

Effect of gibberellic acid (GA₃) on rootstock on the evolution of the mandarin tree induction "Nadorcott" in the Atlantic Coastal Zone, Northern Morocco

Abstract: An application of gibberellic acid (GA₃) was applied to different doses (0; 15; 30 ppm) on **Nadorcott** trees transplanted on five rootstocks (*Macrophylla citrus*, *Carrizo citrange*, *Troyer citrange*, *C35 citrange* and *Swingle citrumelo*) during the fruits coloration shifting. A monitoring of the evolution of the floral induction by the counting of leaves and the flowers/ fruits for the sensitive stages: flowering, fruiting and end physiological drop, with physico-chemical analyzes of the leaves at the physiological dropping stage, of caliber during the magnification stage. The shoots on the *Swingle citrumelo* rootstock had more flowers than the Citranges and even more than the *Macrophylla citrus*. This classification is still valid for fruits during fruiting, results of a standard drop for the different rootstocks with the dominance of leaved inflorescence with unique flower. On the other hand, the physiological drop was different from rootstock to the other, more accentuated in *Swingle citrumelo*, *Troyer* and *Carrizo citrange*, and moderate in *C35 citrange* and *Macrophylla citrus*. While the GA₃ treatment did not significantly affect either the floral induction or the drop in its two phases. It is noted that with the evolution of the flowering there is a significant positive effect of the treatment and of the GA₃ dose on the fruits hooking, with a 23% increase for the dose of 30 ppm GA₃ compared to the control. However, the variation in size is better for fruits on leaved inflorescence with unique flower, as well as on the *Macrophylla citrus* rootstock, followed by Citrange (C35 followed by Carrizo and Troyer), and finally *Swingle citrumelo* (10% less in comparison with *Macrophylla citrus*).

Key words: **Nadorcott**, rootstock, gibberellic acid, fruit coloring, floral induction, type of inflorescence, caliber.

INTRODUCTION

In Morocco, the citrus fruit sector plays an important socio-economic role and it covers in 2016 a surface area of 124.5 kha, of which 109.8 kha is in production. It produces 2.36 MT on average and exports 0.5 MT the equivalent of almost 3 billion DH / year in foreign currency [Maroc Citrus, 2016]. However, the citrus sector generates nearly 21 million working days per year (60% in orchards and 40% in agribusiness) [MAPM, 2016].

The main varieties of Moroccan citrus fruits are Clementine (39%), Maroc Late (29%), Navel (22%), Half season oranges (5%) and other varieties [MAPM, 2016]. The Nadorcott variety gained in importance, with an area of 6.6 kha, of which 4.6 kha in production, with a production of 0.18 MT mainly destined for export [Maroc Citrus, 2016].

This study focuses on the « **Nadorcott** » highly efficient mandarins, with high quality fruits, without seeds, under certain isolation conditions, easy to peel, and whose maturity is later than that of Clementine, which can be spread out between the end of December until mid-March [Nadori, 2004; Nadori, 2005].

In order to spread this harvesting and commercialization periods, there are many means that can be used such as: the diversification of rootstocks [Fellers, 1985], the spraying of the gibberellins during the period spreading between the end of September and the beginning of July. [Coggins, 1973; Garcia-Luis and al., 1985; El Otmani and al., 1990].

The treatment aiming to delay the harvesting of fruits right before the fruit twist [Agusti and al., 1981a and b; El Otmani and Tejjini, 1990a; El Otmani and al., 1990b; El Otmani and al., 2000a] happens before or in the beginning of the presumed period of the installation of the floral induction process [Iwahori and Oohata, 1981], occurs in sub-tropical zones during winter as a response to low temperatures [Moss, 1969]. This fact is even more important than the effect of gibberellic acid has on the floral induction, a marked influence depending on the concentration and the application period [Guardiola and al, 1977; Iwahori and Oohata, 1981], and inhibiting effect over the flowering of the next spring [Guardiola and al., 1982; Wilkie and al., 2008; Bangerth, 2009].

Several studies have shown that floral induction is influenced by environmental conditions and endogenous factors [Garcia-Luis and al., 1986; Southwick and al., 1986; Garcia and al., 1995; Valiente and al., 2004; Gravina and al., 2012; Takeno, 2012]. The axillary buds that have undergone floral induction (physiological process)

followed by floral differentiation (formation of drafts of the flower organs) will give the spring inflorescences (flowers and floral shoots). The buds that have not undergone floral induction will give vegetative shoots [Davenport, 1990].

The picking period is even more reduced with the use of renowned rootstock for their efficiency and the production of big caliber fruits (e.g. *Macrophylla citrus*, *Volkameriana lemon*) [Velarde, 1982; Salazar-Saenz, 2001; Agusti, 2003; Orduz and al, 2009]. By using these rootstocks, the quality declines (low proportion of acids and sugars with a rather tasteless savor) [Forner, 1979; Forner and al, 1996]. However, the hydric stress, under certain conditions, allows a significant amelioration of fruits quality by the increase in dry soluble and titrable acidity [Sánchez-Blanco and al., 1989; González-Altozano and Castel, 2000; Verreynne and al., 2001; García-Tejero and al., 2010; Navarro and al, 2010; Ballester and al., 2013; Pérez- Pérez and al., 2008, 2009, 2014]. Moreover, the coloration and maturity are more advanced [Navarro and al., 2015], the **Nadorcott** fruits in the coastal Atlantic zone, must be harvested between mid-December and mid-January, which is almost the same time as the clementine [Nadori, E.B., unpublished data]. Within these coastal zones and the cited above rootstocks, a delayed picking gives as a result a rapid deterioration of fruits quality, especially after raindrop.

On the other side, in adequate rootstock, the maturity of the **Nadorcott** variety is reached in January and the picking could last until mid-February. The fruits remain stiff, and have a satisfying quality [Errachid, R., 2014].

This article is a study on the interactions between the rootstock and the application of gibberellic acid, at the stage of coloration turning over the evolution of floral induction of the **Nadorcott** variety. These interactions have never been studied under the Moroccan conditions.

MATERIALS AND METHODS

The studied testing has been carried out during the 2011-2012 campaign in the experimental station of the SEBNAK domain (Maroc-Merchich-Lambert Nord: X=414 559m; Y=464 524m; Z=49 m), in BAHARA OULED AYAD, which is within the Atlantic coastal zone of GHARB in Morocco. On a sandy soil (Table 1) and a good physio-chemical quality of irrigation water (Table 2).

Table 1: Physico-chemical soil properties.

	Soil (0-30 cm)	Soil (30-60 cm)
Argil (%)	-	-
Silt (%)	5,00	5,00
Sand (%)	95,00	95,00
pH	7,64	7,87
EC (μS/cm)	240,43	177,13
CaCO ₃ (%)	0,50	0,50
MO (%)	1,10	0,74
N Dumas (ppm)	562,87	395,53
P (ppm)	34,87	52,60
Ca (meq/100g)	4,02	4,46
Mg (meq/100g)	0,69	0,57
K (meq/100g)	0,17	0,10
Na (meq/100g)	0,89	0,83

Table 2: Physico-chemical properties of irrigation water.

NO ₃ ⁻	SO ₄ ²⁻	HCO ₃ ⁻	Cl ⁻	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺
meq/l	meq/l	meq/l	meq/l	meq/l	meq/l	meq/l	meq/l
0,95	0,24	2,11	1,55	3,57	0,43	1,7	-
pH	EC	B	Fe	Mn	Cu	Zn	
	μS/cm	ppm	ppm	ppm	ppm	ppm	
7,85	509	-	-	-	-	-	

The region has only received 227mm/year of raindrop, with a soft weather, not exceeding 41°C during summer, with a limited risk of frost during winters (Figure 1).

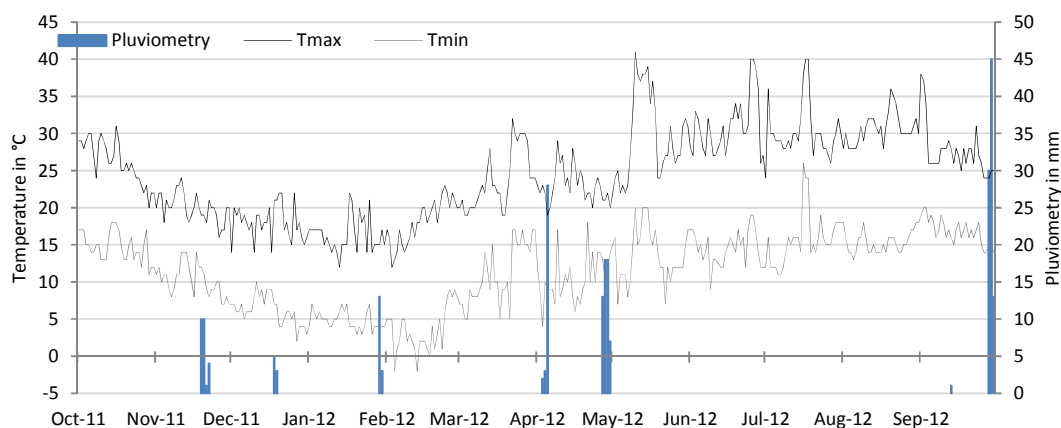


Figure 1: Weather data of the experimental station in SEBNAK domain.

The plantation is five years old, with a density of 2x6 m (833 trees/hectare) and around 3,5m in height. The seeds are transplanted over 5 rootstocks, which are: *Macrophylla citrus* (MC), *Carrizo citrange* [*C. sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] (CC), *Troyer citrange* (TC), *C35 citrange* [*C. sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] (C35C) and *Swingle* or *4475 citrumelo* [*C. paradisi* Macf. x *P. trifoliata* (L.) Raf.] (SC).

Monitoring of floral induction

The experimental plan is a 3-level split plot, with a complete random block disposition in the first level (Figure 2), where the rootstock is the main factor, GA₃ foliar spraying as secondary factor and the stage is the tertiary factor, using 6 complete random blocks. However, the experimental unit is composed of 4 trees, and only the middle 2 are taken into account, as the two trees in the extremities are discarded due to the boundary effect. Three doses of GA₃ (Fengib, Sipcam Inagra S.A., Valencia, Spain, 0,5% p/v d'GA₃) have been tested (0, 15, 30 ppm) in the stage of fruit coloration turning (October 26th, 2011), mixed with a non-ionic admixture (Agral 90, Syngenta S.A., Rabat, Morocco, 90% Nonyl phenol ethoxylated) with a rate of 0.5 l /1000 l. Furthermore, five branches were chosen on each orientation, East and West, at the height of a normal man, for the two central trees...

Bloc 05					Bloc 03					Bloc 01				
R ₂ T ₂	R ₅ T ₂	R ₄ T ₂	R ₁ T ₂	R ₃ T ₂	R ₄ T ₃	R ₅ T ₃	R ₃ T ₃	R ₁ T ₃	R ₂ T ₃	R ₅ T ₂	R ₄ T ₂	R ₃ T ₂	R ₂ T ₂	R ₁ T ₂
R ₂ T ₁	R ₅ T ₁	R ₄ T ₁	R ₁ T ₁	R ₃ T ₁	R ₄ T ₁	R ₅ T ₁	R ₃ T ₁	R ₁ T ₁	R ₂ T ₁	R ₅ T ₃	R ₄ T ₃	R ₃ T ₃	R ₂ T ₃	R ₁ T ₃
R ₂ T ₃	R ₅ T ₃	R ₄ T ₃	R ₁ T ₃	R ₃ T ₃	R ₄ T ₂	R ₅ T ₂	R ₃ T ₂	R ₁ T ₂	R ₂ T ₂	R ₅ T ₁	R ₄ T ₁	R ₃ T ₁	R ₂ T ₁	R ₁ T ₁
Bloc 06					Bloc 04					Bloc 02				
R ₂ T ₃	R ₅ T ₃	R ₄ T ₃	R ₁ T ₃	R ₃ T ₃	R ₄ T ₁	R ₅ T ₁	R ₃ T ₁	R ₁ T ₁	R ₂ T ₁	R ₅ T ₁	R ₄ T ₁	R ₃ T ₁	R ₂ T ₁	R ₁ T ₁
R ₂ T ₂	R ₅ T ₂	R ₄ T ₂	R ₁ T ₂	R ₃ T ₂	R ₄ T ₂	R ₅ T ₂	R ₃ T ₂	R ₁ T ₂	R ₂ T ₂	R ₅ T ₃	R ₄ T ₃	R ₃ T ₃	R ₂ T ₃	R ₁ T ₃
R ₂ T ₁	R ₅ T ₁	R ₄ T ₁	R ₁ T ₁	R ₃ T ₁	R ₄ T ₃	R ₅ T ₃	R ₃ T ₃	R ₁ T ₃	R ₂ T ₃	R ₅ T ₂	R ₄ T ₂	R ₃ T ₂	R ₂ T ₂	R ₁ T ₂

R₁: *Macrophylla citrus*; R₂: *Carrizo citrange*; R₃: *Troyer citrange*; R₄: *C35 citrange*; R₅: *Swingle citrumelo*;
T₁: 0 ppm d'GA₃; T₂: 15 ppm d'GA₃; T₃: 30 ppm d'GA₃;

Figure 2. Experimental Plan.

The comments are done in three periods: Flowering (FLO): April 8th, 2012, Fruiting (FRU): May 12th, 2012. End of physiological drop (EPD): 16th July, 2012. We count on each shoot of last summer, the number of leaves, flowers or fruits for every inflorescence.

Monitoring of the physiological drop

The studied factor is the rootstock in a completely random disposition with 6 repetitions. The elementary parcel contains two trees. Five rootstocks have been studied (MC, CC, TC, C35C, SC). The test has been realized between (FRU) and (EPD).

The samples have been taken on a daily basis, by counting the dropped fruits, which are recovered using a net installed under the two threes, with heightened boundaries in order to avoid any interferences with neighboring trees.

Nutritional state in the physiological drop stage

The test has only used four blocks (1, 2, 3 and 4) as shown in the plan in figure 2, this is a Randomized Complete Block Design. The samples are taken in the physiological drop stage (May 21st, 2012), at the average height of a man around the tree, with a representative sample of 100 leaves, on mature 3 months old shoots (spring shoots).

Once taken, the samples are directed towards the laboratory, where they are cleaned using distilled water (Milli-Q Water), drained, weighted, dried using a stove (Labtech) at 80°C for 8 hours, crushed using a hammer mill, sift using a 2mm sieve and then re-weighted. A 1-g porcelain crucible is weighted and its concentration of N analyzed following the Dumas process: this method consists of dry combustion up to 950°C, using oxygen as an accelerator. The products of this combustion (H₂O, CO₂, SO₂, NO_x, N₂) are filtered. NO_x is reduced into N₂ using copper, and is swept by gaseous helium through a sensor with thermal conductivity where the concentration is measured (DNA 701 VELD). Another one is introduced into an oven (Labtech) at a temperature of 450°C. After a calcination during 2 hours, we remove the crucible and we let it cool down at ambient temperature, then we add a few drops of Milli-Q water to fix the ashes, then we add 2ml of concentrated nitric acid. After filtration, we take the solution into a 100ml flask and fill it up with Milli-Q water. We transverse the obtained solution into a plastic bottle. The obtained solution is to be used for an analysis of chloride by spectrophotometry (Jenway), of phosphorus by colorimetry (Jenway), and of metals by spectrometry of inductive plasma atomic emissions (Agilent Technology 700 ICP OES Series). A computer program allows us to compare every new result to the referential values stored in the database. The measures are taken in an inorganic laboratory, while determining the chemical compositions of the foliar samples (N, P, K, Ca, Mg, Cl, Na, Zn, Mn, Cu, Fe and B).

Monitoring of the caliber

By the end of the physiological drop (July 16th, 2012), we have identified the type of 270 inflorescences with flowers/fruits [Sauer, 1951; Moss, 1969; Agusti, 2003; Rebolledo, 2012], of three trees for five rootstocks (MC, CC, TC, C35C, SC), following the counting methodology.



VI: Vegetative Inflorescence; LSFI: Leafy Single Flowered/Fruited Inflorescence; LMF: Leafy Multi-Flowered/Fruited Inflorescence; LsSFI: Leafless Single Flowered/Fruited Inflorescence; LsMFI: Leafless Multi-Flowered/Fruited Inflorescence.

Inflorescence types.

Under the experiment's conditions, we note the dominance of inflorescences with a unique fruit (Table 3).

Table 3: Effect of rootstock on inflorescence type.

Inflorescence type	Rootstock				
	TC	C35C	SC	CC	MC
LSFI	78% A ^z a ^y	75% Aa	75% Aa	77% Aa	77% Aa
LMFI	0% Ca	0% Ca	0% Ca	0% Ca	0% Ca
LsSFI	22% Ba	25% Ba	25% Ba	23% Ba	23% Ba
LsMFI	0% Ca	0% Ca	0% Ca	0% Ca	0% Ca

^z for a given rootstock, the percentages followed by the same capital letter are not significantly different (SNK, 5%).

^y for a given type of inflorescence, the percentages followed by the same tiny letter are not significantly different (SNK, 5%).

The framing method consists of counting the number of each inflorescence type with the help of a square frame with the length of a side being 33,3 cm. The results show also that only LSFI and LsSFI carry the production of the current year (Table 4).

Table 4: Effect of the rootstock and AG on the inflorescence type.

Dose GA ₃	Inflorescence Type	ROOTSTOCKS				
		TC	C35C	SC	CC	MC
0 ppm	LSFI	95%	90%	96%	100%	95%
	LsSFI	5%	10%	4%	0%	5%
15 ppm	LSFI	100%	100%	97%	100%	100%
	LsSFI	0%	0%	3%	0%	0%
30 ppm	LSFI	100%	89%	88%	88%	100%
	LsSFI	0%	11%	13%	13%	0%

Thereby, for every experimental unit, we follow the caliber, with the help of a numbered sliding foot, of 10 fruits per direction East and West, for the two inflorescence types: LSFI and LsSFI. The caliber recordings have started after the end of the physiological drop (August 8th, 2012) and have been measured regularly every week until the end of autumn (December 29th, 2012). The experimental plan is a slit plot (Figure 2) with 4 completely random blocks (1,2,3 and 4).

The relative growth rate (RGR) has been calculated using the following equation [1995, Tattini]:

$$RGR = \frac{D2 - D1}{t2 - t1}$$

RGR is the relative growth rate in mm/day, **D** is the measured value of the growth parameter (equatorial diameter of the fruit) at instant **t** (in mm) and **t** is the time between measures in days.

The statistical analysis has been carried using the help of the IBM SPSS Statistics program (21st version; IBM Corporation), we use the Duncan test for the means comparisons at P=5%.

RESULTS

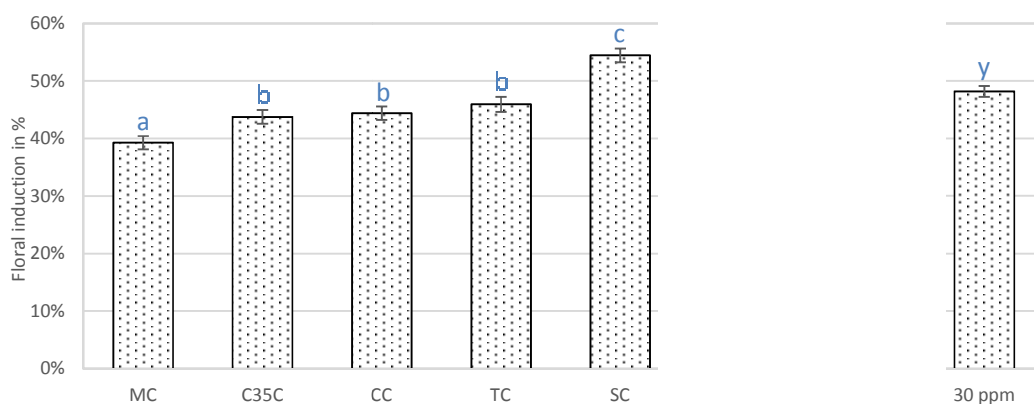
Monitoring of floral induction

The Floral Induction variable (FI) is calculated by the formula:

$$FI (\%) = \frac{\text{Number of inflorescences with flowers}}{\text{Total number of inflorescences}} \times 100$$

The results are expressed as a percentage in order to compensate for the size differences between branches chosen for the counting.

The variances analysis shows that there is no effect of the interaction of the rootstock and the GA₃ treatment over the floral induction. However, it shows a very significant effect of both rootstocks and GA₃.



The reported sticks of the same tiny letter are not significantly different (Duncan, 5%).

Figure 3. Effect of rootstock and GA₃ treatment on floral induction by percentage.

Table 5: Rootstock and GA₃ treatment effect over the inflorescence type and flowering.

Parameters		Rootstock						GA ₃ Treatment			
		SC	TC	CC	C35C	MC	Sig	0 ppm	15 ppm	30 ppm	Sig
% of inflorescences	Vegetative inflorescence	45% a	53% b	55% b	56% b	61% c	***	57% a	53% b	51% b	**
	Floral inflorescences	55% a	47% b	45% b	44% b	39% c	*	43% a	47% b	49% b	***
	Mixt inflorescences	43% a	37% b	35% bc	35% bc	32% c	***	32% a	38% b	39% b	***
	Single flowered	41% a	35% b	34% b	34% b	31% b	**	31% a	36% b	37% b	***
	Multi-flowered	2%	2%	1%	2%	1%	NS	1%	1%	2%	NS
	Generative inflorescences	12% a	10% ab	10% ab	8% bc	7% c	*	10%	9%	9%	NS
	Single flowered	9%	8%	8%	7%	6%	NS	8%	8%	8%	NS
	Multi-flowered	2%	2%	1%	1%	1%	NS	2%	1%	1%	NS
Flower's number/ 100 inflorescence	Floral inflorescences	63 a	54 b	48 cd	49 bc	42 d	**	48 a	51 ab	55 b	*
	Mixt inflorescences	48 a	41 b	37 cd	39 bc	34 d	***	35 a	40 b	44 c	***
	Single flowered	41 a	35 b	34 b	34 b	31 b	***	31 a	36 b	37 b	**
	Multi-flowered	8	6	2	6	3	NS	4	4	6	NS
	Generative inflorescences	15	13	11	10	9	NS	13	11	11	NS
	Single flowered	9	8	8	7	6	NS	8	8	8	NS
	Multi-flowered	5	5	3	3	2	NS	4	3	3	NS

The percentages or the numbers followed by the same tiny letter are not significantly different.

Sig: Significance (Duncan: NS, *, **, *** Not significant or significant at P = 0.05, 0.01, 0.001, respectively).

Figure 3 shows that the *Macrophylla citrus* rootstock has a low floral induction, followed by the Citranges group (C35C, TC, CC) and at the end the *Swingle citrumelo* rootstock.

However, the GA₃ treatment has had a slight positive effect on the floral induction, insignificant, of the GA₃ dose. This being said, GA₃ treatment has an inhibitory effect over the flowering during the floral induction. This allows us to suggest that the floral induction process either has not yet started, or there are other factors that reversed or cancelled the inhibitory effect of GA₃.

In order to better understand each rootstock behavior and how the GA₃ treatment acts on the flowering process, we have tried to deepen our study, by examining the effect of different rootstocks alongside with GA₃ treatment on every type of flowering. The results are shown in table 5. For floral inflorescences, we notice the dominance of mixt inflorescences, notably those with a unique flower, this type being the most influenced by the rootstock and GA₃ treatment.

Our study is also interested in the flowering evolution, and more specifically the hooking rate around the end of the physiological drop, which is conditional for the production. To do so, it is better to study both the flower drop (Drop I) and the fruits drop (Drop II): physiological drop or June drop.

The variances analysis shows that there is neither a rootstock nor a GA₃ treatment effect on Drop 1, no matter the inflorescence type, while the statistical results show that there is a highly significant effect of rootstock on Drop II.

Figure 4 and table 6 show that the shoots on the *Swingle citrumelo* rootstock have had more flowers than the Citranges group and much more than the *Macrophylla citrus*. This ranking is also valid for fruits during the fruit set, result of a standard drop for the different rootstocks, with a domination from the leaved inflorescence with a unique flower. On the other side, the physiological drop is different from one rootstock to another, more pronounced on the *Swingle citrumelo*, the *Troyer citrange* and the *Carrizo citrange*, while being moderate on the *C35 citrange* and *Macrophylla citrus*. It has also led to an important hooking on the *Swingle citrumelo* and the *C35 citrange*, followed by *Macrophylla citrus* and at the end the *Carrizo* and the *Troyer citrange* rootstocks.

In general, we notice that the drop of *Macrophylla citrus* is the least one comparing with other rootstocks, especially with *Swingle citrumelo* reaching a difference of around 89%.

although there is no significant effect between GA₃ treatment and fruit drop in these two phases. It is observed that with the flowering evolution there is a positive and significant effect of treatment and of the GA₃ dose on the fruits hooking, with a 23% increase for the 30 ppm GA₃ dose compared to the control.

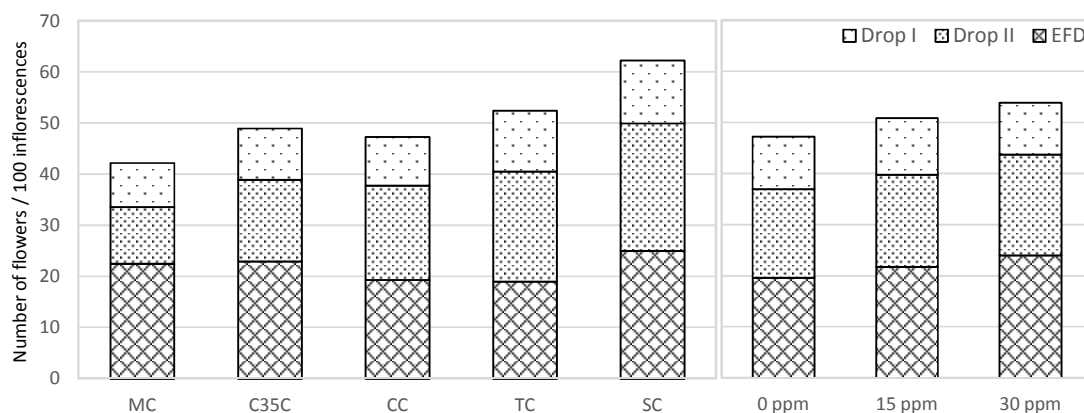


Figure 4. Effect of rootstocks and GA₃ treatments on fruit drop

Table 6: Effect of rootstock and GA₃ treatment on flowering evolution

Stages	Rootstock						GA ₃ Treatment (ppm)			
	MC	C35C	CC	TC	SC	Sig	0	15	30	Sig
FLO	42 a	49 b	47 b	52 b	62 c	**	47 a	51 ab	55 b	**
FRU	34 a	39 b	38 b	41 b	50 c	***	37 a	40 a	44 b	***
EFD	22 b	23 bc	19 a	19 a	25 c	**	20 a	22 b	24 c	***
Drop I	9 a	10 a	10 a	12 a	12 a	NS	10 a	11 a	10 a	NS
Drop II	11 a	16 b	18 bc	22 cd	25 d	**	17 a	18 a	20 a	NS

The numbers followed by the same tiny letter are not significantly different.

Sig: Significance (Duncan: NS, *, **, *** Not significant or significant at P = 0.05, 0.01, 0.001, respectively).

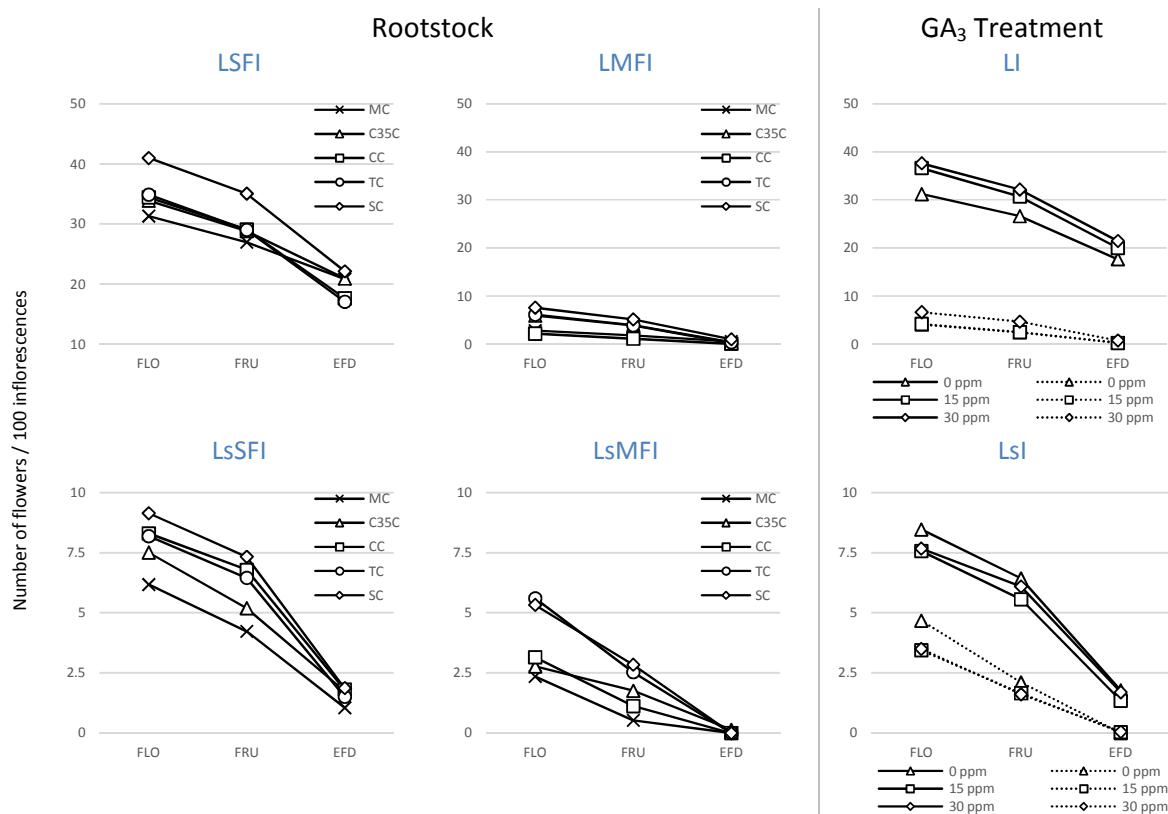


Figure 5. Evolution of the number of flower by 100 inflorescences for every GA₃ treatment and each rootstock between flowering, fruit set and physiological drop.

Under the experiment condition, and the results in Figure 5, we notice that a great number of flowers coming from multiple flowers inflorescences have the tendency to completely drop by the end of the physiological drop, which confirms the results of table 3. The flowers coming from unique flower inflorescences have the tendency to hook more.

Figure 5 show the drop rhythm during the two phases, for the whole types set of inflorescences, brought to light by the existence of two rootstock groups, the first consists of *Macrophylla citrus* and *C35 citrange*, which have a slower pace of drop, while the second group consists of *Swingle citrumelo*, *Carrizo* and *Troyer citrange*.

Monitoring of the physiological drop

The monitoring of the drop only concerns the second drop (Drop II), recognized by the physiological drop. Statistical analysis unveils a highly significant effect of the rootstock on the number of fruits per tree by the end of the physiological drop. Also, a very highly significant effect of rootstock on the percentage of the physiological drop of fruits (PFD).

The PFD variable is calculated as follows:

$$PFD(\%) = \frac{\text{NUMBER OF DROPPED FRUITS (DROP II)}}{\text{NUMBER OF FRUITS (FRU)}} \times 100$$

Figure 6 and table 7 show that the number of fruits dropper is approximately the same for all five rootstocks. In terms of percentage, we find the highest rate in the group containing TC, CC and SC, while the lowest percentages are found in MC and C35C.

The *Macrophylla citrus* could hook the maximum of fruits, followed by the *C35 citrange* and then the other rootstocks.

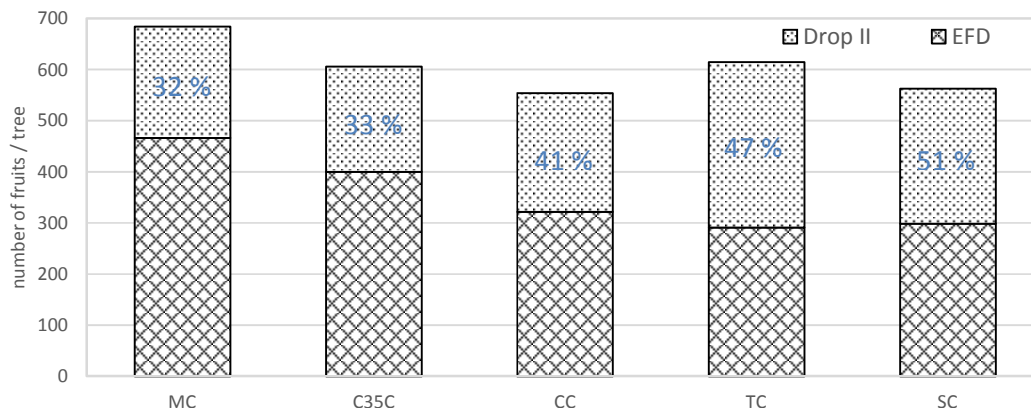


Figure 6. Effect of rootstocks on the number of fruits at physiological drop.

Table 7. Effect of the rootstock on the evolution of the number of fruits at physiological drop.

	Rootstock					
	MC	C35C	CC	SC	TC	Sig
FRU	684 a	606 a	554 a	563 a	615 a	NS
EFD	467 a	400 ab	322 bc	299 c	291 c	**
DROP II	218 a	207 a	233 a	265 a	324 a	NS
PFD	32% a	33% a	41% b	47% bc	51% c	***

The numbers followed by the same tiny letter are not significantly different.

Sig: Significance (Duncan: NS, *, **, *** Not significant or significant at P = 0.05, 0.01, 0.001, respectively).

Nutritional state in the physiological drop stage

The results of physio-chemical analysis of *Nadorcott*'s leaves on each rootstock are presented in table 8. The results show a satisfying level of most of mineral elements, except for very low foliar levels of B and Cu, and low levels of Ca, Mg and P in the *Macrophylla Citrus*; P in *Troyer* and *Carrizo citrange*, N in *Macrophylla Citrus* and *Carrizo citrange*. however, we notice very high levels of S, Zn, Ma and Na.

The Variance analysis results have not shown any significant effect neither of the interaction of rootstock-GA₃ treatment nor of the GA₃ treatment on the mineral composition of leaves. Moreover, they unveil a significant effect of rootstocks on the P and Zn, a highly significant effect on Ca and P and a very highly significant effect on Mg and S.

Table 8. Effect of rootstocks on physio-chemical composition of leaves at physiological drop.

Parameters		Rootstock					Sig
		MC	C35C	CC	TC	SC	
Ca	%	2,99 a	3,49 b	3,59 b	3,53 b	3,74 b	**
Mg	%	0,25 a	0,34 b	0,37 b	0,35 b	0,35 b	***
K	%	1,3 bc	1,28 bc	1,14 a	1,19 ab	1,36 c	**
P	%	0,16 a	0,18 b	0,18 b	0,17 ab	0,18 b	*
S	%	0,34 a	0,37 ab	0,39 b	0,38 b	0,43 c	***
N	%	2,27	2,31	2,28	2,38	2,36	NS
Zn	ppm	91 c	86 bc	67 a	71 ab	78 abc	*
Mn	ppm	101	96	75	84	86	NS
B	ppm	31	30	34	30	33	NS
Fe	ppm	138	182	174	156	147	NS
Cu	ppm	1,17	1,25	1,28	1,4	1,63	NS
Cl	ppm	399	348	471	416	429	NS
Na	ppm	675	696	803	689	739	NS

The numbers followed by the same tiny letter are not significantly different.
Sig: Significance (Duncan: NS, *, **, *** Not significant or significant at P = 0.05, 0.01, 0.001, respectively).

Table 8 shows that the *Macrophylla citrus* has low foliar levels of Ca and Mg when compared to the other rootstocks. *Carrizo citrange* displays the lowest levels when it comes to Zn and P, followed by *Troyer citrange* and *C35 citrange*, *Swingle citrumelo* and then *Macrophylla citrus*.

Caliber monitoring

The variance analysis results show a significant effect of the interaction between the rootstock and the inflorescence type in the initial date (DI: August 4 and 5, 2012). Otherwise, at the final date (DF: December 29 and 30, 2012), we find a very highly significant effect as for the rootstock as for the inflorescence type.

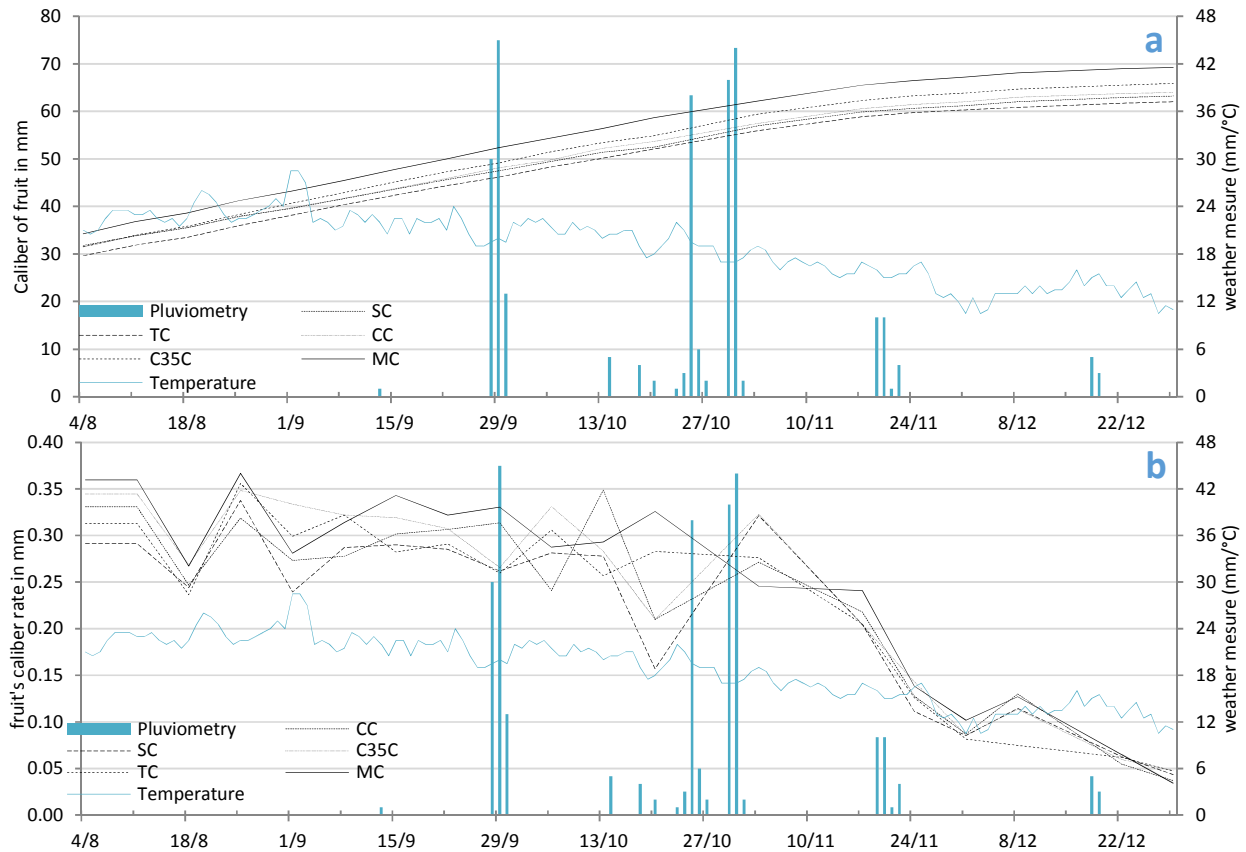


Figure 7. Fruit caliber progress (a) and fruit caliber evolution rate (b) of Nadorcott on each rootstock, alongside with weather data.

The caliber measurements at the initial date represent a result of the tree's equilibrium after the physiological drop, while the measurements at the final date represent the fruit bulking consequence, thus pushing us towards studying the caliber evolution.

The variance analysis of the caliber evolution (CE) between the initial and final dates, show a significant effect of the interaction between the rootstock and inflorescence type.

Table 9. Rootstock and inflorescence type effect on caliber evolution.

Date	Rootstock						Inflorescence type		
	MC	C35C	CC	TC	SC	Sig	LSFI	LsSFI	Sig
ID	34,2 _c	31,6 _b	31,5 _b	29,7 _a	31,8 _b	***	33,6 _b	29,9 _a	***
FD	69,2 _d	65,8 _c	64,0 _b	62,0 _a	63,2 _b	***	66,8 _b	62,9 _a	***
CE	35,0 _d	34,3 _c	32,5 _b	32,4 _b	31,4 _a	***	33,2	33,0	NS

The numbers followed by the same tiny letter are not significantly different.

Sig: Significance (Duncan: NS, *, **, *** Not significant or significant at P = 0.05, 0.01, 0.001, respectively).

The above table shows that there is a highly significant effect of the inflorescence type over the fruit caliber, between the beginning of the bulking until maturity, while there is no significant effect on the caliber evolution. We note again that the leaved inflorescences with unique flowers give bigger fruits than non-leaved inflorescences with a difference of 6,1% at the final stage.

However, the rootstock has a highly significant effect on both the fruit caliber at initial and final dates and the fruit evolution. At the initial date, we notice that the *Troyer citrange* gives the smallest caliber, followed by *Carrizo citrange*, *C35 citrange*, *Swingle citrumelo*, thus leaving the best caliber to the *Macrophylla citrus* rootstock. At the final date, we notice that the *Troyer citrange* is the one that always has the smallest caliber, this time followed by *Swingle citrumelo* then the *Carrizo citrange* and *C35 citrange*, to leave the best caliber to *Macrophylla citrus*. This ranking readjustment is mainly due to the fact that the caliber evolution rhythm differs from one rootstock to another. To know that the best caliber evolution rate come back to *Macrophylla citrus* followed by *Carrizo citrange* (*C35 citrange* followed by *Carrizo* and *Troyer citrange*), and at the end the *Swingle citrumelo* (10% less comparing to *Macrophylla citrus*).

The final caliber profile is a discounted result, the latest being commercially standardized by various groups of calibers for the *Nadorcott*, with a specific weight, as shown in the table below.

Table 10. Measurements and number of fruits for every commercial caliber in 10kg packaging.

Caliber	Range	Number of fruits	Caliber	Range	Number of fruits
HC	< 44	-	C 1 X	68 - 71	75
C 6	44 - 46	225	C 1 XX	72 - 73	68
C 5	47 - 50	196	C 1 XX	74 - 77	60
C 4	51 - 54	168	C 1 XXX	78 - 81	53
C 3	55 - 58	140	C 1 XXX	82 - 84	42
C 2	59 - 64	108	C 1 XXX	≥ 85	36
C 1 X	65 - 67	90			

The below figure shows that the *Macrophylla citrus* rootstock the most important caliber, followed by the *C35 citrange*.

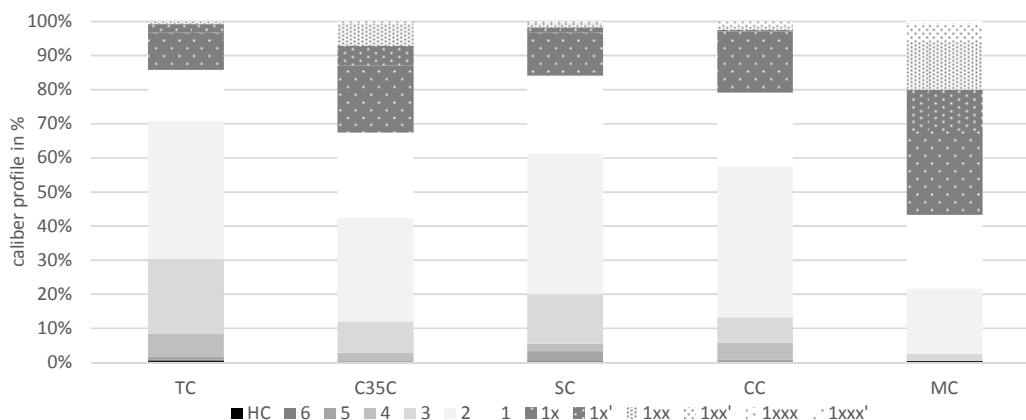


Figure 8. Caliber profile for fruits from each rootstock.

According to figure 8 and table 10, we can deduct that the fruit average weight for each rootstock will be different, which will directly affect the yield.

From the results shown in table 9 and the statistical analysis results, we have shown a highly significant correlation between the temperature and the caliber evolution rate for every rootstock ($r>0.8$). The caliber evolution rate oscillates between 0.24 and 0.36 mm/day with temperatures varying from 20° to 29°C, and afterwards, the rate slows down when the temperatures are below 20°C, while stabilizing at temperatures below 12.5°C.

DISCUSSION

The GA₃ treatment on the mandarin *Nadorcott* transplanted on five different rootstocks was realized at the beginning of floral induction process period (Databases non published). Thereby, an inhibiting effect over the flowering of the following spring should be expected [Wilkie and al., 2008; Bangerth, 2009]. The results in figure 3 show that the gibberellic acid increases the floral induction during the flowering in the following season. The gibberellins (GAs) are responsible for the activation of the cell division process and the stretching of the shoots [Talon and al., 1991], and consequently, the growing of the shoots [Talon and Zeevaart, 1992], or the control of both the growing and stretching of the shoots [Fagoaga and al., 2007; Tata and al., 2015] ... However, as was found on the field, the treatment was coincident with temperatures favorable to the development of the autumn shoot, which actually favored the growth thereafter. The trees that were treated with Gibberellic Acid had more developed shoots and could accumulate more carbon hydrates during winter [Iwahori and al., 1990; Al-Rawi and al., 2016], which have positively influenced the floral induction [Haldankar and al., 2014], and followed by the flowering of the next campaign [Davenport, 1990] especially before the drop of temperatures [Garcia and al., 1995] at an early date [Garcia and al., 1986]. However, the GA₃ treatment serves to increase the number of leaved inflorescences [El-Otmani and al., 2000b; Gravina, 2007] especially with unique flowers instead of minimizing the number of non-leaved inflorescences [Guardiola and al., 1982]. This is maybe further explained by the non-coincidence of the GA₃ application with the two peaks of sensitivity to the response of the citrus plant to the gibberellins. [Guardiola, 1981; Guardiola and al., 1982; Garcia-Luis and al., 1986]: The first is timed with the beginning of the translocation of an unknown flowering signal from the leaves to the buds [Moss, 1976] and the second coincides with the beginning of the morphological flowers differentiation [Iwahori, 1978; Guardiola and al., 1982].

The rootstock influences the vegetative growth, the charge, the size and the fruits quality [Wutscher, 1979, 1988; Syvertsen and al., 2000; Barry and al., 2004; Mesquita and al., 2016]. In our experiment, the rootstock has also significantly influenced the floral induction, which is explained probably by the fruits charge in the going season, or the lack of carbohydrates or the release of safety compounds, essentially the gibberellins [Garcia-Luis and al., 1986; Koshita and al., 1999]. Nonetheless, *Macrophylla citrus* has produced many more vegetative shoots followed by the Citranges group, then the *Swingle citrumelo*, in total harmony with the ongoing season charge.

In our experiment's conditions, the rootstock did not only influence the flowering evolution by the floral induction control, but also the fruit drop, namely in the physiological drop phase (Drop II). This drop has been considered as an autoregulation mechanism that adjusts the number of fruits to the capacity of the tree's procurement metabolite [Guardiola, 1988].

The first wave of abscission (Drop I) is tightly linked to the hormonal equilibrium, particularly the gibberellins [Talon and al., 1990, 1992]. As a result, two flows of GA₃ during the flowering have most likely destroyed any significant effect of rootstock or GA₃ treatment on the drop I.

Other than the environmental conditions, such as the high temperature [Sato and al., 2010], rain, insufficient light that also affect the physiological drop, most of researchers [Sanz and al., 1987; Byers and al., 1990; Mehouchi and al., 1995, 2000; Talon and al. 1997; Gomez-Cadenas and al., 2000; Ruiz and al., 2001; Iglesias and al., 2003; Xi Li and al., 2016] indicate that the abscission during physiological drop is particularly dependent on metabolic factors (carbohydrates). The carbohydrates competition between the fruits, or between fruits and shoots (inexistent in our case), probably causes a fruit drop when the fruits cannot reach a certain carbohydrates concentration threshold [Mehouchi and al., 1995], explains the drop of most of the fruits located on non-leaved inflorescences, followed by multiple flowers inflorescences. The rootstock affects the tree vigor [Barry and al., 2004], thus affecting the carbohydrates development, which means that it directly contributes to the physiological drop control. Even though the *Macrophylla citrus* rootstock, known by its vigor [Salazur-Saenz, 2001], have had a low drop in fruits percentage, followed by C35 citrange and then the other

rootstocks, explained by the number of leaves and the inflorescences foliar area [Ruiz et Guardiola and al., 1994], especially for unique flower leaved inflorescences, the statistical results show a significant effect of the rootstock on the number of leaves in the fruit set stage, with a number superiority for the *Macrophylla citrus*. Otherwise, by the mineral absorption rhythm.

The citrus rootstock affects the yield by influencing the nutritional status of the tree [Georgiou, 2002] by the water absorption capacity [Olien and Lakso, 1986] and the minerals [Wutscher, 1979], by taking in consideration the other parameters such as the edapho-climatic conditions and the cultural practices which simulate the rootstock behavior [Georgiou, 2002].

The rootstock had a significant effect on the foliar levels of Ca, Mg, K, P, S, Zn, matching with Georgiou's results [1999]. However, the rootstock effect on Mg is already verified, the "Lemons" (*Macrophylla citrus*), on the contrary to the Citranges have generally problems with the Mg absorption [Georgiou, 1999], thus explaining the low levels. More than Mg, *Macrophylla citrus* also have low foliar levels when it comes to Ca and P, while as Sanz [1987] says P decreases during the flowering and then increases progressively in the following months, this being associated to a high demand by the hooked fruits, producing an accentuated deficit. Nevertheless, K is at low levels among Carrizo and Troyer citrange.

A level varying from normal to low of N, as reported by Georgiou [2002], is maybe a result of the conducting of a fertilization/irrigation process or of the dilution effect [Com. Pers. A. Ait Houssa, 2014]. We also note low foliar levels of B and Cu, essentially due to the low levels of this elements in the soil and the water. Highly to very highly levels of sodium are caused by a bad management of potassium fertilizers [RACHDI, unpublished data]. Moreover, *Macrophylla citrus* shows a relatively high level in Mn, as it has been described by Wutscher [1979].

Finally, the rootstock effect evaluation on the trees nutritional states, cannot be independently studied of its biotic and non-biotic surroundings, such as the stock levels of nutritive elements, weather condition or cultural practices....

The fruit caliber evolution is mainly influenced by the rootstock and the type of inflorescence from a side, and by the temperature on the other side. However, the rootstock *Macrophylla citrus* allows the most important evolution to the fruit, with a more advanced caliber profile, followed by C35 citrange.

From the gathered data, we could predict the yield (YLD), the number of spring shoots (NSS) for each rootstock. The yield in tons/hectare is computed from the number of fruits per tree and the average weight of the fruit (AWF), Also the spring shout from the number of drop in fruits per tree and per shoot.

Table 11. Estimated yield for each rootstock in tons/hectare.

	Rootstock					
	MC	C35C	CC	SC	TC	Sig
AWF(g)	129	111	103	99	94	-
NSS	2126 a	2120 a	1531 b	1040 c	1386 b	***
YLD	50 a	37 b	28 c	25 c	23 c	***

The numbers followed by the same tiny letter are not significantly different.

Sig: Significance (Duncan: NS, *, **, *** Not significant or significant at P = 0.05, 0.01, 0.001, respectively).

The drop study should always englobe the number of fruits per shoot as the number of shoots, and if these conditions are not met, we will have false results.

The components of the yield are as follows: the number of fruits per shoot, the number of shoots and the average weight of a fruit. Therefore, the tree always finds rise of compensation between the different components, taking into account the biotic conditions, for each physiological stage.

To conclude, the application of gibberellic acid during the coloration turn of the fruit, especially with the existence of autumn shoots does not represent any risk on the floral induction and the flowering evolution of the previous year, nor on the nutritional state of the trees, while we could reach very satisfying results by increasing the dose.

we intend to favor the Citrange C35 rootstock as an appropriate choice for the Atlantic coastal zone of northern Morocco

The diversification of rootstocks is always researched in order to face the complex set of risks we can encounter. In the experiment condition, **we count to favor the C35 citrange rootstock as an appropriate** choice for the coastal zones in north of Morocco, known by a sandy soil, giving us a good adaptation potential, yield and fruit caliber, while focusing on the fruit quality and the production lateness.

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