

Mg leaf content was affected by the cultivar only, and its values ranged between 0.29% in supernova in 2019 to 0.19% in Lauranne®Avijor in the same year of investigation. In addition, the average Mg content expressed as mean of cultivars did not show any noteworthy differences between the two study years (mean values of 0.23-0.24% DW).

Table 3.5a. Macronutrients content (% DW) in almond leaves collected at 90 DAFB over the years 2019-2020. (N = nitrogen, C = carbon, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium). Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar (90 DAFB)	**Year**	**N (% DW)**	**C (% DW)**	**P (% DW)**	**K (% DW)**	**Ca (% DW)**	**Mg (% DW)**
Genco	**2019**	2.01 ± 0.02 e-h	44.23 ± 0.36 cd	0.05 ± 0.004 i	1.11 ± 0.10	1.62 ± 0.16 a	0.24 ± 0.01 b-e
	2020	3.18 ± 0.20 a	42.89 ± 0.12 fg	0.08 ± 0.003 ed	0.86 ± 0.03	0.97 ± 0.26 b-e	0.20 ± 0.01 fg
Mean value		*2.59 ± 0.64 ab*	*43.56 ± 0.76*	*0.07 ± 0.02*	*0.98 ± 0.15*	*1.30 ± 0.40 a*	*0.22 ± 0.02*
Guara	**2019**	1.74 ± 0.17 ghi	44.31 ± 0.12 cd	0.07 ± 0.009 gh	0.86 ± 0.09	1.14 ± 0.05 bcd	0.21 ± 0.02 d-g
	2020	2.45 ± 0.38 cd	42.31 ± 0.30 ghi	0.09 ± 0.007 bc	0.84 ± 0.16	0.89 ± 0.12 def	0.25 ± 0.02 abc
Mean value		*2.09 ± 0.47 cd*	*43.31 ± 1.09*	*0.08 ± 0.02*	*0.85 ± 0.12*	*1.01 ± 0.16 b*	*0.23 ± 0.03*
Lauranne® Avijor	**2019**	1.87 ± 0.02 f-i	44.43 ± 0.02 bc	0.07 ± 0.003 fgh	0.93 ± 0.04	1.06 ± 0.05 bcd	0.19 ± 0.01 g
	2020	2.85 ± 0.01 b	42.96 ± 0.01 fg	0.11 ± 0.003 a	0.94 ± 0.08	0.95 ± 0.05 c-f	0.28 ± 0.02 ab
Mean value		*2.36 ± 0.52 bc*	*43.70 ± 0.80*	*0.09 ± 0.02*	*0.94 ± 0.06*	*1.01 ± 0.08 b*	*0.23 ± 0.05*
Penta®	**2019**	1.88 ± 0.02 f-i	44.29 ± 0.18 cd	0.06 ± 0.005 hi	1.06 ± 0.08	1.22 ± 0.07 b	0.21 ± 0.01efg
	2020	2.31 ± 0.20 cde	42.60 ± 0.38 gh	0.10 ± 0.006 ab	0.89 ± 0.08	0.72 ± 0.02 ef	0.22 ± 0.01 d-g
Mean value		*2.09 ± 0.26 cd*	*43.41 ± 0.95*	*0.08 ± 0.02*	*0.98 ± 0.11*	*0.97 ± 0.27 b*	*0.21 ± 0.01*
Soleta	**2019**	1.59 ± 0.04 i	44.99 ± 0.04 ab	0.09 ± 0.01 cd	0.90 ± 0.07	1.17 ± 0.28 bc	0.25 ± 0.06 a-d
	2020	2.17 ± 0.10 def	41.06 ± 0.13 j	0.08 ± 0.002 def	1.11 ± 0.40	1.04 ± 0.34 bcd	0.26 ± 0.01 abc
Mean value		*1.88 ± 0.32 d*	*43.03 ± 2.28*	*0.08 ± 0.01*	*1.01 ± 0.28*	*1.11 ± 0.29 ab*	*0.25 ± 0.04 fg*
Supernova	**2019**	1.72 ± 0.01 hi	43.71 ± 0.08 de	0.07 ± 0.01 efg	1.01 ± 0.35	1.59 ± 0.40 a	0.29 ± 0.06 a
	2020	2.06 ± 0.27 efg	41.76 ± 0.44 i	0.09 ± 0.005 cd	1.02 ± 0.17	1.07 ± 0.15 bcd	0.23 ± 0.01 c-f
Mean value		*1.89 ± 0.26 d*	*42.73 ± 1.09*	*0.08 ± 0.02*	*1.01 ± 0.25*	*1.33 ± 0.39 a*	*0.26 ± 0.05*
Tuono	**2019**	2.42 ± 0.63 cd	44.27 ± 0.84 cd	0.07 ± 0.004 gh	0.90 ± 0.05	1.18 ± 0.11 bc	0.21 ± 0.01 efg
	2020	2.44 ± 0.09 cd	42.18 ± 0.13 hi	0.10 ± 0.005 b	0.84 ± 0.10	1.08 ± 0.01 bcd	0.24 ± 0.02 cde
Mean value		*2.43 ± 0.42 bc*	*43.23 ± 1.25*	*0.08 ± 0.02*	*0.87 ± 0.08*	*1.13 ± 0.09 ab*	*0.23 ± 0.02*
Vialfas	**2019**	2.51 ± 0.28 bc	45.29 ± 0.15 a	0.07 ± 0.001 fgh	0.98 ± 0.01	1.20 ± 0.06 bc	0.24 ± 0.04 b-e
	2020	3.39 ± 0.22 a	43.43 ± 0.47 ef	0.11 ± 0.004 a	0.87 ± 0.02	0.71 ± 0.03 f	0.22 ± 0.01 c-g
Mean values		*2.95 ± 0.52 a*	*44.36 ± 1.05*	*0.09 ± 0.002*	*0.93 ± 0.06*	*0.96 ± 0.26 b*	*0.23 ± 0.03*
Average cultivars 2019		1.97 ± 0.39 b	44.44 ± 0.56 a	0.07 ± 0.01 b	0.97 ± 0.15	1.27 ± 0.26 a	0.23 ± 0.04
Average cultivars 2020		2.60 ± 0.50 a	42.40 ± 0.86 b	0.09 ± 0.01 a	0.92 ± 0.18	0.93 ± 0.20 b	0.24 ± 0.03

Almond leaf micronutrients detected at 90 DAFB showed significant differences related to the effect of cultivar, year of investigation and their interaction, as shown in Table 3.5b. Particularly, the leaf content of Fe showed wide variability ranged between 130.31 µg g^{-1} DW recorded in 2020 for cv Soleta to 50.15 µg g^{-1} DW in Lauranne®Avijor in 2019. Furthermore, its mean value was higher in 2020 than in 2019 (87.5 µg g^{-1} DW vs 69.7 µg g^{-1} DW), meanwhile the cultivars Guara, Penta® and Vialfas showed high stability of leaf content of Fe over the two years of investigation. Manganese in mid-June showed highest mean values in Penta® (30.73 µg g^{-1} DW in 2019) and the lowest ones in Soleta (14.19 µg g^{-1} DW in 2020). High mean values of this nutrient were also detected for Vialfas (28.43 µg g^{-1} DW) and Tuono (25.87 µg g^{-1} DW) in 2019 for both cultivars, whereas the Mn leaf content expressed as mean of cultivars was significantly higher in 2019 than in 2020.

Referring to the leaf content of B, its values were consistently higher in 2019 for all cultivars with the sole exception of Supernova, where values measured in 2019 were nearly the same as in 2020 (32.1 vs 26.6), as well as partially for Tuono (16.79 vs 10.30). Moreover, although the nutrient was characterized by wide variability in content, its values were in line with the reference values proposed by Mills et al. (1996). The highest mean values were observed in Soleta in 2019 (75.96 µg g^{-1} DW), while the lowest ones were recorded in Tuono in 2020 (10.30 µg g^{-1} DW).

Copper (Cu) in almond leaves at mid-June was the only micronutrient that did not show statistical significance related to the cultivar effect, although showing wide variability in content. Furthermore, its value as mean of cultivars recorded in 2019 was much lower than in 2020 (4.52 µg g^{-1} DW vs 16.42 µg g^{-1} DW, respectively). The highest Cu leaf content was observed in Genco in 2020 (20.84 µg g^{-1} DW), while the lowest content was expressed in leaves of Lauranne®Avijor in 2019 (1.27 µg g^{-1} DW).

Contrariwise to Cu, the micronutrient Zn showed contents as mean of cultivars recorded in 2019 significantly higher than those detected in 2020, with mean values of 47.27 µg g^{-1} DW and 29.58 µg g^{-1} DW, respectively (Table 3.5b). Guara was the cultivar characterized by lower content of Cu in the leaves with mean values expressed as mean of year investigation of 6.66 µg g^{-1} DW, whereas cultivar Supernova showed the higher leaf content with mean values of 58.65 µg g^{-1} DW.

Table 3.5b. Micronutrients content (µg g^{-1} DW) in almond leaves collected at 90 DAFB over the years 2019-2020. (Fe = iron, Mn = manganese, B = boron, Cu = copper, Z = zinc). Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar (90 DAFB)	**Year**	**Fe** (µg g^{-1} DW)	**Mn** (µg g^{-1} DW)	**B** (µg g^{-1} DW)	**Cu** (µg g^{-1} DW)	**Zn** (µg g^{-1} DW)
Genco	**2019**	68.13 ± 7.92 e	18.68 ± 1.80 fgh	50.43 ± 14.60 b	7.20 ± 2.98 ef	33.41 ± 4.72 cd
	2020	101.00 ± 5.98 bc	17.79 ± 1.28 ghi	12.58 ± 2.25 gh	20.84 ± 4.05 a	43.61 ± 0.49 abc
Mean value		*84.57 ± 18.73 ab*	*18.23 ± 1.52 cd*	*31.50 ± 22.43 abc*	*14.02 ± 8.00*	*38.51 ± 9.30 ab*
Guara	**2019**	71.55 ± 20.35 de	22.70 ± 1.07 cde	34.27 ± 10.04 cd	3.23 ± 0.18 gh	28.60 ± 16.44 cde
	2020	60.34 ± 10.44 e	17.92 ± 0.67 gh	13.10 ± 2.25 gh	15.12 ± 0.88 cd	6.66 ± 0.44 e
Mean value		*65.95 ± 16.13 bc*	*20.31 ± 2.69 bcd*	*23.68 ± 13.17 bc*	*9.18 ± 6.38*	*17.63 ± 14.92 c*
Lauranne® Avijor	**2019**	50.15 ± 1.46 e	20.24 ± 2.54 e-h	43.40 ± 17.57 bc	1.27 ± 0.10 h	49.52 ± 18.73 abc
	2020	99.00 ± 13.34 bcd	23.53 ± 1.30 cde	21.86 ± 4.60 efg	16.75 ± 5.57 bc	43.47 ± 10.47 abc
Mean value		*74.58 ± 27.55 abc*	*21.89 ± 2.57 abc*	*32.63 ± 16.55 ab*	*9.01 ± 7.07*	*46.50 ± 14.42 ab*
Penta®	**2019**	52.06 ± 3.34 e	30.73 ± 2.17 a	50.74 ± 7.27 b	1.70 ± 0.62 h	48.57 ± 6.58 abc
	2020	56.40 ± 2.77 e	17.41 ± 1.01 hi	19.31 ± 20.5 fgh	17.29 ± 0.66 bc	18.57 ± 7.70 de
Mean value		*54.23 ± 3.67 c*	*24.07 ± 6.29 ab*	*35.03 ± 17.52 ab*	*9.50 ± 7.53*	*33.57 ± 17.35 bc*
Soleta	**2019**	66.75 ± 19.95 e	20.04 ± 5.21 e-h	75.96 ± 1.69 a	3.41 ± 0.41 gh	56.92 ± 16.98 a
	2020	130.31 ± 28.61 a	14.19 ± 1.09 i	20.11 ± 5.52 fgh	12.71 ± 0.69 d	12.06 ± 1.65 de
Mean value		*98.53 ± 30.93 a*	*17.12 ± 5.18 d*	*48.04 ± 20.09 a*	*8.06 ± 5.00*	*34.49 ± 26.45 bc*
Supernova	**2019**	112.85 ± 32.77 ab	24.93 ± 1.33 bcd	32.10 ± 7.87 de	4.21 ± 1.11 fgh	56.18 ± 31.20 ab
	2020	69.94 ± 21.65 e	16.84 ± 0.39 hi	26.60 ± 4.99 def	14.46 ± 2.92 cd	61.12 ± 19.84 a
Mean value		*91.40 ± 40.82 ab*	*20.88 ± 4.42 a-d*	*29.34 ± 6.77 bc*	*9.33 ± 5.85*	*58.65 ± 24.35 a*
Tuono	**2019**	60.83 ± 16.12 e	25.87 ± 1.41 bc	16.79 ± 3.67 fgh	9.02 ± 0.21 e	43.60 ± 6.16 abc
	2020	111.27 ± 19.44 ab	21.64 ± 1.90 def	10.30 ± 4.39 h	15.60 ± 1.64 bcd	34.13 ± 14.51 bcd
Mean value		*86.05 ± 31.63 ab*	*23.75 ± 2.74 ab*	*13.54 ± 5.11 c*	*12.31 ± 3.68*	*38.86 ± 11.49 ab*
Vialfas	**2019**	75.25 ± 1.66 cde	28.43 ± 5.92 ab	54.55 ± 11.34 b	6.09 ± 1.11 efg	61.37 ± 31.17 a
	2020	71.49 ± 6.42 e	21.27 ± 2.30 efg	13.69 ± 3.68 gh	18.61 ± 1.06 ab	17.05 ± 3.47 de
Mean value		*73.36 ± 4.68 abc*	*24.85 ± 5.54 a*	*34.12 ± 20.13 ab*	*12.35 ± 6.77*	*39.21 ± 21.35 ab*
Average cultivars 2019		69.70 ± 26.95 b	23.95 ± 5.06 a	44.78 ± 19.19 a	4.52 ± 2.79 b	47.27 ± 20.16 a
Average cultivars 2020		87.47 ± 28.98 a	18.82 ± 3.06 b	17.19 ± 6.32 b	16.42 ± 3.45 a	29.58 ± 20.28 b

Leaf macronutrients detected in mid-July (120 DAFB) were reported in Table 3.6a. Content of N was significantly influenced by the year of investigation and interaction cultivar x year, while the nutrient was not influenced by the effect of cultivar. Its mean content was higher in 2020 for all cultivars tested and its values ranged between 3.39% DW recorded in Vialfas in 2020 to 1.72% DW detected in Soleta in 2019. Furthermore, Lauranne®Avijor was the cultivar characterized by a higher N content stability within the two-year research in comparison to the other cultivars studied.

Similarly to N, also C detected at 120 DAFB was influenced by the year of investigation and interaction cultivar x year. Nevertheless, this leaf trait was very stably for all cultivars and years of investigation, as shown by its values that were always ranked within 43-45% DW.

Phosphorus (P) ranged between mean values of 0.12% DW observed in Lauranne®Avijor to 0.07% DW detected in Genco, Supernova and Tuono in the first year of research for all cultivars. This macronutrient was influenced by both effects and their interaction, as shown by statistical analysis. Furthermore, leaf sampling at 120 DAFB showed on general P values slightly lower in comparison to the reference values reported in the literature (Mills et al., 1996).

The leaf content of K at mid-July in the cultivars studied showed significant differences only for year and interaction of cultivar x year with the highest average values being observed in Lauranne®Avijor in 2019 (2.12% DW) and the lowest ones in Soleta in 2020 with (0.29% DW). The nutrient was also much higher during the first research-year, showing values expressed as mean of cultivars of 1.36% DW compared with 0.38% DW in 2020.

Cultivars Vialfas and Guara showed the higher Ca leaf concentration at 120 DAFB expressed as mean of two years, with similar values between them (1.65% and 1.63% DW respectively), meanwhile the highest absolute values of Ca in the almond leaves were detected for Penta® in 2020 (1.86% DW). Contrariwise, lowest content was observed in Supernova in 2020 that showed mean values of 1.10% DW. For this nutrient no differences were observed in relation to the year of the investigation.

Mg leaf content was affected by the effect of interaction between cultivar and year of investigation only, and its values ranged between 0.28% DW in Penta® in 2019 to 0.20% DW in Tuono in the second research-year. In addition, the Mg content expressed as mean of cultivars weas the same over the two years of investigation, as shown in Table 3.6a.

Table 3.6a. Macronutrients content (% DW) in almond leaves collected at 120 DAFB over the years 2019-2020. (N = nitrogen, C = carbon, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium). Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar (120 DABF)	Year	N (% DW)	C (% DW)	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)
Genco	**2019**	1.80 ± 0.01 i	45.05 ± 0.04 bcd	0.07 ± 0.01 f	1.68 ± 0.17 b	1.52 ± 0.19 bcd	0.22 ± 0.02 efg
	2020	3.03 ± 0.33 abc	44.48 ± 0.23 def	0.11 ± 0.02 bc	0.31 ± 0.03 g	1.67 ± 0.13 abc	0.26 ± 0.03 abc
Mean value		*2.42 ± 0.69*	*44.76 ± 0.34*	*0.08 ± 0.02 b*	*1.00 ± 0.74*	*1.60 ± 0.17 ab*	*0.24 ± 0.03*
Guara	**2019**	2.28 ± 0.12 gh	45.39 ± 0.36 bc	0.09 ± 0.01 e	0.77 ± 0.75 ef	1.73 ± 0.11 abc	0.23 ± 0.03 b-g
	2020	2.71 ± 0.52 cde	43.77 ± 0.53 fgh	0.09 ± 0.01 de	0.34 ± 0.04 g	1.56 ± 0.33 bcd	0.27 ± 0.02 ab
Mean value		*2.49 ± 0.42*	*44.58 ± 0.96*	*0.09 ± 0.01 b*	*0.55 ± 0.54*	*1.65 ± 0.24 a*	*0.25 ± 0.03*
Lauranne® Avijor	**2019**	2.33 ± 0.04 fgh	46.58 ± 0.28 a	0.12 ± 0.02 a	2.12 ± 0.31 a	1.51 ± 0.25 bcd	0.25 ± 0.02 a-e
	2020	2.54 ± 0.19 d-g	43.40 ± 0.28 gh	0.10 ± 0.01 bc	0.41 ± 0.01 g	1.25 ± 0.38 efg	0.23 ± 0.03 c-g
Mean value		*2.44 ± 0.17*	*44.99 ± 1.72*	*0.11 ± 0.01 a*	*1.26 ± 0.94*	*1.38 ± 0.33 bc*	*0.24 ± 0.03*
Penta®	**2019**	2.21 ± 0.07 gh	45.62 ± 0.05 b	0.09 ± 0.01 de	1.20 ± 0.12 d	1.34 ± 0.04 d-g	0.28 ± 0.02 a
	2020	2.97 ± 0.09 bc	43.02 ± 1.35 hi	0.09 ± 0.01 de	0.48 ± 0.08 fg	1.86 ± 0.11 a	0.22 ± 0.04 d-g
Mean value		*2.58 ± 0.42*	*44.32 ± 1.64*	*0.09 ± 0.01 b*	*0.84 ± 0.41*	*1.60 ± 0.29 ab*	*0.25 ± 0.04*
Soleta	**2019**	1.72 ± 0.07 i	45.80 ± 0.27 ab	0.10 ± 0.01 cde	1.28 ± 0.18 cd	1.40 ± 0.07 def	0.24 ± 0.04 b-f
	2020	2.66 ± 0.71 c-f	42.28 ± 1.38 i	0.09 ± 0.01 cde	0.29 ± 0.02 g	1.23 ± 0.08 efg	0.25 ± 0.04 a-d
Mean value		*2.19 ± 0.68*	*44.04 ± 2.09*	*0.09 ± 0.01 b*	*0.78 ± 0.45*	*1.32 ± 0.12 c*	*0.24 ± 0.04*
Supernova	**2019**	1.82 ± 0.01 i	44.75 ± 0.11 cde	0.07 ± 0.01 f	1.23 ± 0.02 cd	1.47 ± 0.04 cde	0.22 ± 0.01 efg
	2020	2.90 ± 0.34 bcd	43.55 ± 0.47 gh	0.11 ± 0.02 ab	0.43 ± 0.03 g	1.10 ± 0.11 g	0.23 ± 0.01 c-g
Mean value		*2.36 ± 0.62*	*44.15 ± 0.71*	*0.09 ± 0.02 b*	*0.83 ± 0.33*	*1.28 ± 0.21 c*	*0.22 ± 0.01*
Tuono	**2019**	2.03 ± 0.04 hi	45.17 ± 0.59 bcd	0.07 ± 0.01 f	1.05 ± 0.07 de	1.41 ± 0.05 def	0.23 ± 0.02 c-g
	2020	3.08 ± 0.15 ab	43.32 ± 0.84 gh	0.10 ± 0.01 bcd	0.42 ± 0.04 g	1.21 ± 0.021 fg	0.20 ± 0.03 g
Mean value		*2.55 ± 0.57*	*44.25 ± 1.21*	*0.09 ± 0.01 b*	*0.73 ± 0.34*	*1.31 ± 0.17 c*	*0.21 ± 0.03*
Vialfas	**2019**	2.51 ± 0.05 efg	45.76 ± 0.04 ab	0.09 ± 0.01 e	1.56 ± 0.39 bc	1.53 ± 0.14 bcd	0.21 ± 0.02 fg
	2020	3.39 ± 0.10 a	44.17 ± 0.24 efg	0.09 ± 0.01 cde	0.35 ± 0.02 g	1.74 ± 0.22 ab	0.24 ± 0.01 b-f
Mean value		*2.95 ± 0.48*	*44.96 ± 0.87*	*0.09 ± 0.01 b*	*0.95 ± 0.54*	*1.63 ± 0.21 a*	*0.23 ± 0.02*
Average cultivars 2019		2.08 ± 0.28 b	45.51 ± 0.59 a	0.09 ± 0.02 b	1.36 ± 0.49 a	1.49 ± 0.16	0.23 ± 0.03
Average cultivars 2020		2.91 ± 0.41 a	43.50 ± 0.96 b	0.10 ± 0.01 a	0.38 ± 0.07 b	1.45 ± 0.34	0.24 ± 0.03

Table 3.6b describes the content of micronutrients in the leaves of the cultivars studied at 120 DAFB. Iron (Fe) in the almond leaves ranged between values of 131.32 µg g^{-1} DW recorded in Penta® in 2019 to 50.55 µg g^{-1} DW in Guara in 2020. On average, Penta® showed the highest mean value for the nutrient when expressed as mean of two research-years (100.47 µg g^{-1} DW) contrariwise to Guara, Genco, and Supernova that were characterized by the lowest mean values (65.07, 67.62 and 68.81 µg g^{-1} DW, respectively). In addition, its mean values expressed as mean of cultivars were significantly higher in 2019 (93.26 µg g^{-1} DW) than in 2020 (63.70 µg g^{-1} DW).

Mn was significantly influenced by the effect of cultivar and interaction cultivar x year of investigation. Highest mean values at 120 DABF were observed for Vialfas in both years of investigation (22.40 µg g^{-1} DW in 2019 and 22.01 µg g^{-1} DW in 2020) and for Lauranne®Avijor in 2020 (22.37 µg g^{-1} DW), meanwhile the lowest ones were recorder for Soleta in 2020 (11.09 µg g^{-1} DW).

Similarly to Mn also B showed significant differences only for cultivar and interaction of cultivar x year. The micronutrient at 120 DAFB was characterized by relevant variability of leaf content, ranging from mean values of 42.10 µg g^{-1} DW in Vialfas to 7.03 µg g^{-1} DW in Tuono detected for both cultivars at the second research-year. Furthermore, Vialfas and Genco showed the highest content of B in the leaves when expressed as mean of two-year investigation (mean values of 30.13 and 27.65 µg g^{-1} DW, respectively) in contrast to Tuono and Guara that were characterized by lowest mean values (about 15-16 µg g^{-1} DW).

The content of Cu was influenced by the effect of cultivar, year of investigation and their interaction (Table 3.6b). Its contents, expressed as mean of cultivars, were double in values in 2020 in comparison to those detected in 2019 (30.81 vs 15.06). The highest Cu foliar content was observed in Guara in 2020 (51.45 µg g^{-1} DW), while the lowest one was recorded in Tuono in 2019 (11.25 µg g^{-1} DW).

The highest foliar Zn content were observed in Genco in 2019 with mean values of 45.26 µg g^{-1} DW. Penta® was the cultivar that showed the lowest Zn content in the leaves particularly at mid-July 2020, with values of 7.41 µg g^{-1} DW. Notable variability in content for the micronutrient was highlighted by its mean values expressed as mean of cultivars, that were significantly higher in 2019 (20.48 µg g^{-1} DW) than in 2020 (11.58 µg g^{-1} DW), as shown in Table 3.6b.

Table 3.6b. Micronutrients content (μg g^{-1} DW) in almond leaves collected at 120 DAFB over the years 2019-2020. (Fe = iron, Mn = manganese, B = boron, Cu = copper, Z = zinc). Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar (120 BAFB)	Year	Fe (μg g^{-1} DW)	Mn (μg g^{-1} DW)	B (μg g^{-1} DW)	Cu (μg g^{-1} DW)	Zn (μg g^{-1} DW)
Genco	**2019**	68.45 ± 8.79 def	13.72 ± 0.47 ef	39.84 ± 14.53 a	19.21 ± 4.55 d-g	45.26 ± 26.75 a
	2020	66.80 ± 16.93 def	19.70 ± 2.98 abc	15.46 ± 0.83 e	37.68 ± 4.28 bc	9.50 ± 1.99 c
Mean value		*67.62 ± 12.52 b*	*16.71 ± 3.76 c*	*27.65 ± 16.14 ab*	*28.44 ± 8.68 ab*	*27.38 ± 16.11*
Guara	**2019**	79.60 ± 5.36 bcd	19.99 ± 0.51 bcd	18.51 ± 2.71 de	13.54 ± 3.52 efg	12.98 ± 0.25 bc
	2020	50.55 ± 13.04 f	17.82 ± 4.09 bcd	13.16 ± 3.04 ef	51.45 ± 19.32 a	9.66 ± 3.57 c
Mean value		*65.07 ± 18.06 b*	*17.90 ± 2.70 bc*	*15.83 ± 3.91 c*	*32.49 ± 20.11 a*	*11.32 ± 2.94*
Lauranne® Avijor	**2019**	93.51 ± 6.89 b	17.22 ± 3.26 cd	16.59 ± 2.33 de	21.12 ± 1.87 def	10.72 ± 1.04 bc
	2020	67.40 ± 6.21 def	22.37 ± 0.74 a	28.88 ± 11.10 b	26.34 ± 1.91 d	7.46 ± 0.91 c
Mean value		*80.45 ± 15.22 ab*	*19.79 ± 3.51 ab*	*22.73 ± 9.92 abc*	*23.73 ± 3.29 abc*	*9.09 ± 1.97*
Penta®	**2019**	131.32 ± 1.48 a	17.87 ± 0.27 bcd	27.29 ± 5.76 bc	13.65 ± 3.24 efg	19.01 ± 9.95 bc
	2020	69.62 ± 10.32 de	19.08 ± 2.00 bc	17.92 ± 6.62 de	28.33 ± 6.63 cd	7.41 ± 1.22 c
Mean value		*100.47 ± 33.68 a*	*18.47 ± 1.47 bc*	*22.60 ± 7.62 abc*	*20.99 ± 9.22 abc*	*13.21 ± 9.03*
Soleta	**2019**	90.51 ± 19.34 bc	13.07 ± 1.51 ef	21.02 ± 1.92 b-e	15.09 ± 4.12 efg	12.30 ± 0.66 bc
	2020	52.65 ± 9.94 ef	11.09 ± 1.93 f	15.35 ± 0.59 e	22.89 ± 3.17 de	10.10 ± 2.29 c
Mean values		*71.58 ± 24.74 b*	*12.08 ± 1.92 d*	*18.17 ± 3.32 bc*	*18.99 ± 5.39 bc*	*11.20 ± 2.28*
Supernova	**2019**	64.96 ± 6.65 def	15.32 ± 0.46 de	19.85 ± 2.48 cde	12.14 ± 0.61 fg	13.63 ± 3.37 bc
	2020	72.67 ± 16.46 cd	17.14 ± 1.35 cd	13.93 ± 4.02 ef	19.32 ± 2.54 d-g	28.85 ± 13.36 ab
Mean value		*68.81 ± 12.33 b*	*16.23 ± 1.35 c*	*16.89 ± 4.42 c*	*15.73 ± 4.21 c*	*21.24 ± 12.15*
Tuono	**2019**	129.50 ± 32.21 a	18.15 ± 1.29 bc	23.60 ± 7.14 bcd	11.25 ± 0.57 g	37.29 ± 10.86 a
	2020	61.94 ± 2.03 def	20.48 ± 1.75 ab	7.03 ± 0.50 f	16.02 ± 3.72 efg	11.55 ± 1.11 bc
Mean value		*95.72 ± 41.84 a*	*19.32 ± 1.89 b*	*15.32 ± 9.02 c*	*13.63 ± 3.54 c*	*24.42 ± 15.51*
Vialfas	**2019**	88.22 ± 2.56 bc	22.40 ± 2.35 a	18.16 ± 0.22 de	14.48 ± 5.06 efg	12.64 ± 1.21 bc
	2020	67.97 ± 1.53 def	22.01 ± 1.68 a	42.10 ± 2.33 a	44.48 ± 13.62 ab	8.09 ± 1.45 c
Mean value		*78.10 ±11.32 ab*	*22.20 ± 1.91 a*	*30.13 ± 12.89 a*	*29.48 ± 15.64 ab*	*10.36 ± 2.73*
Average cultivars 2019		93.26 ± 26.91 a	16.47 ± 3.15	23.11 ± 9.08	15.06 ± 4.39 b	20.48 ± 12.08 a
Average cultivars 2020		63.70 ± 12.38 b	18.31 ± 3.95	19.23 ± 11.42	30.81 ± 13.33 a	11.58 ± 7.13 b

Table 3.7 shows values of the ∑DOP calculated from the average reference values (C_{ref}) proposed by Mills et al. (1996) for each cultivar, year of investigation and DAFB. Significant differences related to the year were observed for ∑DOP at 120 DAFB, while the interaction cultivar x year was noticed to be statistically significant for both ∑DOP at 90 and 120 DAFB.

At 90 DAFB the ∑DOP index ranged from 551 in Vialfas in the 2020 to 392 in Genco in 2019. Considering that lower values of ∑DOP indicate high balance in macro- and micronutrients in the leaves, thus indicating a suitable nutritional status of the plant, the cultivar that performed similarly Genco was Lauranne®Avijor in 2020, and contrariwise to 2019 where this last cultivar showed one of the worst performances (mean values of 510). Even Tuono showed low values of ∑DOP at 90 DAFB both in 2019 and 2020. Furthermore, at 90 DAFB this leaf nutritional trait was quite stable during the two research-year as shown by the values expressed as average of cultivars. The ∑DOP calculated at 120 DAFB showed high variability in values, and it was significantly higher in 2020 (mean values of 657) versus 2019 (mean value of 449), when expressed as mean of cultivars. Additionally, all cultivars showed an increase in value of ∑DOP between the first and second year of the trial. Cultivar Guara showed the highest ∑DOP values mid-July in 2020 (845) in comparison to other cultivars, while in 2019 had mean values approximately half then those calculated for the second research-year. Conversely, the lowest ∑DOP were obtained for Penta® and Tuono in 2019 with values of 355 and 384, respectively.

Table 3.7. Values of ∑DOP calculated by adding the absolute values of the DOP index determined for every nutrient. Single nutrient DOP indexes were obtained comparing mean values to the average C_{ref} proposed by Mills and Jones (1996). Data are presented as mean ± standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$).

Cultivar	Year	∑ DOP (90 DAFB)	∑ DOP (120 DAFB)
Genco	**2019**	392 ± 27 d	525 ± 88 fgh
	2020	508 ± 19 ab	718 ± 47 bc
Mean values		*540 ± 65*	*622 ± 122*
Guara	**2019**	479 ± 51 bc	468 ± 40 gh
	2020	519 ± 25 ab	845 ± 155 a
Mean values		*499 ± 43*	*656 ± 227*
Lauranne® Avijor	**2019**	510 ± 11 ab	531 ± 42 fgh
	2020	395 ± 66 d	587 ± 39 def
Mean values		*452 ± 75*	*559 ± 48*
Penta®	**2019**	430 ± 17 cd	355 ± 18 i
	2020	501 ± 20 ab	627 ± 56 de
Mean values		*466 ± 42*	*491 ± 150*
Soleta	**2019**	505 ± 90 ab	456 ± 11 gh
	2020	430 ± 19 cd	635 ± 36 cd
Mean values		*468 ± 73*	*565 ± 99*
Supernova	**2019**	434 ± 50 cd	446 ± 2 h
	2020	425 ± 35 cd	540 ± 78 efg
Mean values		*430 ± 41*	*493 ± 72*
Tuono	**2019**	436 ± 35 cd	384 ± 42 i
	2020	425 ± 48 cd	578 ± 19 def
Mean values		*430 ± 40*	*463 ± 127*
Vialfas	**2019**	429 ± 36 cd	461 ± 38 gh
	2020	551 ± 20 a	732 ± 105 b
Mean values		*490 ± 70*	*596 ± 162*
Year 2019 (*Mean values*)		452 ± 56	449 ± 74 b
Year 2020 (*Mean values*)		469 ± 62	657 ± 118 a

3.6 Pearson correlation matrix among almond leaf traits analyzed.

Tables 3.7a and 3.7b show the Pearson correlation matrix between almond leaf traits analyzed as interaction between detected almond leaf nutrients, eco-physiological and biometrical parameters, at 90 and 120 DAFB, respectively. The ∑DOP was excluded from the correlation matrix as it did not show any significant correlation with the other leaf traits.

According to Pearson correlation at 90 DAFB, almond leaf nitrogen content showed a strong positive correlation with copper (0.808) moderate correlation with total chlorophyll (0.616), NBI (0.594), phosphorous (0.540) and LDMC (0.527), whereas moderate negative correlations were observed with calcium (-0.593), boron (-0.607) and quit low negative correlation with Ant (-0.498). Several studies have shown as the lack of N in leaves limits the growth of the plant due to a low supply of photosynthates between shoots and root system (Hermans et al., 2006; Farhat et al., 2016; Chen et al., 2018). Deficiency of N in leaves is also reflected in a lower net photosynthesis rate and poor stomatal conductance, which reduces photosynthetic efficiency. This is due to a poor functioning of the photosynthesis system II (PSII) (Huang et al., 2004). However, it increases the concentration of carbon dioxide in leaf tissue (Mak and Yeh, 2001; Cechin and De Fátima Fumis, 2004; Marchese et al., 2005).

Leaf inorganic carbon content at the same phenological stage showed quite high positive correlation with B (0.741), moderate correlation with manganese (0.662) and zinc (0.515) as well as it showed medium-high negative correlation with Cu (-0.706) and moderate with iron (-0.511)

Phosphorous showed a high correlation with Cu (0.710) and more moderately with NBI (0.554), as well as it showed a quite strong negative correlation with Ca (-0.768) and moderate with K (-0.539) and B (-0.505), respectively.

Potassium at 90 DABF showed only one positive correlation with Ca of a moderate level (0.576). Studies carried out on other crops, as sweet potato, have shown as in plants bred with K deficiency the photosynthetic rate and stomatal conductance decrease significantly and this physiological disorder was also confirmed by the analysis of chlorophyll fluorescence (Liu et al., 2017).

Calcium showed moderate positive correlations with Zn (0.544) and B (0.523), whereas it was negatively correlated with Cu (-0.650).

Magnesium showed only a moderate positive correlation with Fe (0.585) without showing any significant negative correlations with other leaf traits. Magnesium, thanks to its high mobility, tends to move to the leaves when serious reductions in photosynthesis occurs. Furthermore, Mg-free plants have difficulty in moving sucrose to the roots through the phloem and consequent Mg remobilization affects young leaves to the detriment of adult ones (Hermans et al., 2006; Taiz and Zeiger, 2015; Farhat et al., 2016). Magnesium deficiency also negatively affects the proper leaf absorption of other nutrients as confirmed by Ye et al. (2019). The authors observed as in seedlings of *Citrus sinensis* with low leaf concentrations of Mg, the content of Fe increased mainly in the middle and upper leaves of the shoots and simultaneously decreased in those inserted in the lower shoot portion.

Iron, as well as zinc, Flav and NBI when considered main traits at 90 DAFB showed no significant correlation with all other leaf traits analyzed (Table 3.7a). Nevertheless, several studies demonstrated that phosphorus interferes with zinc absorption and often Zn deficiencies are due to the effect of high P levels in the soil (Das et al., 2005; Salimpour et al., 2010; Mousavi et al., 2012; Mousavi, 2011).

Manganese showed quite high positive correlation with SLA (0.696), whereas it was negatively correlated with LDMC (-0.529) and Cu (-0.500) in a low-moderate way in both cases.

Boron was only positively correlated to Zn (0.579) and highly negatively correlated with Cu (-0.786), whereas copper was positively correlated with Chl, LDMC and NBI with moderate extent (0.622, 0.621 and 0.610, respectively).

Analyzing the almond leaf eco-physiological parameters which showed significance in the Pearson correlation matrix, when considered as main parameters, Chl was very strongly positively correlated with NBI (0.950) and highly negatively correlated with Anth (-0.775), whereas Anth was strongly negatively correlated with NBI (-0.819) and LDMC was negatively correlated with SLA (-0.550) in a moderate way.

The correlated analysis of almond leaf traits determined at 120 DAFB over the two research-years is shown in Table 3.7b. Nitrogen had high positive correlation with Chl (0.721) and more moderately with NBI (0.664), Cu (0.634) and Mn (0.558), whereas it was highly negatively correlated with K (-0.702) and moderately with C (-0.590).

Inorganic carbon content in almond leaves at 120 DAFB showed high positive correlation with K (0.838) and moderate correlation with Fe (0.649) without any negative correlation with the other leaf traits of the matrix.

Potassium showed positive correlation with Fe (0.505) and negative correlation with Cu (-0.539) to a moderate entity in both cases.

While iron in mid-July showed only one moderate negative correlation with Cu (-0.532), this last micronutrient showed at the same time only a moderate positive correlation with Chl (0.521).

Manganese was positively correlated with Chl and NBI (0.700 and 0.672, respectively) and negatively with Anth (-0.695) in a moderate-high manner for all cases.

Leaf eco-physiological parameters which showed significance in the Pearson correlation matrix at 120 DAFB, when considered as main parameters, where Chl, Flav, Anth and LDMC. The first one was very strongly positively correlated with NBI (0.972) also at this time of investigation, whereas was highly negatively correlated with Anth (-0.862). Flav showed moderate positive correlation with LDMC (0.650) and negative correlation with SLA (0.580), whereas Anth had highly correlated to NBI (-0.892) and moderately with SLA (-0.493), in a negative way in both cases. Furthermore, LDMC was moderately negatively correlated with SLA (-0.500).

Finally, phosphorous, calcium, magnesium, boron, zinc, and NBI did not show any significative correlation with all other leaf traits analyzed, when considered main traits at 120 DAFB, as shown in Table 3.7b.

Table 3.7a. Pearson correlation matrix between the analyzed variables. Data from leaf diagnostics, content of total chlorophyll, flavonol, anthocyanin and nitrogen balance index and leaf biometrics recorded at 90 DAFB were compared. The correlation between the variables is highlighted in bold. Significance is expressed as *, **, ***, at $p<0.05$, 0.01, 0.001, respectively.

(Level of correlation: 0.9–1.00 very high; 0.7–0.9 high; 0.5–0.7, moderate; 0.3–0.5, low; 0–0.3: negligible correlation)

Variables	N	C	P	K	Ca	Mg	Fe	Mn	B	Cu	Zn	Chl	Flav	Anth	NBI	LDMC	SLA
N	**1**																
C	-0.282	**1**															
P	**0.540****	-0.450															
K	-0.382	-0.034	**-0.539****	**1**													
Ca	**-0.593****	0.422	**-0.768*****	**0.576****	**1**												
Mg	-0.109	-0.237	0.228	0.291	0.302	**1**											
Fe	0.192	**-0.511****	0.200	0.172	0.131	**0.585****	**1**										
Mn	-0.087	**0.662*****	-0.310	0.032	0.329	-0.029	-0.227	**1**									
B	**-0.607****	**0.741*****	**-0.505****	0.388	**0.523****	0.045	-0.325	0.359	**1**								
Cu	**0.808*****	**-0.706*****	**0.710*****	-0.350	**-0.650*****	0.068	0.330	**-0.500****	**-0.786*****	**1**							
Zn	-0.349	**0.515****	-0.298	0.204	**0.544****	0.095	-0.024	0.496	**0.579****	-0.475	**1**						
Chl	**0.616****	-0.473	0.439	0.095	-0.244	0.265	0.344	-0.150	-0.467	**0.622****	-0.178	**1**					
Flav	0.036	0.078	-0.130	-0.023	0.243	0.113	0.190	-0.338	-0.058	0.032	-0.178	-0.198	**1**				
Anth	**-0.498***	0.257	-0.146	-0.041	0.134	-0.004	-0.197	-0.189	0.435	-0.389	-0.055	**-0.775*****	0.473	**1**			
NBI	**0.594****	-0.493	**0.554****	-0.026	-0.374	0.258	0.334	-0.060	-0.483	**0.610****	-0.100	**0.950*****	-0.405	**-0.819*****	**1**		
LDMC	**0.527****	-0.399	0.419	0.086	-0.340	0.088	0.240	**-0.529****	-0.179	**0.621****	-0.039	0.425	0.231	-0.094	0.354	**1**	
SLA	-0.125	0.296	-0.242	-0.004	0.394	-0.054	-0.113	**0.696****	-0.016	-0.237	0.457	0.046	-0.396	-0.442	0.099	**-0.550****	**1**

Table 3.7b. Pearson correlation matrix between the analyzed variables. Data from leaf diagnostics, content of total chlorophyll, flavonol, anthocyanin and nitrogen balance index and leaf biometrics recorded at 120 DAFB were compared. The correlation between the variables is highlighted in bold. Significance is expressed as *, **, ***, at p<0.05, 0.01, 0.001, respectively.

(Level of correlation: 0.9–1.00 very high; 0.7–0.9 high; 0.5–0.7, moderate; 0.3–0.5, low; 0–0.3: negligible correlation)

Variables	N	C	P	K	Ca	Mg	Fe	Mn	B	Cu	Zn	Chl	Flav	Anth	NBI	LDMC	SLA
N	**1**																
C	**-0.590****	**1**															
P	0.420	0.015	**1**														
K	**-0.702*****	**0.838*****	-0.074	**1**													
Ca	0.139	0.217	-0.216	0.049	**1**												
Mg	0.009	0.127	0.205	-0.092	0.047	**1**											
Fe	-0.435	**0.649*****	-0.100	**0.505****	-0.077	0.209	**1**										
Mn	**0.558****	0.001	0.200	-0.225	0.243	-0.200	0.068	**1**									
B	-0.167	0.171	-0.375	0.158	0.230	0.005	0.137	0.123	**1**								
Cu	**0.634*****	-0.411	0.168	**-0.539****	0.428	0.409	**-0.532****	0.274	0.121	**1**							
Zn	-0.456	0.214	-0.416	0.379	-0.250	-0.170	0.269	-0.358	0.284	-0.384	**1**						
Chl	**0.721*****	-0.270	0.232	-0.260	0.163	-0.016	-0.083	**0.700*****	0.167	**0.521****	-0.392	**1**					
Flav	0.173	-0.271	-0.406	-0.379	0.017	-0.056	-0.399	-0.036	0.225	0.173	0.222	-0.139	**1**				
Anth	-0.477	0.050	-0.038	-0.002	-0.242	0.049	-0.178	**-0.695*****	-0.214	-0.330	0.094	**-0.862*****	0.266	**1**			
NBI	**0.644*****	-0.195	0.306	-0.152	0.149	-0.012	0.001	**0.672*****	0.118	0.461	-0.367	**0.972*****	-0.354	**-0.892*****	**1**		
LDMC	0.277	-0.137	-0.048	-0.176	0.289	0.159	-0.422	-0.058	0.250	0.481	0.108	0.003	**0.650*****	0.044	-0.132	**1**	
SLA	-0.117	0.215	0.088	0.325	-0.127	-0.1430	0.465	0.432	0.077	-0.319	0.051	0.232	**-0.581****	**-0.493****	0.373	**-0.500****	**1**

3.7 Principal component analysis (PCA)

The data on leaf diagnostics and eco-physiological parameters recorded at 90 and 120 DAFB were analyzed using principal component analysis (PCA) to assess and clusterize the different behavior of the cultivars (Figure 3.5). PCA is used to reduce the size of the original data by limiting the loss of information through the calculation of a new set of variables called principal components (PCs). The samples (scores) and variables (loadings) are projected onto the new principal component (PC) in the calculated space. The first two principal components were selected such as characterized by eigenvalues greater than 1 (F1:4; F2: 1.9), based on the Kaiser criterion. This ensures 66.7% explained variability (Table 3.8). The variables nitrogen (N), phosphorus (P), copper (Cu), total chlorophyll and NBI contribute majorly to the first PC. In contrast, the variables manganese (Mn), boron (B) and anthocyanin (Anth) contribute most to the characterization of the second PC (Table 3.8).

The first PC (47.10%) divides the data according to a timeline (Figure 3.5). On the right-hand side of the graph, most of the samples collected at 120 DAFB, in both 2019 and 2020, are clustered. Similarly, on the left-hand side of the graph, most of the samples taken at 90 DAFB, in both sampling years, are clustered.

Along the second PC (19,60%), the samples collected in the two years of investigation are differentiated according to leaf eco-physiological traits (Figure 3.5). The cultivars Vialfas, Lauranne®Avijor, Penta® and Tuono were characterized by high values of the variables N, Chl and NBI. These cultivars showed high values of these parameters in both samplings. The variables Zn, B, Mn, and K highly characterized the leaves of Tuono, Penta®, Genco and Lauranne®Avijor at 90 DAFB. In contrast, the cultivars Soleta, Guara and Supernova were more characterized by the variable Anth, both at 90 and 120 DAFB.

Table 3.8. Loadings contribution (%) of the original variables to the first two principal components (in bold: highest loading values).

Variable	F1	F2
N	**14.93**	0.25
P	**8.32**	5.71
K	3.31	0.13
Mn	0.30	**33.80**
B	7.86	**13.63**
Cu	**14.12**	1.70
Zn	8.77	**14.02**
Chl	**15.11**	9.81
Anth	11.02	**14.12**
NBI	**16.22**	6.80
Eigenvalues	*4.71*	*1.96*
Variability (%)	*47.10*	*19.59*
Cumulative explained variance (%)	*47.10*	*66.69*

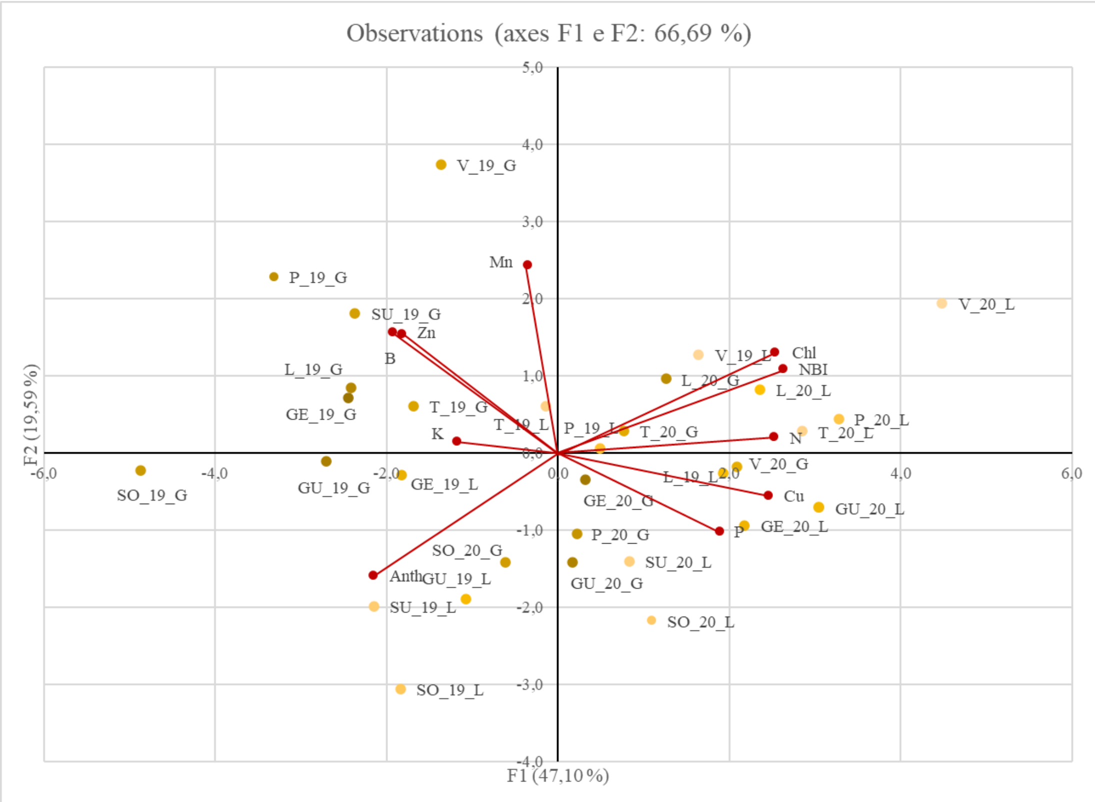

Figure 3.5. Principal component analysis (PCA) comparing the results of leaf diagnostics and leaf eco-physiological parameters of the eight almond cultivars sampled at 90 and 120 DAFB during years of investigation 2019-2020.

4. Conclusion

The analysis of the data acquired during the trial revealed differences in foliar nutrient content due to both almond cultivar and phenological stage of the plant.

The correlation of the analyzed foliar traits also highlighted the reciprocal influences between some leaf nutrients, including those between certain nutrients and eco-physiological leaf parameters. This assessment is in accordance with the finding obtained through the Pearson correlation matrix that confirmed as almond leaf nitrogen content had medium-high positive correlation with NBI either at 90 or 120 DABF. This eco-physiological index, which can be easily investigated using proper field instruments, may be used for a preliminary screening of the plant's nutritional status.

The cultivars Lauranne®Avijor, Penta®, Tuono and Vialfas showed higher stability in leaf diagnostic in the study environment. Furthermore, the sum of deviation of optimum percentage index (∑DOP) expressed as mean of cultivars at 90 DAFB was quite stable during the two research-year whereas at 120 DAFB it was significantly higher in 2020.

Finally, according to the main objective of this study, which was to obtain specific reference values of leaf nutrients for single almond cultivars to be applied during the nutritional management of the crop, Table 3.9 shows the new proposed reference values for foliar diagnostics at 90 and 120 DAFB in central Italy for the cultivars studied.

Findings of this study also helps to develop novel environmental-friendly almond nutrition and fertigation strategies supported by proper cultivar-site specific foliar diagnostics which also consider punctual cultivar nutritional requirements during the growing season.

Table 3.9. Proposed references of almond leaf macro- and micronutrient content, expressed as minimum and maximum values, for foliar diagnostics at 90 and 120 DAFB of the main intermediate and late-bloom cultivars recently introduced in central Italy.

90 DAFB											
Cultivar	**N** % DW	**C** % DW	**P** % DW	**K** % DW	**Ca** % DW	**Mg** % DW	**Fe** μg g^{-1} DW	**Mn** μg g^{-1} DW	**B** μg g^{-1} DW	**Cu** μg g^{-1} DW	**Zn** μg g^{-1} DW
Genco	1.90-3.25	42.80-44.30	0.05-0.10	0.75-1.05	0.90-1.70	0.20-0.25	65.80-100.00	16.70-20.00	9.00-55.00	6.00-22.00	29.00-50.00
Guara	1.60-2.60	42.20-44.40	0.06-0.10	0.70-1.00	0.85-1.20	0.20-0.26	50.00-82.00	17.00-23.00	10.50-36.85	2.80-15.50	10.00-32.50
Lauranne® Avijor	1.80-2.90	42.90-44.50	0.07-0.12	0.80-1.00	0.90-1.10	0.15-0.30	47.0-102.00	19.00-25.00	16.10-50.00	1.90-16.00	32.00-61.00
Penta®	1.80-2.50	42.45-44.35	0.06-0.10	0.80-1.10	0.70-1.25	0.20-0.22	50.60-58.00	17.80-30.50	17.50-52.50	2.00-17.00	16.20-50.00
Soleta	1.60-2.20	40.75-45.30	0.07-0.10	0.70-1.30	0.80-1.40	0.20-0.30	67.60-30.00	12.00-22.30	28.00-68.00	3.00-15.00	8.00-60.00
Supernova	1.60-2.20	41.70-43.70	0.06-0.10	0.75-1.30	0.90-1.75	0.20-0.30	50.60-132.00	16.50-25.30	22.50-36.10	3.50-15.20	34.30-83.00
Tuono	2.00-2.90	42.00-44.50	0.06-0.10	0.80-1.00	1.00-1.25	0.20-0.25	54.00-118.00	21.0-26.50	8.50-18.70	8.70-16.00	27.00-50.00
Vialfas	2.40-3.50	43.30-45.40	0.07-0.12	0.90-1.00	0.70-1.25	0.20-0.30	70.00-80.00	19.30-30.40	14.00-55.00	5.60-20.00	18.00-60.50
120 DAFB											
Cultivar	**N** % DW	**C** % DW	**P** % DW	**K** % DW	**Ca** % DW	**Mg** % DW	**Fe** μg g^{-1} DW	**Mn** μg g^{-1} DW	**B** μg g^{-1} DW	**Cu** μg g^{-1} DW	**Zn** μg g^{-1} DW
Genco	1.75-3.10	44.40-45.10	0.06-0.10	0.25-1.75	1.45-1.80	0.20-0.30	55.00-80.00	12.90-20.50	14.50-40.50	18.00-40.00	11.20-43.50
Guara	2.1-2.90	43.60-45.50	0.08-0.10	0.20-1.10	1.40-1.90	0.20-0.30	47.00-85.00	15.20-20.60	12.00-20.00	18.50-46.50	8.50-14.20
Lauranne® Avijor	2.30-2.60	43.30-46.70	0.10-0.12	0.30-2.20	1.10-1.70	0.20-0.30	65.00-95.00	16.50-23.50	12.80-32.00	18.50-30.00	5.50-13.50
Penta®	2.15-3.00	42.70-45.90	0.08-0.10	0.40-1.25	1.30-1.90	0.20-0.35	78.00-125.00	17.00-20.00	15.00-30.00	12.00-30.50	4.50-22.20
Soleta	1.60-2.90	41.90-46.10	0.08-0.10	0.20-1.30	1.20-1.45	0.20-0.35	47.00-100.00	10.00-15.00	15.00-21.50	13.50-25.00	9.00-13.50
Supernova	1.75-3.00	43.50-44.80	0.07-0.12	0.40-1.25	1.10-1.50	0.20-0.25	57.00-80.00	15.00-18.00	12.50-21.50	11.50-20.00	9.00-33.50
Tuono	2.00-3.10	43.00-45.50	0.08-0.10	0.40-1.10	1.15-1.50	0.18-0.25	75.00-120.00	17.50-21.20	10.50-25.50	10.00-18.00	8.50-40.00
Vialfas	2.50-3.50	44.10-45.90	0.08-0.10	0.25-1.65	1.45-1.80	0.20-0.25	67.00-90.00	20.30-24.20	18.00-43.00	14.00-45.00	7.50-15.00

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5 Conclusions and future perspectives

In late 1980s, 'sustainable development' was first mentioned in the 'Brundtland Report'. The document explains that sustainable development is defined as "economically viable, ecologically sound and socially acceptable development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Latrouffe et al., 2016).

In agriculture, the process of agroecosystem intensification is seen by many as negative, especially by those outside the sector. Agricultural systems are human-modified ecosystems and the modern agro-ecosystems require a lot of energy, still mainly supplied by fossil fuels, which is directed both inside and outside the system. In the coming years, the reduction of resources such as water and fertile soil will have a greater influence on agricultural systems (Pretty et al., 2014).

Sustainable intensification means using intensive and more productive agriculture through better management of agricultural land, according to the fact that ecosystem degradation is itself a reduction in agricultural productivity (Buckwell et al. 2014), which can be translated as “producing more, using less”. Often, when talking about sustainability, reference is made only to ecological aspects. But sustainability also must be interpreted from an economic and social perspective. This is one of the goals that agricultural research is trying to achieve. In the tree fruit crop sector, there are many ways to increase the level of sustainability. For instance, to reduce the use of limited available resources will require the use of sensors and handheld instruments that in combination with traditional monitoring and cultural practices such as water and nutrient balances will identify when the availability of water or nutrients needs to be modified through external inputs.

In this research, we present the studies carried out on the almond tree introduced in central Italy to verify the adaptability of the species to the new growing environment and to provide growers who have invested in this nut crop with indications for sustainable management of the almond orchard. According to these targets, eco-physiological and agronomic investigations were carried out concerning the most commonly used rootstocks and almond cultivars and some agronomic management practices such as nutrition.

In Chapter I, two trials are described. In the first, the phenological and agronomic influences induced by the clonal rootstock GF677 on the grafted cultivars Tuono, Supernova and Genco were evaluated, and compared to those induced by the peach seedling rootstock. In the second trial, the effect of three different clonal rootstocks (GF677, Rootpac®20 and Rootpac®R) on the Spanish cultivar Guara was evaluated. During the period of investigation, the rootstock GF677 conferred greater vigour than clonal rootstocks of the Rootpac® series, as already demonstrated by other authors in Spanish environment (Miarnau et al. 2010, and Romero et al.,2018), confirming its suitability to establish new specialized almond plantings.

In Chapter II, the effect of foliar nutrition on a commercial almond orchard was evaluated with the aim of developing a sustainable protocol for nutrition in almond orchards, limiting the input of fertilizers to be applied in the soil. Foliar analysis and the use of the DOP index confirmed the effectiveness of the foliar treatments. The plants subjected to the foliar treatments did not show any stress due to nutritional deficiencies, which was also confirmed by the eco-physiological parameters detected in the leaves.

Furthermore, the behavior of the main Italian, Spanish and French almond cultivars characterized by late blooming was evaluated in Chapter III. The aim was to give better indications to growers to guide the cultivar choice in the Latium region. The study was carried out through the phenological observations, agronomical traits, and development of leaf eco-physiological indicators and leaf diagnostics. A proper adaptation was observed for the cultivars Tuono, Lauranne®Avijor, Penta® and Vialfas. The latter two, catalogued as extra-late flowering cultivars (Dicenta et al., 2018) were characterized by having escaped the latest late frosts and are strongly recommended for the establishment of new plantings. At the end of the work, new cultivar-site specific reference values of leaf macro and micronutrient contents were released to encourage the use of foliar diagnostics to monitor the plant health status in order to provide the right amount of nutrients at the right time, according to “sustainable intensification” approach.

6 References of general introduction, conclusions and future perspectives

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