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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

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1. Introduction

The almond tree (*Prunus dulcis* (Miller) D.A. Webb) is the most widely cultivated and economically important nut species in the world [1]. About 30% of human nut consumption concerns almonds, followed by walnuts (20%), cashews, and pistachios [2]. According to FAO data [3], 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 [4].

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 [5], their high adaptability in this environment, and to their storage over long periods [6]. 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 [1].

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 [7].

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 [8].

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 [9]; 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 [10].

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 [11,12], 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 [13]. Thus, the agronomic approach to the use of grafted plants involved almond cultivation as well as other nut species such as walnut [14], 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 [15].

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* [16]. 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 [11].

The red leaf rootstock 'Garnem', released by CITA (Zaragoza-Spain) in 2000 [17], is characterized by similar vigor to 'GF677' [18], 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 [11,19,20].

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) [21].

The most used clonal rootstocks for SHD almond orchards are the 'Rootpac®' series, derived from the hybridization of *p. besseyi* × *P. cerasifera* [22,23] 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 [21]. Furthermore, it seems to be tolerant to calcareous soils and asphyxia, and moderately resistant to *Meloidogyne* spp. [16].

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 [8].

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' [24–26].

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. 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.

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 [27], and with the support of field-captured images. These phenological remarks allowed the development of specific cultivar-site blooming phenograms and ripening calendars.

Table 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

2.3. 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 [28,29]. 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. [30], when the plants were at stage 81 (fruit at full size) of the BBCH scale [27].

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 [17].

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

3.1. Blooming and Ripening Phenograms

Figures 1 and 2 present the blooming and ripening phenograms of trials A and B, respectively.

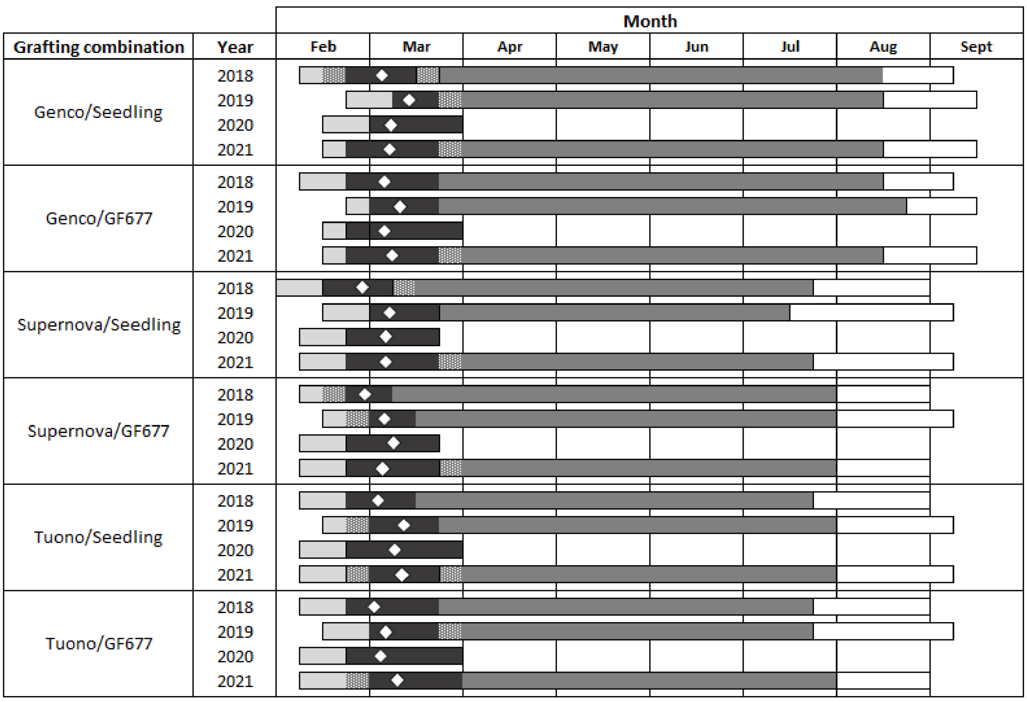

Figure 1. Phenograms of blooming and ripening recorded over four years of investigation (2018–2021) for cultivars 'Genco', 'Supernova' and 'Tuono' grafted on clonal rootstock 'GF677' and seedling rootstocks, respectively (light grey histogram = beginning flowers emergence; dark grey histogram = blooming;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

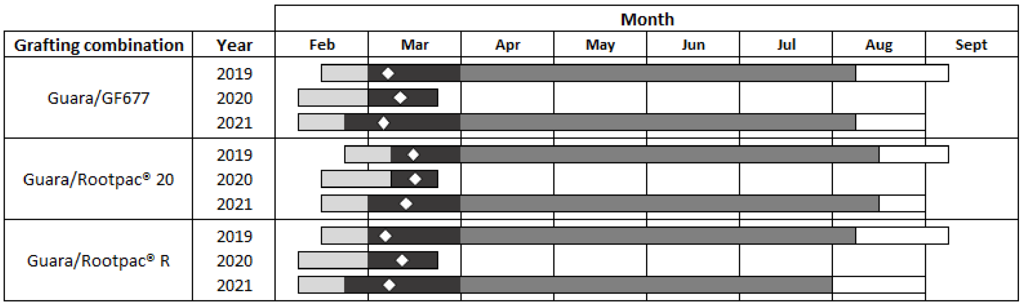

Figure 2. Phenograms of blooming and ripening recorded over four years of investigation (2018–2021) for cultivars 'Guara' grafted on clonal rootstock 'GF677', 'Rootpac® 20' and 'Rootpac® R', respectively (light grey histogram = beginning flowers emergence; dark grey histogram = blooming; white dots = overlapping of successive phenological stages; mid-dark grey histogram = fruit development; white histogram = fruit maturity: 1% hull split).

Referring to the first 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).

Concerning the trial B (Figure 2), 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.

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 (trial A, Table 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 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 μg cm^{-2}	Flav μg cm^{-2}	Anth μg cm^{-2}	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
	GF677 (3-year average)		26.78 ± 3.97	2.25 ± 0.10	0.08 ± 0.03	12.04 ± 1.92
	Seedling (3-year average)		26.64 ± 6.83	2.26 ± 0.10	0.10 ± 0.04	11.85 ± 3.33
	Rootstock	**Year**	**Chl μg cm^{-2}**	**Flav μg cm^{-2}**	**Anth μg cm^{-2}**	**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
	GF677 (3-year average)		27.55 ± 4.89	2.23 ± 0.13	0.09 ± 0.03 b	12.35 ± 2.41
	Seedling (3-year average)		27.16 ± 7.87	2.24 ± 0.11	0.11 ± 0.05 a	12.11 ± 2.83
	Rootstock	**Year**	**Chl μg cm^{-2}**	**Flav μg cm^{-2}**	**Anth μg cm^{-2}**	**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
	GF677 (3-year average)		29.55 ± 5.14 a	2.14 ± 0.16 b	0.07 ± 0.03 b	13.89 ± 2.64 a
	Seedling (3-year average)		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.

Referring to trial B, all parameters were significantly influenced by rootstock, as reported in Table 3. 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.

Table 3. 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 μg cm^{-2}	Flav μg cm^{-2}	Anth μg cm^{-2}	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
GF677 (3-year average)		28.9 ± 4.26 b	2.21 ± 0.14 a	0.08 ± 0.03 b	13.3 ± 2.35 a
Rootpac® 20 (3-year average)		29.33 ± 5.06 a	2.17 ± 0.15 b	0.09 ± 0.04 b	13.81 ± 2.90 a
Rootpac® R (3-year average)		28.18 ± 5.42 b	2.19 ± 0.15 ab	0.11 ± 0.06 a	12.89 ± 3.14 b

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.

3.3. Fruit Set Incidence

The incidence of fruit set, determined following the literature guidelines [27,30], was estimated in late April 2021 for both trials, as reported in Table 4 (trial A) and Table 5 (trial B), respectively.

Table 4. 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	Rootstock	Number of Flowers	Number of Fruits at Full Size	Fruit Set Incidence (%)
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

Table 5. 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 $p < 0.05$). (2021).

Rootstock	Number of Flowers	Number of Fruits at Full Size	Fruit Set Incidence %
GF677	584	182	31.25 ± 8.91
Rootpac® 20	550	143	26.31 ± 6.72
Rootpac® R	579	172	29.71 ± 7.12

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 4).

Table 5 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'.

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 6. 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 6. 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/Nut Ratio %
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 similar to 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 3 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.

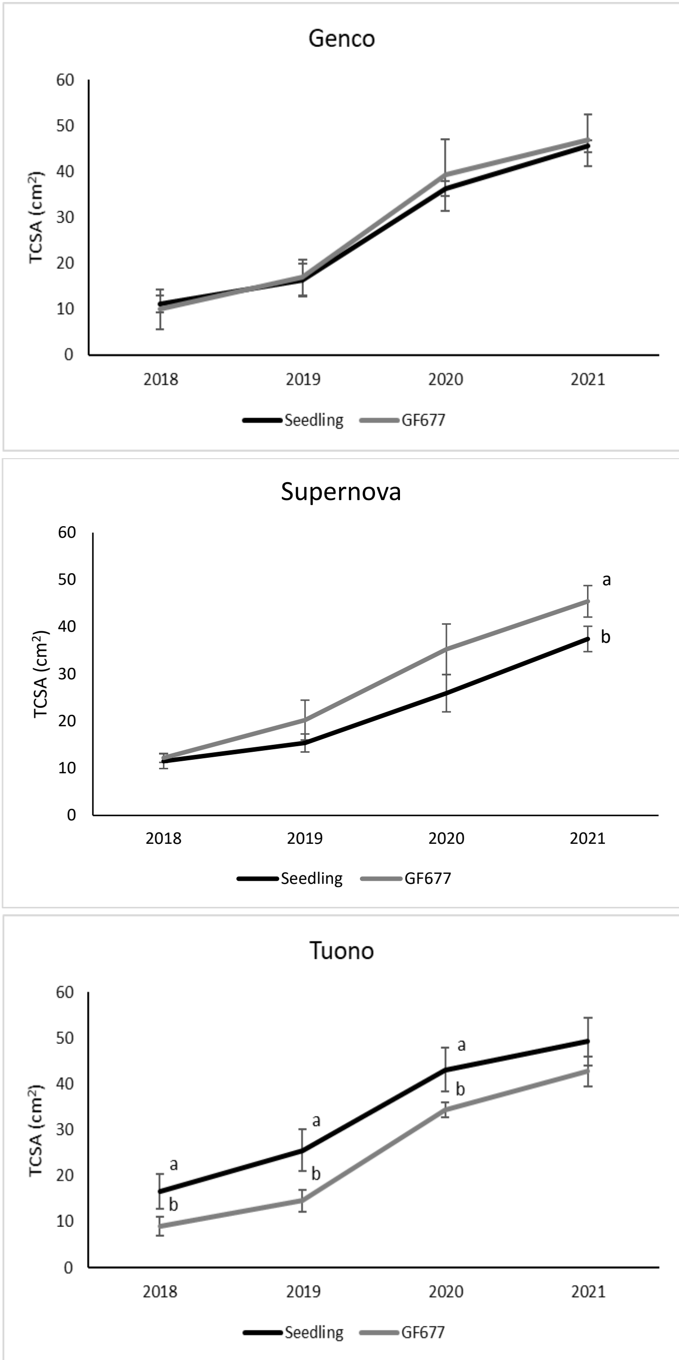

Figure 3. Comparison of annual mean values of trunk cross-sectional area (TCSA) computed at 30 cm above the ground, and recorded at the end of growing season over the period 2018–2021 for cultivars 'Genco', 'Supernova' and 'Tuono', grafted on 'GF677', and on peach seedling rootstocks (trial A). Lowercase letters on the graph mean values indicate significant differences between rootstock per year (Fisher's test $p < 0.05$).

As confirmed by the Figure 3, 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.

Table 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 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 $p < 0.05$).

Rootstock	Yield Per Plant kg	YE kg/cm^2	Kernel/Nut Ratio %
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 [31].

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 4.

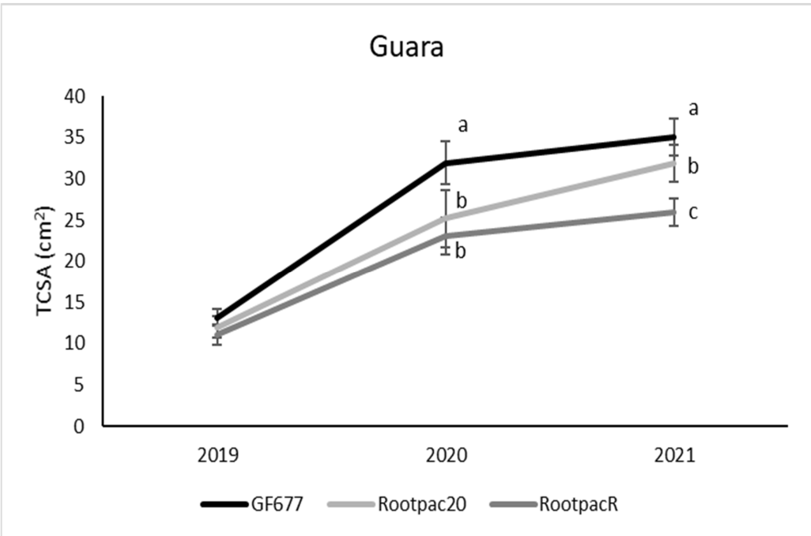

Figure 4. Comparison of annual mean values of trunk cross-sectional area (TCSA) computed at 30 cm above the ground, and recorded at the end of growing season over the period 2019–2021 for cultivars 'Guara' grafted on clonal rootstocks 'GF677', 'Rootpac® 20' and 'Rootpac®' (trial B). Lowercase letters on the graph mean values indicate significant differences between rootstock per year (Fisher's test $p < 0.05$).

4. 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 [32], which is consistently influenced by the scion genotype [33], whereas other trials carried out using different almond cultivars showed the rootstock influence on flowering [34]. 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 [35], 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 [13]. 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. [13]. 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 [36], 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 [37].

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 [38].

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 [39]. 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 [40–42], 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 [43].

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 [30].

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 [44,45].

Furthermore, referring to trial B, it is also possible to assert that, in the new environment of almond introduction, the rootstock 'GF677' imposed more vigor to the cultivar 'Guara' than the tested clonal rootstocks 'Rootpac® 20' and 'Rootpac® R', as confirmed by Miarnau et al. [46] for Spanish environments. Furthermore, it has been observed by Romero et al. [34] that less vigorous rootstocks offer lower quality nuts than more vigorous ones. However, in recent studies [21], 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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