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The dwarfing mechanism of citrus rootstocks F&A 418 and #23 is related to competition between vegetative and reproductive growth

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Summary The annual development of Navelina (*Citrus sinensis* (L.) Osbeck) trees budded on three hybrid citrus rootstocks was studied. Two rootstocks, named #23 and #24, were obtained from the cross of Troyer citrange (*C. sinensis* × *Poncirus trifoliata* (L.) Raf.) × Cleopatra mandarin (*C. reshni* Hort. ex Tan.). The third rootstock, named F&A 418, came from a cross of Troyer citrange × common mandarin (*C. deliciosa* Ten.). Rootstocks #23 and F&A 418 are dwarfing rootstocks and reduce the size of the scion by about 75%. Rootstock #24 yields a standard size scion. Major growth differences that influenced tree size were apparent during the first summer after grafting and appeared to be related to fruit productivity, because defruiting the dwarfed scions caused a significant increase in vegetative shoot development, including summer sprouting. The reduced growth of the dwarfed scions was not restored by hormone application, indicating that a hormonal deficiency is unlikely to be the primary reason for scion dwarfing, although differences in gibberellin concentrations were found in actively growing shoots. Leaf photosynthesis was similar in scions on all three rootstocks, but the carbohydrate accumulation in fruits and fibrous roots during the summer sprouting period was significantly greater in the dwarfed trees than in the standard trees. Our results suggest that the dwarfing mechanism induced by the F&A 418 and #23 rootstocks is mediated by enhanced reproductive development and fruit growth, resulting in reduced vegetative development in the summer. Thus, a change in the pattern of assimilate distribution appears to be one of the main components of the dwarfing mechanism.

Keywords: benzyladenine, carbohydrate distribution, fruit, gibberellins, photosynthetic capacity, summer sprouting.

Introduction

Fruit trees are generally cultivated on rootstocks that interact with the scions and modulate several aspects of canopy performance. An intriguing effect of certain rootstocks is the ability to dwarf shoot growth of scion genotypes that are normally tall. In citrus, the mechanism underlying rootstock dwarfing has not been identified, although several mechanisms have been proposed for a range of woody species. In addition to alterations with a possible pathological basis (Mendel and Cohen 1967, Rabe et al. 1992, Ermel et al. 1997), hormonal mechanisms (Richards et al. 1986, Steffens and Hedden 1992, Cutting and Lyne 1993, Wang and Faust 1993, Bertling and Lovatt 1997), anatomical mechanisms (McKenzie 1961, Soumelidou et al. 1994*a*) and nutritional mechanisms (Jones 1976, Schechter et al. 1991) have been postulated. Moreover, several of these mechanisms may interact (Dana et al. 1962, Lockard and Schneider 1981, Soumelidou et al. 1994*b*). The dwarfing influence of the rootstock over growth of the scion can have several characteristics. First, there are rootstocks that induce intrinsic dwarfing effects over the scion (Cheng and Roose 1995). In this case, the self-rooted rootstocks also show a reduced size. A second model implies an indirect effect that depends on the interaction between both rootstock and scion (McKenzie 1961, Lockard and Schneider 1981). Here, the dwarfing ability varies with the scion variety and disappears when self-rooted rootstocks are used. Third, some rootstocks are postulated to modify canopy development as a result of a negative influence of reproductive development on vegetative growth (McKenzie 1961, Lenz 1967). Other physiological aspects, such as photosynthetic capacity, nutrient metabolism and water relationships, may also be involved (Carlson 1974, Syvertsen and Graham 1985, Schechter et al. 1991). Lastly, it is well known that citrus scion growth can be influenced by pathological agents such as the exocortis viroid on susceptible rootstocks (Rabe et al. 1992).

In this work, the effects of three citrus rootstocks, F&A 418, #23 and #24, on tree size, hormonal changes, carbohydrate distribution, and vegetative and reproductive growth of Navelina orange scions were investigated.

Materials and methods

Plant material

All the measurements and samples used in this work were taken from Navelina orange (*Citrus sinensis* (L.) Osbeck) budded on three hybrid rootstocks. Two rootstocks, #23 and #24, come from a cross between Troyer citrange (*C. sinensis* ×

Poncirus trifoliata (L.) Raf.) × Cleopatra mandarin (*C. reshni* Hort. ex Tan.). The third rootstock (Troyer citrange × common mandarin (*C. deliciosa* Ten.)) has been registered in the European Union as F&A 418. All rootstocks were obtained by J.B. Forner at the I.V.I.A. (Moncada, Valencia, Spain) as described in Forner et al. (2000).

The #23 and F&A 418 hybrids are dwarfing rootstocks and reduce the size of the budded scion by 75%. Rootstock #24 maintains a normal scion growth. Seventeen-year-old trees, located in Càrcer (Valencia, Spain) were used for all the experiments. Measurements of canopy volume were made on the same trees when they were 13 and 17 years old.

In some experiments, the hybrid rootstocks were budded onto the commercial citrus roostock, Carrizo citrange, in an experimental plot located in Moncada (Valencia, Spain). Canopy volume of 17-year-old trees was measured. Data from *Poncirus trifoliata* cv. 'Flying Dragon,' a well known dwarfing citrus rootstock, were included for comparison.

For analyses, representative samples of fruit and fibrous roots were collected from trees of each graft type, lyophilized, ground and stored at –20 °C until analyzed.

Vegetative growth

Tree canopy size was estimated by measuring the height of the canopy and two horizontal and perpendicular diameters. The canopy was assumed to be a sphere and its volume calculated from the mean data. At least three trees were used in each of these determinations.

Intensity of vegetative sprouting was estimated by randomly placing a 42-cm diameter ring on the tree canopy and counting and measuring every new shoot within the ring. This operation was repeated four times for each canopy. From the data, the number and total length of shoots on a canopy area basis (m^2) were calculated. The same trees were measured in two consecutive years and the measurements were summed to account for any possible alternate-year vegetative growth pattern.

Hormonal applications

To test the effects of hormones on the induction of sprouting, latent buds were soaked in one of four different aqueous solutions (20 ppm gibberellic acid (GA_3), 20 ppm 6-benzyladenine (BA), 20 ppm indoleacetic acid (IAA), and a water control, all containing 2% Tween 20). Tween 20 was added after the hormones had been dissolved in distilled water. For each treatment, several drops of the hormonal solution were painted on the bud with a small paintbrush. All buds of 20 terminal twigs (from the last spring's sprouting) around the tree were used. All four treatments were applied on the same tree. Treatments started on May 30 and continued every 5 or 6 days (a total of 17 applications) until bud burst, at the beginning of September. In November, 2 months after bud sprouting, the lengths of new shoots on the treated twigs were measured.

GA extraction and analysis

To quantify GA, actively growing shoots ranging between 3 and 6 cm in length were collected from three trees of each graft type. Lyophilized tissue (3 g) was homogenized with a Polytron homogenizer (Kinematica, Littau-Luzerne, Switzerland) and extracted with 300 ml of cold 100% methanol. The extract was filtered and the residue resuspended in 100 ml of 80% aqueous methanol at 4 °C overnight. The following GAs were added to combined filtrates as internal standards: [17, 17-$^2H_2$]GA_{19} (95.9% enrichment), [17, 17-$^2H_2$]GA_{20} (99.6% enrichment), [17, 17-$^2H_2$]GA_{29} (99.8% enrichment), [17, 17-$^2H_2$]GA_1 (99.6% enrichment) and [17, 17-$^2H_2$]GA_8 (99.7% enrichment). The extracts were then reduced to the aqueous phase in vacuo and an equal volume of 0.1 M potassium phosphate buffer, pH 8.2, was added. The extract was centrifuged at 500 *g* for 10 min and the supernatant was partitioned against *n*-hexane (1×, 1:1, v/v) and diethyl ether (3×, 1:1, v/v). The aqueous phase was acidified to pH 2.5 and purified on a charcoal:Celite (1:2, w/w) column. The extract was loaded on the column in 10 ml of 0.1 M potassium phosphate buffer, pH 8.2, and the GAs were eluted with 150 ml of 80% acetone. The acetone was removed in vacuo and the aqueous phase was adjusted to pH 8.5 and then purified by PVPP (polyvinylpolypyrrolidone) chromatography (Glenn et al. 1972). The extract was absorbed to a column containing 2 g of PVPP and eluted with 30 ml of potassium phosphate buffer. The eluate was then adjusted to pH 2.5 and partitioned against ethyl acetate (3×, 1:1, v/v). The organic phase was washed with water at pH 2.5 (2×, 20:1, v/v) and evaporated to dryness. The dried extract was dissolved in several drops of methanol and water, adjusted to pH 8.5 and purified by QAE-Sephadex A-25 (Sigma, St. Louis, MO) anion exchange chromatography. The column was prepared with 10 ml of Sephadex powder in 1 M acetic acid and equilibrated with pH 8.5 water (Talon and Zeevaart 1990). Samples were applied to the column and washed with 30 ml of pH 8.5 water. The GAs were eluted from the column with 40 ml of 1 M acetic acid and purified on C18 Sep-Pak cartridges, previously activated with methanol. After several rinses with 1 M acetic acid, the GAs were eluted with 80% methanol (Talon et al. 1990). The methanol was reduced to dryness in vacuo and the dry residues were dissolved in 2.5 ml of 20% methanol for further fractionation by reversed-phase high-performance liquid chromatography (HPLC). One µg of abscisic acid was then added as an internal standard to ascertain the reproducibility of retention times. Abscisic acid was detected by absorbance at 254 nm. Samples were filtered through a 0.45-µm diameter nylon filter and injected into a Waters-Millipore HPLC system (Milford, MA). The instrument was equipped with an analytical column (25 × 0.46 cm I.D.) packed with Hypersil C18 and attached to a C18 Guard-Pak precolumn. A 40-min linear gradient of 20–100% methanol in 1% aqueous acetic acid at a flow rate of 1 ml min^{-1} was used. The HPLC fractions were collected at 1-min intervals and conveniently grouped. Samples were evaporated to dryness, methylated with ethereal diazomethane and trimethylsilylated at room temperature for at least 1 h with 10 µl of bis-trimethylsilyl-trifluoroacetamide (Ben-Cheikh et al. 1997). The samples were quantified by GC–MS. The gas chromatograph (Model 8000, Fisons, Danvers, MA), which was equipped with a fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness; DB-5MS, J&W Scientific,

Folsom, CA), was coupled to a quadrupole mass detector (800; Fisons). The samples (2 µl) were injected in the splitless mode. Oven temperature was 50 °C and after 1 min was increased at 30 °C min^{-1} to 240 °C and then at 10 °C min^{-1} to 280 °C. The He inlet pressure was 85 kPa and the injector, interface and MS source were set at 250, 250 and 200 °C, respectively. Positive ion electron impact masses at 70 eV were acquired in selected ion monitoring (SIM) mode.

Gibberellin quantification was based on the use of internal standards and the method of sequential extractions (Ben-Cheikh et al. 1997, Mehouachi et al. 2000). The amounts of internal standards to be added to the extractions were successively adjusted in consecutive extractions until similar amounts of deuterated internal standards and endogenous compounds were found in the samples. In this method, values with ratios equal or close to 1 yield the most accurate determinations. The identity of the endogenous/standard mixture of GAs utilized for SIM analysis was previously confirmed by full-scan GC–MS (Ben-Cheikh et al. 1997). Endogenous gibberellin concentrations were determined as follows. First, estimates of the GA concentratons were obtained from full-scan GC–MS and SIM in preliminary extractions without internal standards. Second, for actual quantification, appropriate amounts of internal standards were added to the methanolic extract, based on the previous estimates of endogenous concentrations. Third, the amounts of the internal standards were successively adjusted and the extractions were repeated until the amount of internal standards and the amount of endogenous GAs were about equal. Concentrations of endogenous GAs were quantified by successive extractions, although only data for the last extraction are presented. In all cases, at least two injections of the same sample, which provided nearly identical results, were made.

Reproductive growth

Fruit yield was determined by counting and weighing all fruits from at least three trees. Sixty fruits of each graft type were weighed individually and the mean was calculated. Yield efficiency was estimated on a canopy surface basis, as previously explained.

Thinning experiment

Some trees were completely defruited before the beginning of June fruit drop. At the end of August, vegetative growth was estimated as previously explained.

Photosynthetic measurements

Net CO_2 assimilation rates during the summer were measured regularly in field-grown trees early in the morning to avoid elevated afternoon temperatures and vapor pressure deficits. Photosynthetic rates (A) were determined with an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE) equipped with an 18 cm^3 leaf chamber that carried a gallium arsenide phosphide (GaAsP) photosynthetically active radiation (PAR) sensor. All measurements were performed at a PAR of 900 µmol m^{-2} s^{-1} and a constant flow rate when the system was stable and the coefficient of variation was less than 1%. Measurements were made on a minimum of 15 leaves of each of three leaf types: (1) previous-year leaves; (2) current-year leaves from leafy shoots of the spring flush, and (3) current-leaves from shoots of the spring flush bearing a single fruit.

Carbohydrate analysis

Carbohydrates were analyzed as described by Mehouachi et al. (1995) with some modifications. Briefly, samples were purified sequentially by cation and anion exchange columns and C18 cartridges. One ml of purified extract was filtered through a 0.45 µm membrane (Waters-Millipore, Millford, MA), and 1.5 ml of 100% acetonitrile was added. Sucrose, glucose and fructose were analyzed with a Waters-Millipore HPLC system equipped with a high-performance carbohydrate column (250 mm long, 4.6 mm I.D., Waters-Millipore), and a R401 differential refractometer. The solvent was 80% acetonitrile. The effectiveness of the extractions was ascertained by using fucose as the internal standard. Starch was determined as described by Mehouachi et al. (1995). Starch was gelatinized by autoclaving the samples. Sodium acetate buffer and excess amyloglucosidase (650 units) were added to the extracts, and enzymatic digestions were performed at pH 4.5 for 2 h at 55 °C. After filtration, the released glucose was determined by HPLC as previously described.

Results

Vegetative growth

The citrus rootstocks F&A 418, #23 and #24 reached standard size when grown as self-rooted trees (data not shown). However, differences in size among Navelina trees budded onto these rootstocks were dramatic (Table 1A). Canopy volume of

Table 1. A: Canopy volume (m^3) of Navelina citrus budded on dwarfing rootstocks F&A 418 and #23 and standard rootstock #24. All trees were 13 or 17 years old. Values are means of 3–5 trees. In each column, different letters indicate significant differences ($P \leq 0.05$). B: Canopy volume (m^3) of F&A 418, #24, #23 and 'Flying Dragon' citrus budded on commercial citrus rootstock Carrizo citrange. All trees were 17 years old when measured. In each column, different letters indicate significant differences ($P \leq 0.05$, $n \geq 3$). 'Flying Dragon' is a classic dwarfing citrus rootstock.

	Canopy volume (m^3)	
	13 years	17 years
A: *Rootstock*		
F&A 418	2.68 a	3.53 a
#23	2.41 a	3.05 a
#24	8.01 b	13.88 b
B: *Scion*		
F&A 418		18.62 a
#24		24.87 a
#23		23.66 a
Flying Dragon		3.05 b

17-year-old Navelina trees budded on F&A 418, #23 and #24 was 3.53, 3.05 and 13.88 m^3, respectively. The increase in canopy size from 13 to 17 years old was greatest in trees budded on the #24 rootstock, which grew about 6 m^3 canopy^{-1}, whereas the canopies of dwarfed trees on F&A 418 and #23 grew about 0.85 and 0.64 m^3 canopy^{-1}, respectively (Table 1A).

The dwarfing effect of the F&A 418 and #23 genotypes was observed only when these rootstocks were used (Table 1B). In contrast, 17-year-old trees composed of the F&A 418, #24 and #23 scions and the commercial citrus rootstock, Carrizo citrange, reached standard canopy volumes (18.62, 24.87 and 23.66 m^3, respectively) (Table 1B). This behavior differed from that of 'Flying Dragon,' a classical dwarfing citrus rootstock that also shows a dwarfed phenotype when budded on Carrizo (canopy volume of 3.05 m^3 under the same conditions; Table 1B).

Spring sprouting of the three graft types (F&A 418, #23 and #24) was similar for the parameters studied (Table 2). Flowering intensity was slightly higher in #23, but F&A 418 showed values similar to those of the standard rootstock #24 (data not shown). The main differences among graft types were observed during summer–autumn sprouting, and included sprouting ability (number and total length of new shoots per unit of canopy area). The rootstock F&A 418 induced the lowest values (116 shoots m^{-2} and 427 cm m^{-2}), followed by #23 (197 shoots m^{-2} and 1066 cm m^{-2}) and #24 (349 shoots m^{-2} and 1986 cm m^{-2}) (Table 2).

Hormonal applications on latent buds

Benzyladenine (BA) application significantly increased sprout number in #23 compared with the control treatment (21 versus 5). However, total sprout length in the BA treatment in #23 was much less than for the control treatment in #24 (38.30 versus 86.20 cm), which had a similar sprouting ability (21 versus 22%) (Table 3). Therefore, the stimulation of sprout number by BA did not completely counteract the restrictive effect of rootstock #23 during summer vegetative growth. Furthermore, BA had no detectable effect on sprouting in either F&A 418 trees or #24 trees. In both graft types, sprout number did not increase significantly in response to BA applications (Table 3), although total vegetative growth of #24 increased relative to the control. The IAA and GA_3 treatments had minor effects on sprouting and vegetative growth. The GA_3 application caused a threefold reduction in the number of buds that sprouted in #24 relative to the control treatment (9 versus 27 buds) (Table 3).

The dwarfing rootstocks did not reduce GA concentrations in vegetative shoots appreciably. Concentrations of active GA_1 ranged between 3.5 and 5.0 ng g_{DW}^{-1} in these trees (Table 4), a difference that does not appear to account for the large differences in tree size (cf. Talon et al. 1990).

Reproductive growth

The F&A 418 rootstock induced the highest fruit yield efficiency (7.19 kg m^{-3} canopy), followed by rootstocks #23 (4.75 kg m^{-3}) and #24 (2.41 kg m^{-3}) (Table 5). The number of fruits per unit of canopy volume was also higher for the dwarfing rootstocks than for the standard rootstook. Mean fresh mass per fruit was significantly higher for F&A 418 (234.84 g

Table 2. Number and length of vegetative sprouts produced in the spring and summer–autumn periods for Navelina citrus budded on dwarfing rootstocks F&A 418 and #23 and standard rootstock #24. Values are the mean of measurements taken over two consecutive years. In each column, different letters indicate significant differences ($P \leq 0.05$, $n \geq 3$).

Season	Rootstock	No. of sprouts per m^{-2} canopy	Total length (cm m^{-2} canopy)
Spring	F&A 418	401 a	1742 a
	#24	407 a	2422 a
	#23	350 a	1776 a
Summer–autumn	F&A 418	116 a	427 a
	#24	349 c	1986 c
	#23	197 b	1066 b

Table 3. Number of sprouts and total length of summer shoots that emerged from hormone-treated buds of adult Navelina citrus budded on dwarfing hybrid rootstocks F&A 418 and #23 and standard rootstock #24. All buds from 20 terminal twigs (80–100 buds) per tree were used in each treatment. Abbreviations: BA = 6-benzyladenine; GA_3 = gibberellic acid; and IAA = indoleacetic acid.

Rootstock	Treatment	No. of sprouts per 20 twigs	Total length of shoots (cm)
F&A 418	BA	3	7.70
	IAA	0	–
	GA_3	0	–
	Control	1	2.00
#24	BA	30	131.40
	IAA	20	69.50
	GA_3	9	28.30
	Control	27	86.20
#23	BA	21	38.30
	IAA	8	27.00
	GA_3	2	24.69
	Control	5	14.80

Table 4. Gibberellin concentrations in shoots of summer sprouts of Navelina citrus budded on dwarfing rootstocks F&A 418 and #23 and standard rootstock #24. Samples were taken when shoots were growing actively. The GA determinations were repeated in successive extractions with similar trends.

Rootstock	Gibberellin concentration (ng g_{DW}^{-1})				
	GA_{19}	GA_{20}	GA_1	GA_8	GA_{29}
F&A 418	20.9	16.7	4.0	12.7	27.7
#24	23.2	22.4	5.0	12.6	27.8
#23	21.3	15.0	3.5	8.3	27.0

Table 5. Yield and fruit mass of Navelina citrus budded on dwarfing rootstocks F&A 418 and #23 and standard rootstock #24. Sixty fruits per graft type were used for fruit mass mesurements. In each column, different letters indicate significant differences ($P \leq 0.05$, $n \geq 3$).

Rootstock	No. fruits per m^{-3} canopy	Fruit mass (kg m^{-3} canopy)	Mean fruit fresh mass (g $fruit^{-1}$)
F&A 418	30.93 b	7.19 b	234.84 c
#24	12.01 a	2.41 a	204.66 b
#23	32.93 b	4.75 ab	134.04 a

$fruit^{-1}$) than for #24 (204.66 g $fruit^{-1}$) and #23 (134.04 g $fruit^{-1}$) (Table 5).

Thinning experiment

In trees of Navelina orange on dwarfing rootstocks #23 and F&A 418, early thinning of fruits (before the beginning of the June fruit drop) increased the number of new shoots and the total shoot length per m^2 of canopy surface (Table 6). Thus, the total length of vegetative growth in defruited F&A 418 was about 1000 cm m^{-2} versus 132 cm m^{-2} in fruit-bearing F&A 418. The corresponding values for defruited and fruit-bearing #23 were 841 and 486 cm m^2, respectively.

Photosynthetic measurements

We measured *A* in previous-year leaves (old leaves) and in current-year leaves (young leaves) on leafy shoots and on shoots bearing a single fruit. In general, *A* ranged between 4 and 8 μmol CO_2 m^{-2} s^{-1} (Table 7). Young leaves had higher *A* than old leaves, but no significant differences were induced by the rootstocks or by the presence of fruit (Table 7).

Carbohydrate analysis

Sucrose concentration of Navelina fruits increased during development (Table 8A). A similar trend was found for total soluble carbohydrates (data not shown). Sucrose concentrations were similar across rootstock types on June 10 (about 19 mg g_{DW}^{-1}) and July 28 (92–106 mg g_{DW}^{-1}). However, after summer sprouting (September 4), sucrose concentration was significantly higher in fruits on the dwarfing rootstocks compared with fruits on the standard rootstock (Table 8A). This increase in sucrose concentration was observed not only on a dry mass basis, but also when expressed per volume of canopy, indicating that fruits from dwarf trees contained more sucrose as well as producing higher fruit yields. After summer sprouting, sucrose concentration in fibrous roots was also higher in dwarfing rootstocks than in the standard rootstock (Table 8B). The ratio between sucrose concentrations in fibrous roots and in fruits for each period was correlated for each citrus rootstock (Figure 1). It was also apparent that, after summer sprouting, both of the dwarfing rootstocks had increased amounts of sucrose in roots and fruits, whereas the standard rootstock showed decreased sucrose concentratons in roots.

There was little variation in starch concentrations in fibrous roots between the start of spring sprouting (February 24) and the end of the June drop period (June 25). A decrease in root starch concentration occurred after the summer sprouting (data not shown).

Table 6. Effect of defruiting on summer vegetative sprouts in Navelina citrus budded on dwarfing rootstocks F&A 418 and #23. In general, $n \geq 3$–5 except that only one tree of each dwarfing rootstock was defruited.

Rootstock	Shoot no. m^{-2} canopy	Total shoot length (cm m^{-2} canopy)
F&A 418	40.7 ± 21.0	132.5 ± 83.2
F&A 418 defruited	350.0	1000.1
#23	76.7 ± 9.00	486.1 ± 128.8
#23 defruited	150.0	841.7

Table 7. Photosynthetic rates (900 μmol m^{-2} s^{-1} PFD) of 1-year-old leaves (old leaves) and of fully expanded current-year leaves (young leaves from the spring flush) of field-grown Navelina citrus budded on dwarfing rootstocks F&A 418 and #23 and standard rootstock #24. Photosynthesis was measured between 0800 and 1000 h during the summer. Means ($n \geq 15$) followed by the same letter do not differ significantly at $P \leq 0.05$.

Rootstock	Old leaves	Young leaves on vegetative shoots	Young leaves on shoots bearing a single fruit
F&A 418	5.73 a	7.01 b	6.46 b
#24	4.68 a	7.41 b	6.12 b
#23	4.45 a	6.57 b	6.52 b

Discussion

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Table 8. A: Sucrose concentrations (mg g_{DW}^{-1}; g m^{-3} of canopy) in fruits of Navelina citrus budded onto dwarfing rootstocks F&A 418 and #23 and standard rootstock #24. Means ($n \geq 3$) within each column followed by different letters differ significantly at $P \leq 0.05$. B: Sucrose concentrations (mg g_{DW}^{-1}) in fibrous roots of dwarfing rootstocks F&A 418 and #23 and standard rootstock #24, grafted with Navelina citrus. Means ($n \geq 3$) within each column followed by different letters differ significantly at $P \leq 0.05$.

A:	Sucrose concentration in fruits			
	mg g_{DW}^{-1}			g m^{-3} canopy
Rootstock	June 10	July 28	September 4	September 4
F&A 418	19.95 a	106.03 a	142.50 b	92.07
#24	18.20 a	101.31 a	106.86 a	25.73
#23	19.91 a	92.91 a	134.32 b	51.15

B:	Sucrose concentration in fibrous roots (mg g_{DW}^{-1})		
Rootstock	February 24	June 25	September 4
F&A 418	24.4 a	30.2 b	37.4 b
#24	23.9 a	28.5 b	26.7 a
#23	23.7 a	24.7 a	34.1 b

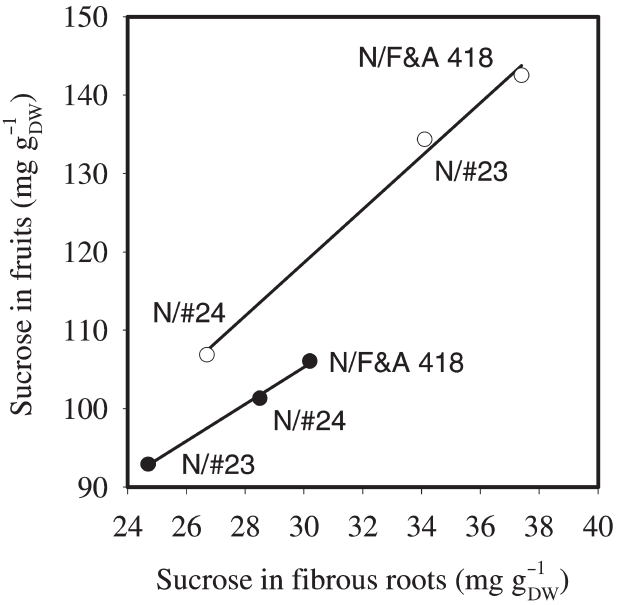

Figure 1. Relationship between sucrose concentration in fibrous roots and fruits in Navelina citrus budded on dwarfing rootstocks F&A 418 and #23 and standard rootstock #24. Samples were taken after the June fruit drop, either just before (●) or after (○) vegetative sprouting in summer.

trees budded on three hybrid citrus rootstocks revealed major growth differences that influenced tree size during the first summer after grafting. The growth differences were mainly attributable to the contribution of summer–autumn sprouts (Table 2). Summer sprouting has previously been suggested to determine the vegetative growth of citrus trees (Spiegel-Roy and Goldschmidt 1996). The data in Table 1B also indicate that these dwarfing effects are likely based on physiological components rather that on genetic factors, because both hybrids, in contrast to 'Flying Dragon' (a "true" citrus dwarf), grow normally on Carrizo citrange. Among possible physiological components, two main causes of growth reductions in citrus are hormonal deficiencies (Talon et al. 1992) and nutritional shortages (Gomez-Cadenas et al. 2000).

To examine the involvement of hormones in the dwarfing phenomenon, we determined the effects of hormone application to latent buds on summer sprouting. Overall, our data (Tables 3 and 4) indicated that growth hormones were not directly involved in the dwarfing mechanism for these rootstocks. It has been suggested that root-produced cytokinin has an essential role in bud break (Jones 1973, Cutting et al. 1991) or shoot growth (Young 1989), particularly when rapid leaf expansion is taking place (Hendry et al. 1982). A single application of BA was enough to promote growth of buds of *Pisum sativum* L. (Pillay and Railton 1983), whereas in other species, subsequent application of hormones such as auxins or gibberellins (Phillips 1975) or sucrose (Giladi et al. 1977) was also needed. We found that applications of BA on latent buds produced similar results in the standard control rootstock and the #23 rootstock (Table 3). Hence, it is possible that there is a deficiency in endogenous cytokinins arriving from roots for these two graft types, although the shortening of shoots and the generally abnormal appearance of the shoots after BA treatment suggest that there are other limiting factors. We note that the endogenous GA concentration in vegetative shoots tended to be lower in dwarfed trees than in control trees (Table 4). However, we conclude that reduced concentrations of gibberellins and IAA do not appear to be the primary causes of dwarfing because their exogenous application did not completely restore vegetative growth to the level observed in the control rootstock trees.

An alternative to the hormonal deficiency mechanism could be alterations in nutritional status. Most of our findings are compatible with the proposal that the pool of carbohydrates for summer shoot growth in dwarf trees is restricted. Thus, in response to defruiting, vegetative summer sprouting was markedly increased and comparable to that of the control trees (Table 6). Because photosynthetic ability was similar for all three rootstocks (Table 7), it is possible that competition for carbohydrates between fruit and vegetative development was responsible for the reduced vegetative growth of the dwarfed trees. This assumption is supported by the finding that fresh fruit mass per unit of canopy volume was higher in dwarfed trees than in standard trees (Table 5). Furthermore, before summer sprouting (July 28), sucrose concentrations in fruits were similar for the three tree types, whereas at the end of the vegetative development (September 4) the sugar concentration was significantly greater in Navelina scions on the dwarfing rootstocks (Table 8A). Fruit carbohydrate concentrations per unit canopy volume were also higher in dwarf trees than in standard trees, even though the dwarf trees had higher yield efficiency (Table 8A). Thus, the dwarf phenotypes had large effects on both reproductive development and carbohydrate accumulation in fruit. Although it has been known for some time that there is a strong dominance of fruit development over vegetative development in citrus (Lenz 1967), it is not known how this successful competition by the fruits over vegetative shoot growth comes about. Furthermore, we have recently demonstrated that the presence of high carbohydrate concentrations in citrus fruits is associated with increased fruit set, subsequent fruit growth and general increases in all aspects of reproductive development (Iglesias et al. 2002).

Sucrose concentrations in fibrous roots after the summer sprouting period were also lower in the standard rootstock than in the dwarf rootstocks (Table 8B, Figure 1). This difference is probably associated with the growth of fruit trees, roots and shoots in flushes that generally alternate from one flush to another (Lockard and Schneider 1981, Bevington and Castle 1985). However, the finding that the standard rootstock contained lower concentrations of sucrose in both fruits and roots may indicate that most of the carbohydrate pool is being utilized for vegetative growth in this less fruitful tree type compared with the dwarfing rootstocks.

Overall, our results suggest that the dwarfing mechanism of F&A 418 and #23 is related to a competition for carbohydrates between vegetative and reproductive growth. This interpretation is incompatible with the idea that an inhibitor released

from the dwarfing roots blocks vegetative growth, because the defruiting experiment demonstrated that vigorous vegetative growth can occur on the dwarfing rootstocks. Similarly, the normal vegetative and reproductive growth of F&A 418 and #23 on non-dwarfing roots and when these rootstocks are self-rooted invalidates the suggestion that fruits secrete a factor the inhibits sprouting.

We conclude that the mechanism responsible for the dwarfing effect induced by the F&A 418 and #23 rootstocks is associated with the distribution of carbohydrates between reproductive and vegetative development during summer–autumn sprouting. Fruit development after the June fruit drop markedly restricts vegetative growth, thereby yielding dwarfed trees. The pattern of assimilate distribution induced by these rootstocks is probably a pivotal component of a complex dwarfing mechanism that likely includes phytohormones, especially cytokinins, as secondary factors in the control mechanism of bud sprouting and elongation.

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