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Influences of Propagation Method, Rootstock, Number of Axes, and Cultivation Site on 'Fuji' Scions Grown as Single or Multi-Leader Trees in the Nursery

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Abstract: The adoption of high-density plantings (HDP) in apple orchards started with the introduction of dwarfing rootstocks from East Malling's (UK) breeding program. A range of spindle-derived training systems have been developed to improve light interception/distribution with a variation in leader numbers. Many of these training systems cannot guarantee early, consistent, and uniform illumination of the entire canopy. For this reason, planar 2-D canopies have been developed with varying numbers of primary axes with numerous second-order shoots. In this trial, carried out at the nursery level, three sites were selected: one in New Zealand (Hawke's Bay Research Centre) and two in Italy (Bologna and Trento). Trees were produced with a single-, bi-, and tri-axis system utilizing three rootstocks ('M27', 'M9', 'MM106'), characterized by an increasing level of vigor. The cultivation site played an important role in modulating early tree performance. Multi-leader trees reduced average shoot length in the Italian sites in the first year after grafting. The number of shoots and total growth developed on multi-leader trees was higher than single-axis trees. This may benefit growers that seek to use canopy architecture manipulation to fill space and control vigor when establishing HDP orchards.

Keywords: apple; growth; planar canopy; high-density planting; cordon system

1. Introduction

The concept of high-density plantings (HDP) in apple orchards began with the launching of the dwarfing rootstocks from the East Malling Research Station's (UK) rootstock development program. The most popular rootstock from the East Malling M-series was the dwarfing, 'M9' [1]. A joint breeding program between the East Malling Research Station and the John Innes Institute in Merton, England, focused on producing woolly apple aphid-resistant rootstocks. As a result of this collaboration, two series of rootstocks were formed: the Merton–Immune (MI) series and the Malling–Merton (MM) series (MM101–115) [2].

The most evident effect of dwarfing rootstocks is the reduction in tree height. For example, the 'M27' rootstock is one of the most dwarfing apple rootstocks, and it can reduce the height of a tree by up to 80% compared to a seedling rootstock. Conversely, 'MM111' only reduces tree size by approximately 20% compared with a seedling rootstock [3]. Beyond vigor control, rootstocks also provide other agronomical advantages, including variation in different aspects such as precocity [4,5], floral induction [4,6–8], fruit set [3],

photosynthetic activity [9,10], disease susceptibility [11], cold tolerance, and resistance to unfavorable soil conditions [12].

High-density plantings depend on many rootstock traits, especially the dwarfing and precocity attributes, allowing an early return of investment for the initial costs of orchard planting and improving profitability. In young trees, production occurs simultaneously with canopy development. This is possible due to more efficient light utilization, as HDP can increase light interception and distribution within the canopy [13–18].

Dwarfing rootstocks have played a central role in training system evolution, leading to the introduction of the spindle bush and the slender spindle in the 1950s and 1960s [19]. The slender spindle was demonstrated to optimize light interception and canopy light availability, especially in growing sites characterized by low vigor, such as Northern Europe or Alpine regions [20]. Alternatively, the slender spindle training and pruning methods were not recommended for high-vigor sites because the top part of the tree may become overgrown [21].

Studies found that many training systems could not guarantee high illumination across the whole canopy, especially early on in the orchard's lifespan [22,23]. For this reason, increasingly planar and 2-D canopies have been developed and evaluated, such as the Geneva Y-trellis [24], Tatura trellis leading to the Washington Vee trellis [23], the bi-axis, or other experimental multi-leader trees [25–28]. Planar canopy systems are obtained generally, with two (bi-axis) or three to six (multi-leader) main vertical axes oriented in the parallel direction of the row, with numerous short lateral limbs [25–28], conferring to the tree a narrow 2-D shape. A more recent concept, the Planar Cordon [29,30] or Guyot [31], uses up to 10 non-branching vertical fruiting stems on a bi-cordon tree. The renewed interest in planar divided-canopy training systems has increased with the availability of preformed, split-branch trees from the nursery, eliminating the need for orchard topping (i.e., heading back the tree) and delayed shoot system formation [25,26,32,33].

In mature apple trees, it has been shown that dwarfing rootstocks influence the length of the growing period, inducing an early set of terminal buds and reducing the number of nodes present on each shoot [5,34–36]. Rootstocks can affect shoot length by affecting the number of nodes, branching density, position, and other branch characteristics [36]. In fact, in two different studies [37,38], the percentage of bud break in axillary buds on a given axis was unaffected by the selected rootstock. Thus, the number of developing axillary structures per branch seems to depend on the number of nodes developed during the previous year [39,40]. For example, Seleznyova et al. [36] demonstrated that dwarfing rootstocks produced fewer nodes and axillary buds when compared to vigorous rootstocks, which contributed to diminished shoot growth in the following year. The same authors demonstrated that these shoots with reduced nodes also have smaller internode lengths [36]. Many trials have reported architectural modifications of the scion in the first year after planting due to the dwarfing rootstock's effects, which include a reduction in shoot length [41,42], number of nodes [39], and number of second-order shoots (i.e., lateral feathers) [43]. This reduction in scion shoot growth in a growing season is primarily due to the earlier shoot extension termination conferred by dwarfing rootstocks [44]. Ultimately, it was shown that dwarfing rootstocks affect the type of growth units produced throughout an annual growth cycle, reducing the number of extension growth units, thus influencing the architecture and vigor of the tree [5]. These dwarfing effects are further enhanced by the dwarfing rootstocks' precocious transition to flowering to help reduce vegetative vigor through early fruiting induction [5], causing a greater number of subsequent sympodial shoots, which typically have fewer nodes. Overall, these results suggest that the effect of the rootstock on scion growth is cumulative and superimposed year after year and may begin as early as the first or second year after planting by modifying tree architecture [4,5].

The overall purpose of this study was to investigate, at both the nursery level and the first year after planting, how multi-leader trees modify the initial scion architecture with respect to shoot node production and length, along with how these traits affect precocity (e.g., the initial occurrence of flowering). Different rootstocks were also used to

observe modifications in vigor, and two propagation methods were tested to assess which was the easiest technique to successfully develop mature trees. In addition, we studied the influence of growing conditions in three different environments: New Zealand and two sites in Italy (Bologna and Trento).

2. Materials and Methods

2.1. Experimental Sites and Design

In 2009, three sites were selected for this trial: the first location was in New Zealand, at the Plant & Food Research Institute Ltd.—Hawke's Bay Research Centre (HB), (39°39′12.1″ S, 176°52′43.7″ E), and the other two were in Italy (Bologna and Trento). In Italy, the experiment was carried out at the University of Bologna (BO)—Cadriano Experimental farm (44°32′55.1″ N, 11°23′10.0″ E), and at the Fondazione Edmund Mach—Istituto Agrario di San Michele all'Adige (SM) Experimental Farm (46°11′19.1″ N, 11°06′17.3″ E).

The Hawke's Bay soil was a silt clay loam, fully cultivated, then fumigated with chloropicrin, and aerated before planting in September 2008. The soil in the Bologna site was not fumigated and had no apple or pear planting history before the present trial. The soil was a deep silt clay loam, with optimal levels of potassium and available magnesium, and was plowed in April 2009. The San Michele all'Adige soil was fertile, with an upper profile of 150 cm of sandy clay loam and sand in the deeper strata, rich in organic matter and potassium. The soil was fumigated with metam potassium (Tamifum Eastman Milano, Italia S.r.l.) at the rate of 340 L/ha of commercial product in early March 2009. A study of nursery tree growth was performed to explore the influences of the site of cultivation, the propagation method, the rootstock, and the number of leaders per tree on the early development of tree architecture for apple cultivar 'Fuji'.

2.2. Fertilization and Irrigation at Experimental Sites

A different fertilization strategy was established for each site in order to optimize the growth according to soil type. In the Hawke's Bay site, calcium ammonium nitrate (CAN, 27% N) was applied to the soil at a rate of 14 g CAN/m^2 four times monthly, starting from the second week of November 2008 and completing the schedule of fertigation in March 2009 (Table S1). This resulted in an application of 1.512 g of N per tree per application, for a total of approx. 6 g over the season. In Bologna and San Michele all'Adige, a complex NPK mineral fertilizer (21-7-14) supplemented with calcium nitrate (15.5% N) was distributed over the season (Table S1). Total amounts of nitrogen per tree were 12.5 and 38.5 g of N per tree in each location, respectively. Trickle irrigation was provided at each site to maintain soil moisture content close to field capacity.

2.3. Tree Production and Planting

For the Hawke's Bay experiment, three apple rootstocks were grafted with 'Fuji' (Nagafu 6). 'Fuji' was selected due to the higher vigor of this cultivar compared to other accessions [45,46]. One-year-old rooted rootstocks were produced in stool beds in a local nursery, and scions were collected from orchards set up at the Hawke's Bay Research Centre. Three different rootstocks were used, providing an increasing vigor scale: 'M27' (very dwarf), 'M9' EMLA (dwarf), 'MM106' (semi-vigorous). Two grafting methods were adopted to establish the multi-leader treatments: 50% of the rootstocks were chip-budded with one, two, or three buds [40,47], and 50% were bench-grafted with four bud scions (one-year-old wood with four buds to increase opportunities for multi-leader development). During the growing season, we selected in the nursery one, two, or three active shoots as they developed. This design provided 18 treatments in a 3 × 3 × 2 factorial design: three rootstocks, three leader systems (single, double, or triple), and two propagation methods (chip budding and bench grafting). Subsequently, trees were planted in the nursery at a spacing of 3.0 m × 0.5 m (6667 trees/ha) in a completely randomized design with 12 replications (trees) per treatment. Trees are commonly planted at 90–100 cm × 10–15 cm, independently of the rootstock vigor in a nursery. To minimize shading and growth inter-

ference from the trees, we quintupled the standard inter-tree within-row spacing to 50 cm, while inter-row spacing was tripled (3.0 m) to reduce light limitations and minimize any border effects. The design was a completely randomized block with 12 blocks containing 1 tree per each of the 18 treatments. The total number of trees in the trial in this specific location was 216 (3 rootstocks × 3 levels of leader × 2 propagation methods × 12 trees per treatment). Grafting height was standardized at 30 cm above the root system for all the trees. Graft unions were protected with tape, and the top of the rootstock/graft was sealed with a pruning wax paste.

Bench-grafted trees were stored in a controlled environment (7–10 °C) with humidity and placed in a wooden bin containing moist untreated soil for at least two weeks to improve the callus tissue formation [33,48]. Planting height was the same for all treatments, with the graft union at 10 cm above the ground.

A single nursery supplied all the tree material used in the two Italian sites (Griba Baumschule, Niederthorstraße 9, I-39018 Terlan BZ, Italy). The 'M27' rootstocks were two years old because it was not possible to obtain one-year-old stools with appropriate caliper quality due to the low vigor of this rootstock.

Trees were bench-grafted by the Italian nursery during spring 2009 (6 April 2009) and planted in the nursery a few days later (15 April 2009 in San Michele all'Adige and 16 April 2009 in Bologna). A different budding strategy was adopted for chip-budded trees compared to the Hawke's Bay experiment in Italy. Three scion buds were budded on the rootstocks for all of the multi-leader treatments to standardize the total area of rootstock bark viable from the budded area among the treatments (i.e., there was a lot of damaged bark because of the budding depending on the number of buds). In this way, there was a similar amount of functional bark on the rootstock in the early stage of development, irrespective of budding treatment. Moreover, the rootstock was not headed at budding but approximately a month after planting to improve survival. Trees at the Italian sites were planted with a spacing of 3.0 m × 0.5 m (6667 trees/ha), the same utilized in NZ. A completely randomized block design with 20 replications (trees) in Bologna (BO) and 18 replications (trees) in San Michele all'Adige (SM) per each treatment was used. In more detail, the design in BO was a completely randomized block with 20 blocks, while in SM, there were 18 blocks. In both cases, a block consisted of 1 tree for each of the 18 treatments under investigation.

The total number of trees in BO was 360 (3 rootstocks × 3 levels of leader × 2 propagation methods × 20 trees per treatment), while that in SM was 324 (3 rootstocks × 3 levels of leader × 2 propagation methods × 18 trees per treatment). Unrequired scion growing shoots were removed from respective axis number treatments when they reached the average length of 15 cm, leaving only the required number per rootstock (single, double, or triple leader) for the experiment. Suckers from the rootstocks were removed throughout the growing season.

2.4. Scion Development Measurements

At the end of the growing season, the trees were evaluated for their shoot growth and graft survival. The proportion of trees presenting different levels of development was determined. We classified these trees into four qualitative categories: good (large), delayed (small), dead, and other (wrong number of axes). The proportion of each tree classification was determined for each treatment combination and averaged for treatment comparisons. In the Italian sites, leaves were removed from the scion before leaf abscission to determine the number of leaves and leaf area (cm^2) per tree using a leaf area scanner (Li-3100, LI-COR Biotechnology, Lincoln, NE, USA). No leaf measurements were carried out at the Hawke's Bay site.

In each site, tree development was measured using a coding method described by Costes et al. [49], which provides the indications to describe different orders of shoot axes, the node they originated from, and their position. Shoot node number, length (cm), and cross-sectional area (cm^2) were also measured. Shoot length (cm) and node numbers were

summed across single and multi-leader trees to evaluate total tree growth. Axis cross-sectional area (ACSA) (cm^2) of each primary axis and shoot cross-sectional area (SCSA) (cm^2) of secondary axes (feathers) were calculated. ACSA was recorded by measuring the axis diameter two cm above the graft, while the SCSA measured the diameters of shoots two cm above their insertion. The average internode length (cm) of shoots was calculated by dividing the length of shoots by the number of nodes. Axillary bud break proportion (feather production) was calculated by dividing the total number of secondary shoots by the total number of bud nodes developed over the first vegetative season.

Within each leader, it was possible to discriminate single growth units (GU) [50]—which are sections of shoot developed during a single growth flush—and multiple growth units. The latter case occurred when the shoot apical meristem temporarily stopped growing or slowed its activity and resumed later. To identify GUs, morphological markers as rings of bud-scale scars or compressed internodes were used [36]. Floral cluster number/tree was counted at full bloom, which occurred on 12 October 2009 in HB, 16 April 2010 in BO, and 29 April 2010 in SM.

2.5. Statistical Analysis

Data were analyzed using the statistical package Statistica (version 9, StatSoft, Tulsa, OK, USA). Data were first checked for homogeneity of variance and a normal distribution. ANOVAs were run to evaluate statistical significance at $p < 0.05$. Data for each site and for each rootstock × axis number combination were first analyzed separately using a general linear model; all the data were then analyzed to study the effect of each variable of the factorial design (three sites, three rootstocks, three-axis numbers, two propagation methods). Relative proportions of tree status were evaluated amongst treatments, with only the first two dominant classes (e.g., good, other, delayed, dead) from each site × propagation method being analyzed by HSD Tukey's mean comparisons. For significant effects or interactions, comparisons of treatment means were made using HSD Tukey's test, and different letters were assigned for differing means based on this test.

3. Results

3.1. Effect of Propagation Method on Multi-Leader Trees' Survival Rate

In Hawke's Bay (HB), a difference in the survival rate between chip-budded and bench-grafted trees was recorded (Figure 1A,B). Many chip-budded trees presented a high proportion of dead cases, and this was observed primarily in combinations with 'M27' (Figure 1A). In contrast, 'MM106' single-leader trees obtained through the bench graft technique generated the highest success rate, along with 'M9', trained to a single- and double-leader system. In general, propagation success was much better in the bench-grafted trees (Figure 1B). More than 70% of the bench-grafted trees trained with a single leader reached a commercially acceptable tree size expected for a spindle nursery tree, irrespective of rootstock (Figure 1B). The proportion of full-sized trees from bench grafts declined as the axis number increased. Furthermore, the proportional decline with higher axis numbers also increased with the relative reduction in vigor of the respective rootstocks (Figure 1B). For both propagation methods, there was quite clear evidence of the difficulty of multi-leader treatments in originating trees with the correct number of leaders; the higher the failure, the greater the number of axes, increasing from one to three.
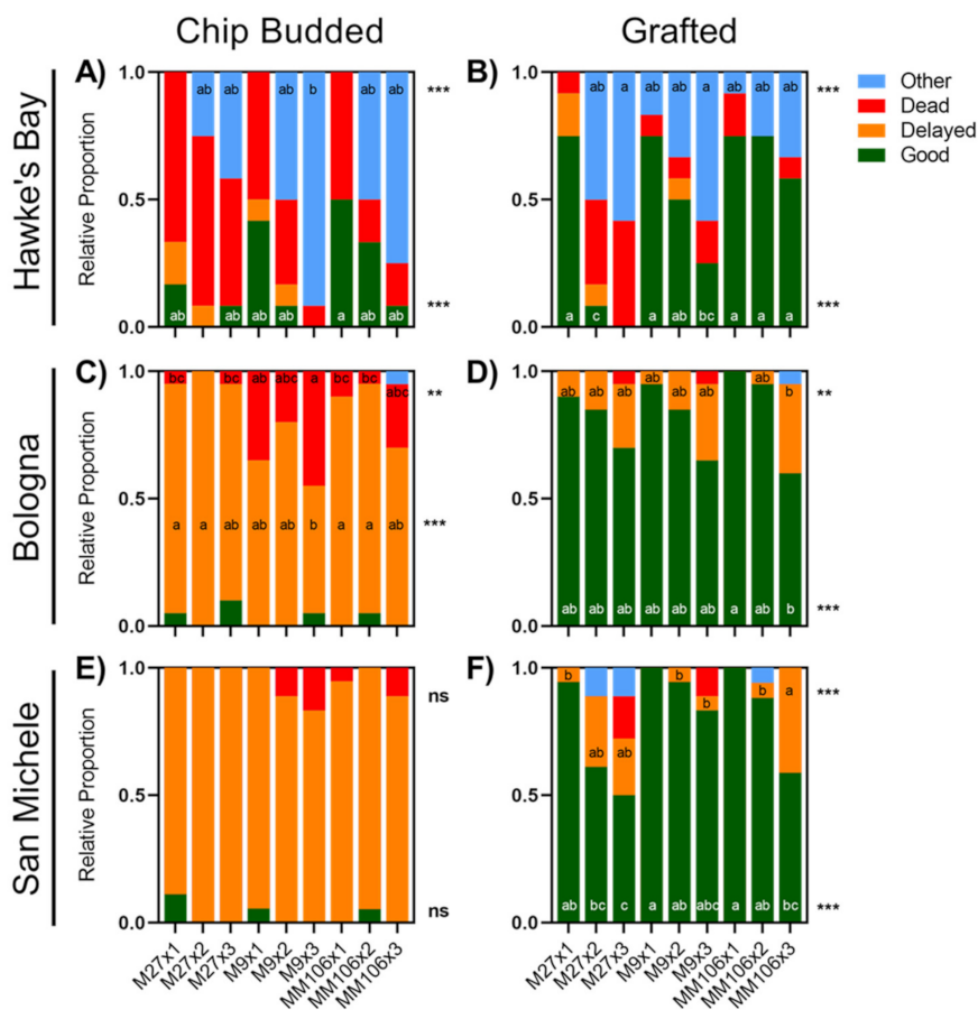
Figure 1. The interaction effect of 'rootstock × axis number' in chip-budded (**A**,**C**,**E**) and bench-grafted 'Fuji' trees (**B**,**D**,**F**) on the relative proportion of development trees grown in New Zealand (**A**,**B**), Bologna, Italy (**C**,**D**), and San Michele, Italy (**E**,**F**). Tree development classifications included: good development (green), delayed development (yellow), tree death (red), or developed a lesser number of axes required for the treatment (i.e., other, blue). On the *X*-axis, 'rootstock × axis number treatments' are listed. ANOVA significance is indicated to the right of each graph, labeled with **, ***, denoting where the model was significant at $p < 0.01$ and 0.001, respectively, across the two primary categories for each 'site × propagation' plate. 'Rootstock × axis number treatment' proportion means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$.

At the Bologna (BO) site, very few chip-budded trees were classified as well-developed (good), while a larger proportion of them presented delayed development or died, with a difference amongst some treatments related to rootstock effects (Figure 1C). The opposite response was found in the bench-grafted trees, where the majority of treatments showed good development (Figure 1D). The highest proportion was observed in 'MM106' single-leader (×1) trees (100% good trees) (Figure 1D). In general, training the trees with two or three leaders reduced the proportion of large-sized trees, with a slightly greater reduction from the more dwarfing rootstocks. In the San Michele all'Adige (SM) site, no statistical difference was detected in chip-budded trees among the treatments (rootstock × no. of leaders) for the four considered variables (good trees (large), delayed (small), dead, other) (Figure 1E). Bench-grafted trees were similar to the corresponding treatment trees in Bologna (Figure 1F).

Each treatment factor (or main effect) evaluated in this study (site, rootstock, number of leaders, and propagation method) showed a significant influence on the proportion of trees successfully developed into high-quality nursery trees (Table 1). Among the treatment factors affecting tree quality and propagation success, the propagation method was the single most influential factor (Table 1).

Table 1. Main effect of the treatments on the proportion of well-developed trees grown over their first season of growth at three different locations.

Category	Treatment	Proportion of Well-Developed Tree
Site	BO [1]	0.43 [a]
	HB [2]	0.34 [b]
	SM [3]	0.41 [a]
	Significance	***
Rootstock	M27	0.35 [b]
	M9	0.42 [a]
	MM106	0.44 [a]
	Significance	***
Axis Number	1	0.51 [a]
	2	0.40 [b]
	3	0.29 [c]
	Significance	***
Propagation Method	Bench Grafting	0.74 [a]
	Chip Budding	0.06 [b]
	Significance	***
Interactions	S × R	***
	S × A	**
	S × P	*
	R × A	*
	R × P	**
	A × P	***
	S × R × A × P	*

[1] Bologna, [2] Hawke's Bay, [3] San Michele; *,**,*** indicates significance at $p < 0.05, 0.01, 0.001$; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$. Non-significant interactions are not presented.

3.2. Main Effects on Leaf Number and Leaf Area

There was a highly significant difference in the number of leaves per axis between the two Italian sites (BO and SM). Trees in BO showed (194 leaves) 3.5 times more leaves than at SM (55 leaves), while the average leaf area was larger in SM, with 22.7 cm^2 of leaf area compared to 20.7 cm^2 of leaf area measured in BO (Table 2).

Rootstock did not influence the number of leaves per axis, although there was an overall increase in the number of leaves with accentuating vigor (Table 2). The average leaf size was statistically similar on dwarfing (22.9 cm^2) and very dwarfing (22.4 cm^2) rootstocks, although they were larger when compared to the vigorous rootstock (19.9 cm^2) (Table 2). The number of leaders per tree reduced the number of leaves and average leaf area per axis (Table 2). Trees grown in SM with one or two leaders had larger leaves than all the other combinations (Table 2). The interaction of cultivation site × rootstock did not affect the average leaf area or number of leaves per leader, although trees in BO grafted on invigorating rootstocks had the greatest number of leaves (Table 2). With the rootstock × axis number interaction, there was a remarkable reduction in the leaf number per axis recorded in multi-leader trees, grafted on 'M27'. Values from SM were smaller than from BO (Table 2). Variations in average leaf area, with respect to the rootstock × axis

number interaction, were not consistent, but values for trees grown in SM were usually higher than for BO (Table 2).

Table 2. Main effects of site of cultivation, rootstock, axis number, and their interactions on number of leaves per axis and average leaf area in 'Fuji' scions.

Category	Treatment	No. of Leaves/Axis	Average Leaf Area (cm^2)
Site (S)	BO [1]	194.0 [a]	20.7 [b]
	SM [3]	55.2 [b]	22.7 [a]
	Significance	***	***
Rootstock (R)	M27	109.3	22.4 [a]
	M9	126.0	22.9 [a]
	MM106	138.6	19.9 [b]
	Significance	ns	**
Axis Number (A)	1	187.3 [a]	22.8 [a]
	2	123.6 [b]	23.5 [a]
	3	104.4 [b]	20.2 [b]
	Significance	***	***
S × R	BO × M27	162.7	22.0
	BO × M9	196.2	21.2
	BO × MM106	223.2	19.0
	SM × M27	55.9	22.7
	SM × M9	55.8	24.6
	SM × MM106	54.0	20.8
	Significance	ns	ns
S × A	BO × 1	299.7	20.5 [b]
	BO × 2	193.5	21.9 [b]
	BO × 3	159.1	20.1 [b]
	SM × 1	74.8	25.1 [a]
	SM × 2	53.6	25.0 [a]
	SM × 3	49.8	20.4 [b]
	Significance	ns	*
R × A	M27 × 1	183.4	22.5 [a,b]
	M27 × 2	103.1	25.2 [a]
	M27 × 3	88.8	20.4 [b,c]
	M9 × 1	187.8	23.8 [a,b]
	M9 × 2	128.4	23.0 [a,b]
	M9 × 3	103.8	22.5 [a,b]
	MM106 × 1	190.6	22.0 [a,b,c]
	MM106 × 2	139.2	22.1 [a,b]
	MM106 × 3	120.8	17.7 [c]
	Significance	ns	*

[1] Bologna, [3] San Michele; ns, *, **, *** non-significant or significant at $p < 0.05$, 0.01, and 0.001, respectively; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$.

The total measured leaf area per tree was very dependent on the site of cultivation (Figure S1). Trees in BO (0.77 m^2) had an average total canopy area that was almost three times greater than the ones in SM (0.28 m^2) (Table 3). There was no difference in total tree leaf area between rootstocks. However, the total tree leaf area was larger in multi-leader trees (0.54 m^2 in the bi-axis and 0.58 m^2 in the tri-axis, respectively) when compared to single-leader trees (0.44 m^2) (Table 3). Interactions among the main factors were not significant for total tree leaf area (Table 3). However, by looking at the three-way interaction of site × rootstock × axis number, it appears that, except for trees grafted on 'M27' in SM

and three leader trees on 'MM106' in BO, the total leaf area tended to increase with an increase in the number of leaders per tree (Figure S1).

Table 3. Main effects of site of cultivation, rootstock, and axis number on total tree leaf area in 'Fuji' scions. Interactions are not included due to non-significant results across all combinations.

Category	Treatment	Total Tree Leaf Area (m^2)
Site	BO [1]	0.77 [a]
	SM [3]	0.28 [b]
	Significance	***
Rootstock	M27	0.52
	M9	0.55
	MM106	0.49
	Significance	ns
Axis Number	1	0.44 [b]
	2	0.54 [a]
	3	0.58 [a]
	Significance	**

[1] Bologna, [3] San Michele; ns, **, *** non-significant or significant at $p < 0.01$ and 0.001, respectively; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$; no significant interactions were detected, so their results are excluded from the table.

3.3. Main Effects on Primary Axis (Leader and Axis) Growth

The cultivation site induced a highly significant effect on all growth attributes of the primary axes. Length, node number, and axis cross-sectional area (ACSA) were higher in BO, whereas the internode length (19.7 mm) was smaller than at the other two sites (21.1 mm in HB and 21.2 mm in SM) (Table 4). There was a reduction in node number and axis cross-sectional area (ACSA) in HB and SM, compared with BO. The axis cross-sectional area (ACSA) was higher in BO (175 mm^2) compared to HB (146 mm^2) and SM (84 mm^2) (Table 4).

The rootstock effect was very influential on the growth of the main axis. Invigorating rootstocks induced higher shoot length, ACSA, and internode length values. No significant difference was recorded for node number with respect to rootstock effect (Table 4).

The effect of the number of axes resulted in a reduction in mean axis length with an increasing number of leaders. The three-axis trees were shorter (1.1 m) when compared to single- (1.3 m) and double- (1.2 m) axis trees (Table 4). This reduction in axis length is explained by the decrease in mean node number, as there was no statistical difference in internode length across the axis number (A) treatments. A reduction in ACSA was detected in multi-leader trees (125 and 116 mm^2) when compared to the single-leader trees (147 mm^2) (Table 4).

The site × rootstock interaction induced significant effects on the main attributes of growth. Minor differences were recorded on measurements of the primary axis of trees grown in SM, while, in the other two sites, the effect of rootstock vigor was pronounced. The site × axis number interaction analysis did not reveal any statistical differences (Table 4). Nonetheless, a reduction in the absolute values of the growth parameters was recorded for multi-leader trees (Table 4). When the interaction of rootstock × axis number was considered, a slight tendency for a reduction in axis length was observed in multi-leader trees for all rootstocks (Table 4). It is important to underline that when adopting the multi-leader training system on 'MM106', the length of each axis was strongly reduced to an average value that was not statistically different to trees grafted on weaker rootstocks and/or trained with one or two axes (Table 4).

Table 4. Main effects of site of cultivation, rootstock, axis number, and their interactions on the growth attributes of the primary axis in 'Fuji' scions.

Category	Treatment	Length (m)	No. of Nodes/Axis	ACSA[4] (mm^2)	Internode Length (mm)
Site (S)	BO [1]	1.4 [a]	69.8 [a]	175 [a]	19.7 [b]
	HB [2]	1.2 [b]	56.6 [b]	146 [b]	21.1 [a]
	SM [3]	1.1 [b]	53.0 [c]	84 [c]	21.2 [a]
	Significance	***	***	***	***
Rootstock (R)	M27	1.1 [c]	58.0	111 [b]	19.4 [c]
	M9	1.2 [b]	58.4	126 [a,b]	20.6 [b]
	MM106	1.3 [a]	59.1	140 [a]	21.9 [a]
	Significance	***	ns	***	***
Axis Number (A)	1	1.3 [a]	62.4 [a]	147 [a]	20.5
	2	1.2 [a]	58.6 [b]	125 [b]	20.6
	3	1.1 [b]	56.1 [b]	116 [b]	21.1
	Significance	***	***	***	ns
S × R	BO × M27	1.2 [b,c]	66.2 [a]	159 [b]	18.6 [c,d]
	BO × M9	1.4 [a,b]	71.0 [a]	164 [a,b]	19.7 [b,c]
	BO × MM106	1.5 [a]	72.2 [a]	202 [a]	20.6 [b,c]
	HB × M27	1.0 [d]	57.3 [b,c]	104 [c]	16.8 [d]
	HB × M9	1.1 [c,d]	54.2 [b,c]	150 [b]	20.9 [b,c]
	HB × MM106	1.4 [a,b]	58.3 [b]	164 [b]	23.3 [a]
	SM × M27	1.1 [c,d]	53.1 [b,c]	84 [c]	21.2 [b]
	SM × M9	1.1 [c,d]	53.9 [b,c]	85 [c]	20.9 [b]
	SM × MM106	1.1 [c,d]	52.1 [c]	85 [c]	21.4 [a,b]
	Significance	***	ns ^	**	***
S × A	BO × 1	1.5	72.5	217	20.2
	BO × 2	1.4	72.0	170	20.0
	BO × 3	1.3	67.5	165	19.3
	HB × 1	1.2	60.6	151	19.8
	HB × 2	1.2	53.8	141	21.5
	HB × 3	1.2	54.5	147	22.4
	SM × 1	1.3	59.5	106	21.9
	SM × 2	1.2	54.5	85	21.5
	SM × 3	1.0	49.2	75	20.7
	Significance	ns	ns	ns	ns
R × A	M27 × 1	1.1	61.0	119 [b,c]	18.4
	M27 × 2	1.2	56.4	100 [c]	20.6
	M27 × 3	1.1	56.6	114 [b,c]	19.5
	M9 × 1	1.3	61.9	149 [a,b]	21.0
	M9 × 2	1.2	58.7	121 [b,c]	20.5
	M9 × 3	1.2	56.2	117 [b,c]	20.5
	MM106 × 1	1.5	64.8	184 [a]	23.2
	MM106 × 2	1.3	60.2	146 [a,b]	22.2
	MM106 × 3	1.2	55.7	117 [b,c]	21.1
	Significance	ns	ns	^	ns

[1] Bologna, [2] Hawke's Bay, [3] San Michele; [4] Axis cross-sectional area; ns, **, ***, ^ refers to non-significant or significant at $p < 0.01$, 0.001, 0.06–0.08, respectively; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$.

3.4. Main Effects on the Growth of Second-Order Shoots (Lateral Feathers)

Analysis of the final growth of second-order shoots (originating from each main axis) did not include data from HB because trees grown in this site produced a minimal number

of such shoots, which could not be statistically analyzed. Only data from BO and SM are reported herein (Table 5).

Table 5. Main effects of site of cultivation, rootstock, axis number, and their interactions on the growth attributes of second-order shoots in 'Fuji' scions.

Category	Treatment	Length (m)	No. of Nodes/Axis	SCSA [5] (mm^2)
Site (S)	BO [1]	0.35	29.6 [a]	30
	SM [3]	0.25	11.7 [b]	21
	Significance	ns	***	ns
Rootstock (R)	M27	0.33	19.4	31.3
	M9	0.31	18.3	28.2
	MM106	0.34	20.4	27.6
	Significance	ns	ns	ns
Axis Number (A)	1	0.41 [a]	24.0 [a]	33.7 [a]
	2	0.34 [b]	20.1 [b]	31.2 [a]
	3	0.25 [c]	15.6 [c]	23.1 [b]
	Significance	***	***	***
S × R	BO × M27	0.32 [a]	19.9 [a]	31.9 [a]
	BO × M9	0.30 [a]	18.5 [a]	27.9 [a]
	BO × MM106	0.37 [a]	22.5 [a]	29.8 [a]
	SM × M27	0.35 [a]	14.9 [a]	25.9 [a]
	SM × M9	0.36 [a]	16.3 [a]	30.9 [a]
	SM × MM106	0.17 [b]	8.4 [b]	14.4 [b]
	Significance	***	**	*
S × A	BO × 1	0.40 [a]	24.9	33.9
	BO × 2	0.35 [a,b]	22.0	31.7
	BO × 3	0.27 [a,b]	17.2	24.9
	SM × 1	0.42 [a]	18.3	32.1
	SM × 2	0.31 [a,b,c]	13.5	23.8
	SM × 3	0.14 [c]	7.4	13.7
	Significance	*	ns	ns
R × A	M27 × 1	0.38	22.5	37.0
	M27 × 2	0.32	18.8	31.2
	M27 × 3	0.27	16.4	24.5
	M9 × 1	0.35	20.4	29.6
	M9 × 2	0.32	19.9	31.5
	M9 × 3	0.26	15.5	24.9
	MM106 × 1	0.47	27.8	34.9
	MM106 × 2	0.38	22.0	31.0
	MM106 × 3	0.24	15.4	21.6
	Significance	ns	ns	ns

[1] Bologna, [3] San Michele; [5] Shoot cross-sectional area; ns, *, **, *** non-significant or significant at $p < 0.05$, 0.01, 0.001, respectively; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$.

Cultivation site accounted for a significant effect on second-order shoot length, with trees in SM developing shoots with 40% fewer nodes than shoots measured in BO (11.7 compared to 29.6, respectively) (Table 5), translating into a numerically greater average shoot length at BO (0.35 m) compared with SM (0.25 m). However, this was not statistically different (Table 5).

Rootstocks did not affect second-order shoot growth attributes (Table 5). A higher axis number reduced the length, node number, and shoot cross-sectional area (SCSA) of secondary shoots (Table 5).

There were significant interactions between site of cultivation × rootstock, but, for all of the variables considered, only the combination of SM × 'MM106' had statistically smaller values than the other combinations (Table 5). The site × axis number interaction showed a significant influence on shoot length. SM trees with three axes had reduced shoot lengths compared with the combinations in BO and the single-axis trees in SM (Table 5).

With respect to the distribution of number of nodes per shoot and shoot cross-sectional area (SCSA) in second-order shoots by cultivation site, it appeared clear that trees grown in SM had a higher proportion of shorter shoots (more than 50% of shoots had less than 10 nodes) than those from BO, where it was possible to find shoots with more than 45 nodes per shoot (data not shown). A similar pattern was detected when SCSA was considered, with a higher proportion of smaller SCSA values in SM than in BO (data not shown).

The number of second-order shoots developed over the first year after grafting was strongly influenced by the cultivation site and the rootstock vigor. The average number of shoots per tree in BO was approximately eleven times higher than the one recorded in SM and increased with increasing rootstock vigor (Table 6). The number of secondary axes per tree was not statistically different among single- (3.1) and double-axis (3.4) trees, but was higher in trees trained with three axes (4.9) (Table 6).

3.5. Main Effects on the Total Growth per Tree

All the main factors had a highly significant effect ($p < 0.001$) on total growth per tree expressed as total length of shoots/tree (Table 7). Sites of cultivation had a substantial impact on total shoot length/tree and node number/tree (Table 7). For both variables, HB registered the smallest means (1.82 m and 86.6 nodes, respectively), due mainly to the lack of development of second-order shoots, which was practically non-existent. At the same time, SM had values approximately 50% higher than the HB site. Trees in BO grew the most, with length and node values nearly three times those of the HB site and two times those of the SM site (Table 7).

Total growth was also directly related to rootstock vigor and the number of axes (Table 7). Rootstock did not affect tree growth in SM, but their effects were evident in HB and BO, as the increased vigor rootstocks increased the total length (Table 7). Moreover, the total shoot length of trees in BO was approximately four times higher than the corresponding combinations in HB. Total shoot length and total node number followed the same trend of enhanced vigor equal to increasing growth values (Table 7).

Site × axis number interactions were also highly significant (Table 7). The effect of rootstock × axis number, although not significant, could be relevant from a more practical perspective. According to the data shown in Table 7, the total length per tree was proportionally related to the number of axes.

3.6. Main Effects on Bud Break and Flowering in the Second Vegetative Season

Information on the proportion of bud break (i.e., buds generating new vegetative shoots or floral buds) registered the year after planting was meaningful only for trees grown in BO because only trees in this site produced many second-order shoots.

Rootstock did not have any statistical influence on the proportion of bud break, although 'M27' showed a smaller proportion of bud break (i.e., less buds developing into shoots or flowers) (data not shown). However, the proportion of bud break was impacted by axis number, with a reduction in trees trained at two or three leaders (data not shown).

All main factors had a highly significant effect on the first occurrence of flowering (Table 8). Large differences were recorded among the different cultivation sites. Trees in SM had, on average, less than one flower cluster per tree, while BO had three, and HB trees developed around twenty-two floral clusters per tree (Table 8). Rootstock comparison exhibited a statistical difference in the average floral cluster number per tree when comparing 'M27' (5) and 'MM106' (5) with 'M9' (11), which had twice as many clusters as the other rootstocks (Table 8). An increased cluster number was detected in trees trained with two leaders (9) when compared to trees with a single axis (7) or three axes (6) (Table 8).

Table 6. Main effects of site of cultivation, rootstock, axis number, and their interactions on the number of second-order shoots developed in the first season after grafting in 'Fuji' scions.

Category	Treatment	No. of Shoots/Tree
Site (S)	BO [1]	8.9 [a]
	SM [3]	0.7 [a]
	Significance	***
Rootstock (R)	M27	2.4 [a]
	M9	3.5 [a]
	MM106	5.4 [a]
	Significance	***
Axis Number (A)	1	3.1 [a]
	2	3.4 [a]
	3	4.9 [a]
	Significance	***
S × R	BO × M27	5.7 [c]
	BO × M9	8.6 [a]
	BO × MM106	12.3 [a]
	SM × M27	0.4 [b]
	SM × M9	0.6 [b]
	SM × MM106	1.2 [b]
	Significance	***
S × A	BO × 1	7.5 [c]
	BO × 2	8.6 [a,b]
	BO × 3	10.5 [a]
	SM × 1	0.6 [d]
	SM × 2	0.3 [d]
	SM × 3	1.3 [d]
	Significance	^
R × A	M27 × 1	2.2 [a]
	M27 × 2	2.5 [a]
	M27 × 3	2.5 [a]
	M9 × 1	3.0 [a]
	M9 × 2	3.2 [a]
	M9 × 3	4.0 [a]
	MM106 × 1	4.6 [a,b]
	MM106 × 2	4.9 [a,b]
	MM106 × 3	7.3 [a]
	Significance	***

[1] Bologna, [3] San Michele; ^, *** represents significant at $p < 0.07$, 0.001, respectively; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$.

In BO and SM, cluster counts/tree were inversely related to the rootstock vigor (Table 8). In HB, this trend was not apparent, as 'M9' had the highest number of floral clusters, followed by 'MM106' and then 'M27', which had consecutively lower floral bud induction (Table 8). In BO and HB, the number of floral clusters was directly related to the number of leaders, with increased clusters with an increased number of axes, although this was not true for SM (Table 8). The site × axis interaction was significant. In HB, which had a high number of clusters, the number of clusters increased with the number of axes for all the rootstocks (Table 8). A similar trend was detectable in BO as well. The rootstock × axis number interaction was not significant.

Table 7. Main effects of site of cultivation, rootstock, axis number, and their interactions on total tree growth in 'Fuji' scions.

Category	Treatment	Length (m)	No. of Nodes/Tree
Site (S)	BO [1]	5.9 [a]	335.1 [a]
	HB [2]	1.8 [c]	86.6 [c]
	SM [3]	2.4 [b]	113.2 [b]
	Significance	***	***
Rootstock (R)	M27	2.5 [c]	132.4 [c]
	M9	3.0 [b]	153.7 [b]
	MM106	3.6 [a]	182.4 [a]
	Significance	***	***
Axis Number (A)	1	2.0 [c]	106.0 [c]
	2	3.3 [b]	166.6 [b]
	3	4.5 [a]	235.9 [a]
	Significance	***	***
S × R	BO × M27	4.5 [c]	254.2 [c]
	BO × M9	5.9 [b]	329.3 [b]
	BO × MM106	7.4 [a]	417.5 [a]
	HB × M27	1.1 [f]	66.1 [f]
	HB × M9	1.7 [e]	83.4 [e]
	HB × MM106	2.4 [d]	104.8 [d]
	SM × M27	2.3 [d]	107.8 [d]
	SM × M9	2.5 [d]	117.6 [d]
	SM × MM106	2.5 [d]	114.2 [d]
	Significance	***	***
S × A	BO × 1	4.9 [b,c]	282.4 [b]
	BO × 2	5.9 [a,b]	328.0 [b]
	BO × 3	7.0 [a]	392.6 [a]
	HB × 1	1.3 [g]	61.8 [f]
	HB × 2	2.2 [e,f]	107.9 [d,e]
	HB × 3	3.5 [c,d,e]	163.4 [c,d]
	SM × 1	1.7 [f,g]	76.2 [e,f]
	SM × 2	2.5 [d,e,f]	113.6 [c,d,e]
	SM × 3	3.3 [d]	155.7 [c]
	Significance	***	***
R × A	M27 × 1	1.7	91.0
	M27 × 2	3.1	157.8
	M27 × 3	4.0	208.5
	M9 × 1	2.0	101.4
	M9 × 2	3.1	159.2
	M9 × 3	4.6	236.4
	MM106 × 1	2.6	129.5
	MM106 × 2	3.7	180.0
	MM106 × 3	4.8	251.8
	Significance	ns	ns

[1] Bologna, [2] Hawke's Bay, [3] San Michele; ns, ***, non-significant or significant at $p < 0.001$, respectively; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$.

Table 8. Main effects of site of cultivation, rootstock, axis number, and their interactions on the number of floral clusters per tree in 'Fuji' scions.

Category	Treatment	No. of Floral Clusters/Tree
Site (S)	BO [1]	3 [b]
	HB [2]	22 [a]
	SM [3]	1 [c]
	Significance	***
Rootstock (R)	M27	5 [b]
	M9	11 [a]
	MM106	5 [b]
	Significance	***
Axis Number (A)	1	7 [b]
	2	9 [a]
	3	6 [b]
	Significance	***
S × R	BO × M27	5 [c,d]
	BO × M9	4 [d]
	BO × MM106	0 [e,f]
	HB × M27	10 [c]
	HB × M9	35 [a]
	HB × MM106	18 [b]
	SM × M27	1 [e,f]
	SM × M9	1 [e,f]
	SM × MM106	0 [f]
	Significance	***
S × A	BO × 1	2 [d,e]
	BO × 2	3 [d,e]
	BO × 3	4 [d]
	HB × 1	15 [c]
	HB × 2	27 [b]
	HB × 3	37 [a]
	SM × 1	1 [d,e]
	SM × 2	0 [e]
	SM × 3	1 [e]
	Significance	***
R × A	M27 × 1	6
	M27 × 2	5
	M27 × 3	3
	M9 × 1	10
	M9 × 2	13
	M9 × 3	10
	MM106 × 1	4
	MM106 × 2	7
	MM106 × 3	5
	Significance	ns

[1] Bologna, [2] Hawke's Bay, [3] San Michele; ns, ***, non-significant or significant at $p < 0.001$, respectively; Treatment means sharing the same letter are not significantly different according to Tukey's post-hoc means comparison test at $p < 0.05$.

4. Discussion

4.1. Effect of Propagation Method on Multi-Leader Trees' Survival Rate

In both Italian sites, chip-budded trees were treated differently at planting compared to the New Zealand site, where the rootstocks were headed back above the grafted bud immediately after planting. This technique was not applied in the Italian sites in order to

aim for a better survival rate. This modification in the timing of heading the rootstocks improved the rate of survival (Figure 1), which delayed the shoots' development. In the Italian sites, the scion started growing more than a month after planting. The chip budding presented a low tree development success rate, given the conditions in this trial (Figure 1). This result was different from what is reported in the literature [51]. There was no difference between the Italian sites regarding tree survival, and they both performed better than the New Zealand site (Table 1).

'M9' and 'MM106' revealed an almost identical proportion of good trees and were statistically larger than 'M27,' probably due to the increased vigor of these rootstocks and the delayed growth of 'M27'. Increasing the number of leaders statistically reduced tree quality (Table 1), contrary to what Yong-Koo et al. [52] reported in a similar study.

4.2. Effect of Treatments on Leaf Number and Leaf Area Measurements

Leaf number and leaf area measurements were only carried out in Italy, and these parameters differed between the two Italian sites (BO and SM). The trees grown in BO had more leaves and a smaller average leaf area when compared to the SM site (Table 2).

Rootstocks did not exhibit differences in the number of leaves. However, there appeared to be an inverse relationship with leaf area and vigor amongst rootstocks grafted with 'Fuji' scions, with 'M9' (dwarfing) and 'M27' (very dwarfing) showing a larger average leaf size than the 'MM106' (vigorous) rootstock. However, this could be an effect of the vigorous rootstock growing later into the season, leading to a higher proportion of late-developed leaves being sampled, which a smaller size could have characterized. Nevertheless, the finding presented herein is in contrast with data on apple and kiwifruit. Anthony et al. [53], in a rootstock trial on mature 'WA 38' trees grafted on Geneva 41®, showed that a more vigorous rootstock induced higher leaf area values than M9-Nic29, which is a less vigorous rootstock. When a comparison amongst eight rootstocks in kiwifruit (*Actinidia chinensis* var. *chinensis* 'Hort16A') was assessed, the most vigorous combination reported the highest leaf area [54]. Both studies were conducted on mature trees in full production, while our data refer to trees immediately released from the nursery. A study across *Prunus* rootstocks did not find any correlation between vigor level and leaf area [55].

An increased number of axes induced a reduction in the number of leaves per tree (Table 2). However, the individual average leaf size showed no difference between single- and double-axis trees, but the tri-axis trees exhibited a reduction in individual leaf size (Table 2). Regarding total canopy leaf area, Van Hooijdonk et al. [56] reported a higher total leaf area per tree in bi-axis trees compared with single-leader trees due to increased primary shoot length and node numbers, as they sustained growth later in the season. However, regarding individual leaves, the area of primary shoot leaves was smaller on bi-axis trees than single-leader trees [56]. Although this reduction was not noted between single leaders and bi-axis trees in the present trial, this decrease in average leaf area was observed in the tri-axis trees (Table 2). In Van Hooijdonk et al. [56], individual leaf area was also affected by shoot type, with the individual leaf area being reduced on sylleptic shoot leaves, which were more prominently developed on single-leader trees when compared with bi-axis trees from the nursery.

4.3. Effect of Treatments on Primary Axis Growth

Cultivation site induced modifications of growth attributes in the primary axes (Table 4). Node number and ACSA were higher, and average internode length was reduced in BO compared to the other sites, proving the strong effect of this location on the vigor of the trees. This is in partial agreement with what was reported in the International Apple Growth Study by Tustin et al. [21]. In general, the primary axis length was reduced with decreasing rootstock vigor, confirming the results of Rao and Berry [41].

Multi-leader trees presented a reduction in the growth parameters in the primary axis. Tri-axis trees induced a "dwarfing" effect on the mean primary leader length compared to the single- and double-leader trees. However, when considering the total tree growth,

three-leader trees maintained the longest total shoot length and highest total number of nodes per tree (Table 7). Therefore, although the multi-leader trees may exhibit larger values of total growth, these trees can distribute this growth across a larger number of leaders, contributing to reduced lengths and node numbers on a per axis basis (Tables 4 and 7). Overall, the trees appear to distribute vigor across multiple leaders, reducing primary leader growth by decreasing the number of nodes per axis.

Rootstocks indicated a dwarfing effect as they induce earlier shoot growth cessation [35], contributing to a reduction in the leader and internode lengths presented herein (Table 4). The average length of the primary axes was reduced progressively by dwarfing ('M9') or very dwarfing ('M27') rootstocks in the BO and HB sites, confirming results obtained by Cannon [42]. In a previous trial set up in New Zealand, Seleznyova et al. [5] observed that the primary shoot growth was not affected by rootstock/interstock in the first season after propagation. This further supports the hypothesis that the innate vigor in a specific growing site also contributes to the growth and/or dwarfing effect induced in the scion, as soon as the first year after planting.

In a comparison between four standard apple cultivars ('Imperial Red Delicious', 'Imperial McIntosh', 'Law Rome', and 'Granny Smith') and their spur-type sport ('Redchief', 'MacSpur', 'Lawspur Rome', and 'Granny Smith spur'), Walsh and Miller [57] demonstrated that the internode length is more indicative of a dwarfing effect, rather than the number of nodes in a shoot. However, the dwarfing mechanism referenced in this study was not due to the rootstock effect but to a shift in hormonal control (e.g., GA responsiveness) in the scion tissue [57]. Similar to this study, rootstock genotype appears to influence the internode length of the scion heavily due to earlier shoot termination, which is a primary rootstock scion dwarfing mechanism [36]. Another rootstock-induced dwarfing mechanism is the stimulation of floral precocity, leading to different types of shoots being subsequently grown (i.e., reducing the relative occurrence of shoots that have more than one growth unit) [5]. This is demonstrated by reducing second-order shoots with increased dwarfing genotypes (Table 6) and an increase in floral clusters per tree, particularly in 'M9,' on average (Table 8).

In sum, vigor control by rootstock appears to heavily influence internode length (via shoot extension termination) and growth unit determination, while vigor diffusion (i.e., average leader growth reduction) with increased canopy architecture complexity influences the number of nodes per axis.

4.4. Effect of Treatments on the Growth of Second-Order Shoots (Lateral Feathers)

The BO site stimulated a higher number of nodes per second-order shoots when compared to the SM site (Table 5). This may be a result of the increased vigor conditions at this site. Previous studies have reported that 'M9' decreased the average annual shoot length due to an earlier cessation of growth when compared to more vigorous rootstocks [58,59]. However, in this study, the rootstocks compared did not demonstrate a significant effect on second-order shoot growth parameters. Therefore, in contrast to what was reported by Van Hooijdonk et al. [44], rootstock did not influence the growth attributes of secondary shoots.

Between the main effects, only canopy architecture significantly impacted all second-order shoot growth parameters (Table 5). In general, the increased number of leaders reduced the average length, number of nodes, and SCSA of second-order shoots (Table 5). This result may support the hypothesis that when there is more than one leader, the hormonal balance and/or nutrient availability may be altered, leading the trees to reduce secondary shoot growth and elongation [60]. This further supports the hypothesis of vigor control via growth distribution, as the growth of individual primary leaders and secondary shoots is reduced, but the total growth of the tree remains high when compared to less complex canopy architectures (Tables 4–7). In short, multi-leader trees reduce the organ size (e.g., axis, shoots, etc.) by modifying the growth distribution within a single plant but generating larger canopies on a total dry weight basis [56].

All main effects demonstrated impacts on bud outgrowth and second-order axillary shoot stimulation (Table 6). A broad difference in the number of lateral shoots was found when comparing sites of cultivation. BO had almost nine laterals per tree, while SM had less than one (Table 6), and there were almost none in the HB site (data not shown). This continues to highlight the natural vigor of the BO cultivation site. It is known that some environmental factors, such as soil temperature and air humidity, can affect the formation of secondary shoots [61]. Moreover, Tromp [62] observed that lateral shoot growth of newly grafted apple trees was higher after exposure to temperatures over 20 °C with respect to lower temperatures. This information may help to explain the differences observed across sites in this experiment, but further research is needed to pursue this possibility since neither canopy nor soil probes were installed in this multi-site trial.

The decreased number of second-order shoots found on dwarfing rootstocks confirms the results described by Volz et al. [43], but it is important to note that, for a given rootstock, the number of lateral shoots increased by raising the number of primary axes (Table 6). Further, it should also be noted that this rootstock trend and bud outgrowth was only demonstrated at the BO site (Table 6). Van Hooijdonk et al. [44] showed that the decrease in the rate of bud outgrowth and the formation of secondary axes induced by dwarfing rootstocks might be restored by applying exogenous cytokinin, suggesting a regulating role and beneficial application of this hormone to improve axillary bud outgrowth break to the desired rate.

4.5. Effect of Treatments on Total Growth per Tree

Total growth appears to be most related to the cultivation site, as trees in BO displayed a total number of nodes that was almost double or triple the average reported for the trees in SM and HB (Table 7). The results reported for the BO site support the idea that tree growth can be modified by enhancing the number of nodes per tree and the incidence of bud outbreak, which contributes to a larger number of second-order shoots (Table 6).

Dwarfing rootstocks ('M27' and 'M9') reduced the total length of growth per tree and number of nodes per tree compared to the most vigorous, 'MM106' (Table 7). Interestingly, the total growth of the tree increased with the number of leaders (Table 7). It is hypothesized that this increased length in the multi-leader trees is the result of the increased number of nodes. This physiological response became more evident when we analyzed the second-order shoots' growth (Table 6). This is significant as nursery trees are often limited in their ability to intercept light, inhibiting their growth, as photosynthesis is linearly related to light interception and leaf area in nursery trees [63]. Therefore, if multi-leader trees can enhance their total growth, node number, and second-order shoot incidence in the first year, then they can promote a higher amount of leaf area, as demonstrated in this study (Table 3). This increase in leaf area in multi-leader nursery trees can then contribute to enhanced light interception, photosynthesis, and dry matter accumulation in apple propagation [63].

4.6. Effect of Treatments on Bud Breaking and Flowering in the First Vegetative Season

Differences in the incidence of floral clusters per tree were recorded between sites. Our data confirm the findings of Tustin et al. [21], where, in a trial with ten locations around the world, New Zealand recorded the highest number of floral buds per limb. In our experiment, HB ranked first, followed by BO and SM, with respect to floral clusters per tree.

Dwarfing rootstocks help to form trees that are more prone to induce the formation of flower buds, as reported by Atkinson and Else [63]. Seleznyova et al. [36] demonstrated that rootstock can induce floral precocity as early as the second year of growth along the scion's primary axis. This is a primary scion–rootstock interaction, where the rootstock reduces scion growth through the induction of floral precocity [4,36,64]. Overall, dwarfing rootstocks shift annual shoot development away from monopodial, vegetative growth to sympodial, floral shoots [36]. Further, it has been observed that short apple shoots generally bear flowers and fruit, while long shoots are mainly vegetative [40,65]. The

analysis of the rootstock effect revealed no difference in flower bud formation between 'M27' and 'MM106', while 'M9' was higher compared to both of the others. This confirms the enhanced flowering effect of 'M9' described by Hirst and Ferree [37,38] and, more recently, by Seleznyova et al. [5], but does not explain the lower flowering rate of 'M27.' This result contrasts with what was reported by Faedi et al. [66] on 'Starking Delicious' grafted on 'M27,' 'M9,' 'M26', and 'MM106,' where 'M27' induced a higher level of flower buds when compared to 'MM106.'

The high average count of floral clusters per tree in 'M9' appears to be an artifact of the cultivation site, as there is a significant interaction between site × rootstock main effects (Table 8). In the Italian sites (BO and SM), the expected trend of increased floral precocity with growth reduction is demonstrated, while in HB, 'M9' demonstrates an exceptionally higher level of floral clusters than 'MM106' and 'M27' (Table 8). These results further show that the propagation site is a significant factor in floral precocity, along with rootstock vigor. The outgrowth of axillary buds to form second-order shoots, especially in BO, perhaps caused a reduction in flowers at the axillary node sites due to these being occupied by second-order vegetative shoots that grew later in the season. Therefore, this growth could have contributed to longer, vegetative shoots rather than short, reproductive ones [40,66]. However, this does not explain why 'M27' retained lower numbers of floral clusters than 'M9' at the HB site. In a study carried out in Havelock (New Zealand), both 'M9' and 'M27' rootstocks increased floral buds' number on the primary axis and reduced the number of sylleptic shoots [6]. In our experiment, the flowering response may have been reduced in 'M27' given the reduced number of nodes and shorter axis lengths (Tables 4 and 7), thus reducing the number of available sites for flowering [36].

5. Conclusions

The wide range of training systems proposed for apple cultivation in the last few decades share some common features: the rapid achievement of high light interception and early cropping, the preservation of optimal light distribution within the canopy over the season and orchard lifespan, and more efficient harvesting due to canopy size control and planar architectures.

The data presented in this research at the nursery level indicate that multi-leader trees seem to be very suitable for guaranteeing rapid canopy development and subsequent good light interception. With three rootstocks of differing vigor, we determined larger total canopy development on newly grafted 'Fuji' trees with multiple leaders compared to single-leader trees. This finding highlights the need for specific rootstock trials to optimize the multi-leader systems.

Further, the cultivation site played an important role in modulating early tree performance. The production of second-order shoots was effective in the Italian sites, while no growth of feathers was observed in the New Zealand site. Multi-leader trees reduced their average shoot length in the Italian sites in the first year after grafting. The number of shoots developed on multi-leader trees was higher than in single-axis trees at the same planting density. This could be of considerable benefit to growers, who can use canopy architecture manipulation (e.g., increased number of axes) as a tool for vigor control when their site of cultivation and/or rootstock selection is too vigorous to easily establish HDP orchards, as well as the newer concepts for 2-D planar fruiting walls.

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