



Article

Abscission of Orange Fruit (*Citrus sinensis* (L.) Osb.) in the Mediterranean Basin Depends More on Environmental Conditions Than on Fruit Ripeness

Hajer Khefifi ^{1,2,3} , Rim Selmane ², Mehdi Ben Mimoun ² , Francisco Tadeo ⁴,
Raphael Morillon ¹ and François Luro ^{3,*}

¹ CIRAD, UMR AGAP, Avenue Agropolis-TA A-75/02, 34398 Montpellier CEDEX 5, France; khefihajer@yahoo.fr (H.K.); raphael.morillon@cirad.fr (R.M.)

² Laboratoire GREEN-TEAM (LR17AGR01), Université de Carthage, INAT, 43, Avenue Charles Nicolle, Tunis 1082, Tunisia; selmanerim@hotmail.fr (R.S.); mehdibenmimoun@yahoo.com (M.B.M.)

³ INRAE, UMR AGAP Corse, Equipe SEAPAG, 20230 San Giuliano, France

⁴ IVIA, Carretera CV-315, Km. 10,7, 46113 Moncada (Valencia), Spain; tadeo_fra@gva.es

* Correspondence: francois.luro@inrae.fr; Tel.: +33-495-595-946; Fax: +33-495-595-937

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Abstract: Orange fruit abscission usually occurs at the final stage of fruit maturation but in some areas of citrus production, in advance of the usual harvest period, and sometimes suddenly and intensely. The reasons for this precocious citrus fruit abscission remains unclear. Therefore, the aim of this study was to try to clarify what the determinants of this phenomenon are. A multi-site experimentation was carried out on six orange cultivars, in Corsica, Spain and Tunisia where the phenomenon of early massive fruit drop varies. Climatic parameters, fruit maturity parameters and fruit detachment force (FDF) were recorded along the fruit maturation period. Respectively to the fruit drop, the FDF decreased in Tunisia and in Spain until the fruit falls, whereas in Corsica, it remained relatively constant throughout fruit maturation. Although data on fruit maturity parameters (rind color, acidity and total soluble solids) differed at the three sites, their evolution was similar during the period of maturation. FDF was not related to changes in any fruit maturity parameters, and more likely depended on changes in temperatures on days when the mean temperature was above 13 °C. Massive fruit abscission could be linked to the earlier more rapid restart of vegetative growth in Tunisia and Spain than in Corsica.

Keywords: fruit detachment force; citrus color index; acidity; total soluble solids; growing degree days

1. Introduction

Citrus is one of the major fruit crops produced in the world [1]. Citrus production is challenged by multiple biotic and abiotic stress factors [2,3]. Fruit development is controlled by phytohormones, but also by rootstock, nutrition and agricultural practices [4], soil and climate [5]. The physiological processes related to tree and fruit development, fruit yield and fruit metabolite contents vary with the production area. Each of which is characterized by different environmental factors [6].

Maturation involves numerous biochemical and physiological changes within the fruit that subsequently affect its commercial quality [7]. Fruit quality is usually characterized by size, rind color, firmness, acidity, sugar content, juiciness, pulp color, and aroma [8]. In mandarins and related cultivars such as clementines or oranges, rind color and pulp acidity are the main criteria of fruit quality or maturity that are the most affected during maturation. After increasing during the second phase of fruit development called ‘fruit enlargement’, acidity declines during the third and last stage called ‘fruit maturation’ [9]. Changes in total soluble sugars [TSS] differ from those in acidity, with a slight

increase during the maturation phase [10]. The ratio between total soluble sugars and acidity is the main indicator of fruit maturity and is usually used to select the harvest period [11].

These fruit quality parameters are under the influence of environmental factors [12–16]. The maturation of citrus fruit is then impacted by climatic factors such as relative humidity, solar radiation and especially temperature [17,18]. In fact, citrus fruit maturation is closely related to thermal summation [17,19]. Depending on the plant investigated, degree days provide estimations of rates of activity of biochemical processes as well as plant growth. It is defined as the sum of mean daily temperatures of intervals between minimum and maximum thresholds [19]. This index is used to predict tree life cycle, phenological stage, harvesting time and also pest activity [20].

During fruit maturation, particularly during its final phase, fruit abscission can occur in some citrus cultivars, before the harvesting period. Abscission is defined as cell separation events resulting in shedding of plant organs [21]. It is induced by developmental, hormonal, and environmental signals. Climatic factors have been proposed to modulate citrus fruit abscission by influencing their internal metabolism [22].

Abscission of mature fruit before the harvest period, also called preharvest abscission, is a serious problem, mainly in sweet oranges. The navel group is particularly sensitive to preharvest abscission, which causes yield losses in many citrus production areas [6,23,24]. The reasons and the different factors responsible for the high sensitivity of Navel oranges have not yet been identified. Tadeo et al. [24] suggested that TSS accumulation during the maturation stage could be a triggering signal involved in the control of the orange abscission.

The evolution of fruit components and pedicel retention force was then followed during the late maturation phase in three different environments along the Mediterranean rim: Corsica, Spain and Tunisia. This study allowed us to clarify whether fruit quality parameters and environment that are different in the three located places may lead to specific changes in the orange abscission phenomenon.

2. Results

2.1. Comparison of Fruit Detachment Force in Corsica, Spain and Tunisia

The fruit detachment force (FDF) was monitored throughout the maturation period in 'Navelina', 'Washington navel', 'Maltaise demi-sanguine' and 'Navelate' at the three sites (Figure 1). From day 220 to day 300 after anthesis, FDF decreased quite rapidly in Spain and Tunisia. In Spain, at 220 days after anthesis, the FDF was higher than other sites (about 100 N). By the end of the measurement period, FDF was reduced by $\approx 50\%$ from their starting values. The latest FDF values in Tunisia were below 20 N for 'Washington navel' and 'Maltaise demi-sanguine' (Figure 1B,C), which was concomitant with a massive fruit drop (no more fruit were available on the tree for the FDF measurement) and led us to stop the FDF measurements. In Corsica, the initial FDF values were relatively low (around 55 N) at day 220 after anthesis but did not change much during maturation, whatever the cultivar. Interestingly, at the time of the last FDF measurement made in Spain and Tunisia, about 50% of fruit remained on the trees in Corsica. Much later at day 438 after anthesis (July 2013), the six orange cultivars had FDF values about 40 N (Figure S1A) and about 10% of the initial fruit yield was still present on the trees.

In Spain, the FDF decreased faster than in Tunisia. Likewise, the FDF threshold to fruit drop was not the same in Spain and Tunisia. In fact, in Spain fruit shedding occurred when the FDF was below 55 N, whereas in Tunisia the threshold was less than 20 N for the majority of cultivars. In the two sites, a FDF averaging below the defined threshold was associated with significant fruit drop. Statistical analyses suggested that abscission estimated based on the FDF values differed with the site (Table 1). FDF decreased significantly in Tunisia and Spain but not in Corsica (Table 2). Interestingly, previous results obtained in Corsica in the 2011/2012 season showed that trends of FDF curves measured on a set of eight orange cultivars were very similar to results obtained in the 2012/2013 season (Figure S1). The FDF of all the cultivars investigated remained almost the same during maturation until May, when

the last measurement was made. In some cultivars including ‘Valencia late’ and ‘Washington navel’, some FDF values measured in May were quite high, 80 N and 60 N, respectively.

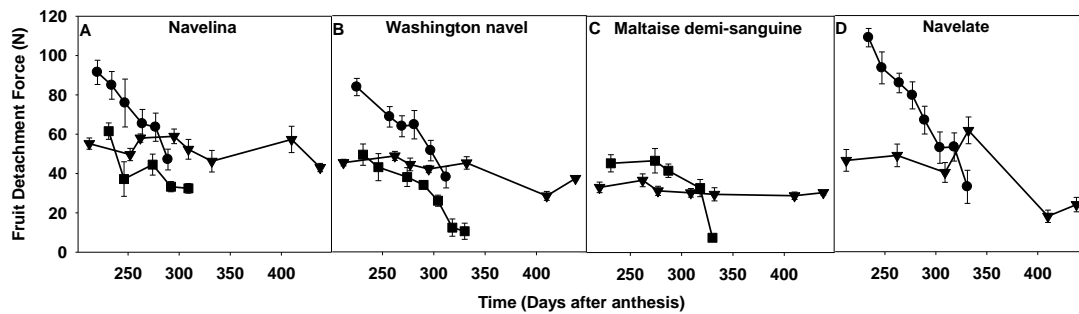


Figure 1. FDF kinetics during fruit maturation in Corsica (▼), Spain (●) and Tunisia (■). FDF was measured in (A) ‘Navelina’, (B) ‘Washington navel’, (C) ‘Maltaise demi-sanguine’ and (D) ‘Navelate’. Values are means ± SE (*n* = 12). Anthesis occurred in the first, second and fourth week of April 2012 in Tunisia, Spain and Corsica, respectively. In the graphs, day 150 after anthesis thus corresponds to 09/26/2012 in Corsica, 09/12/2012 in Spain, and 09/07/2012 in Tunisia.

Table 1. Probability of a site effect on fruit attributes at the three sites (A), in Corsica and Tunisia by the end of January (B) and in Corsica and Tunisia by the end of February (C). Data are means (*n* = 12). *, **, *** indicate significance levels at *p* < 0.01, *p* < 0.001 and *p* < 0 respectively.

	FDF (N)	Fruit Mass (g)	Juice Percentage	Firmness (Kg/cm ²)	TSS (°Brix)	Acidity (g/100 g)	Maturity Index
(A) Corsica/Spain/Tunisia	5.9 × 10 ⁻⁸ ***	2.8 × 10 ⁻⁹ ***	1.1 × 10 ⁻³ **	1.5 × 10 ⁻⁴ ***	2.3 × 10 ⁻¹² ***	<2 × 10 ⁻¹⁶ ***	<2 × 10 ⁻¹⁶ ***
(B) Corsica/Tunisia	5.1 × 10 ⁻¹³ ***	2.2 × 10 ⁻¹³ ***	1.7 × 10 ⁻⁶ ***	1.0 × 10 ⁻⁴ ***	2.5 × 10 ⁻² *	<2 × 10 ⁻¹⁶ ***	<2 × 10 ⁻¹⁶ ***
(C) Corsica/Tunisia	5.9 × 10 ⁻⁸ ***	3.6 × 10 ⁻⁹ ***	1.5 × 10 ⁻¹¹ ***	1.8 × 10 ⁻⁴ ***	1.2 × 10 ⁻¹² ***	2.2 × 10 ⁻⁵ ***	4.0 × 10 ⁻⁵ ***

Table 2. Statistical analysis of variations in fruit parameters during maturation. The different letters indicate statistically significant differences (*p* ≤ 0.05).

Site	Cultivar	Date	Fruit Mass (g)	Juice %	Color Index	FDF (N)	TSS (°Brix)	Acidity (g/100 g)	Maturity Index	Firmness (Kg/cm ²)
Corsica	Maltaise ½ sanguine	Date1	175 ^a	43 ^b	81 ^a	44 ^a	9.3 ^a	2.0 ^b	5 ^a	5 ^a
		Date2	222 ^b	38 ^a	49 ^b	41 ^a	9.6 ^a	0.9 ^a	10 ^b	4 ^a
	Navelate	Date1	208 ^a	39 ^b	1 ^a	57 ^a	8.0 ^a	1.9 ^b	4 ^a	6 ^a
		Date2	257 ^b	35 ^a	42 ^b	57 ^a	9.7 ^b	0.7 ^a	14 ^b	5 ^a
	Newhall navel	Date1	213 ^a	39 ^b	23 ^a	55 ^a	10.0 ^a	1.3 ^b	8 ^a	8 ^a
		Date2	251 ^b	32 ^a	63 ^b	41 ^a	10.4 ^a	0.6 ^a	17 ^b	4 ^b
	Lane late	Date1	325 ^a	37 ^a	-22 ^a	66 ^a	7.5 ^a	1.7 ^b	4 ^a	12 ^b
		Date2	383 ^b	36 ^a	38 ^b	56 ^a	9.4 ^b	0.6 ^a	14 ^b	8 ^a
	Navelina	Date1	223 ^a	38 ^b	35 ^a	59 ^a	9.7 ^a	2.3 ^b	4 ^a	6 ^a
		Date2	280 ^b	34 ^a	54 ^b	46 ^a	10.6 ^b	0.8 ^a	14 ^b	4 ^b
	Washington navel	Date1	291 ^a	41 ^b	15 ^a	61 ^a	8.2 ^a	1.2 ^b	6 ^a	5 ^b
		Date2	362 ^b	27 ^a	44 ^b	60 ^a	9.4 ^b	0.4 ^a	19 ^b	3 ^a
Spain	Navelate	Date1	204 ^a	39 ^a	0 ^a	107 ^b	11.1 ^a	1.7 ^b	6 ^a	-
		Date2	221 ^a	39 ^a	9 ^b	53 ^a	13.8 ^b	0.9 ^a	14 ^a	-
	Navelina	Date1	251 ^a	37 ^a	11 ^a	91 ^b	12.3 ^a	1.4 ^a	8 ^a	-
		Date2	257 ^a	37 ^a	15 ^b	47 ^a	12.9 ^b	1.5 ^a	9 ^b	-
	Washington navel	Date1	224 ^a	38 ^a	5 ^a	111 ^b	11.2 ^a	1.6 ^b	7 ^a	-
		Date2	240 ^a	41 ^b	10 ^b	51 ^a	11.3 ^a	1.2 ^a	9 ^b	-
Tunisia	Maltaise ½ sanguine	Date1	178 ^a	46 ^b	-	60 ^b	8.3 ^a	1.0 ^b	8 ^a	10 ^b
		Date2	181 ^a	43 ^a	-	10 ^a	9.1 ^b	0.9 ^a	10 ^b	6 ^a
	Newhall navel	Date1	197 ^a	47 ^b	-	58 ^b	7.9 ^a	0.7 ^a	13 ^a	9 ^b
		Date2	244 ^b	41 ^a	-	14 ^a	9.8 ^b	0.6 ^a	14 ^b	8 ^a
	Lane late	Date1	195 ^a	39 ^a	-	112 ^b	9.2 ^a	0.8 ^b	11 ^a	11 ^b
		Date2	247 ^b	38 ^a	-	48 ^a	12.5 ^b	0.5 ^a	18 ^b	10 ^a
	Navelina	Date1	184 ^a	40 ^a	-	61 ^b	10.7 ^a	0.5 ^b	19 ^a	7 ^b
		Date2	189 ^a	37 ^a	-	24 ^a	10.8 ^a	0.4 ^a	25 ^b	5 ^a
	Washington navel	Date1	187 ^a	42 ^b	-	66 ^b	10.4 ^a	0.9 ^b	11 ^a	9 ^b
		Date2	206 ^a	39 ^a	-	14 ^a	11.4 ^b	0.6 ^a	17 ^b	7 ^a

(-) no data shown, Date1: 21 November 2012, Date2: 13 February 2013.

Although the FDF measured in Corsica remained constant during maturation. About fifty percent of the fruit remained on the tree showing where fruit drop occurred. Conversely, all fruit dropped in Tunisia and Spain after the last FDF measurement (in March).

2.2. Soil and Climatic Conditions in Corsica, Spain and Tunisia

The climatic data (cumulative degree days, mean temperature, global solar radiation, total rainfall and maximum wind speed) recorded at the three sites are presented in Figure 2. Climatic factors differed considerably at the three sites, especially cumulative degree days (Figure 2A). In October, degree days were higher than 1800 °C in Spain and Tunisia, whereas in Corsica, this sum was only reached in June, i.e., eight months later. It is interesting to note that cumulative degree-day plateau was recorded in November at all three sites. In addition, close to the preharvest drop period (i.e., day 280 after anthesis), trees cultivated in Spain and Tunisia had reached respectively 400 and 900 degree days more than trees cultivated in Corsica (Figure 2A). Comparison of the mean temperature at the three sites showed a difference of 3 °C to 4 °C between Tunisia and Corsica over the year (Figure 2B). Differences between Corsica and Spain ranged from 0 °C to 3 °C. During the fruit drop period, the difference in temperature was 3.5 °C between Tunisia and Corsica and 4 °C between Spain and Corsica.

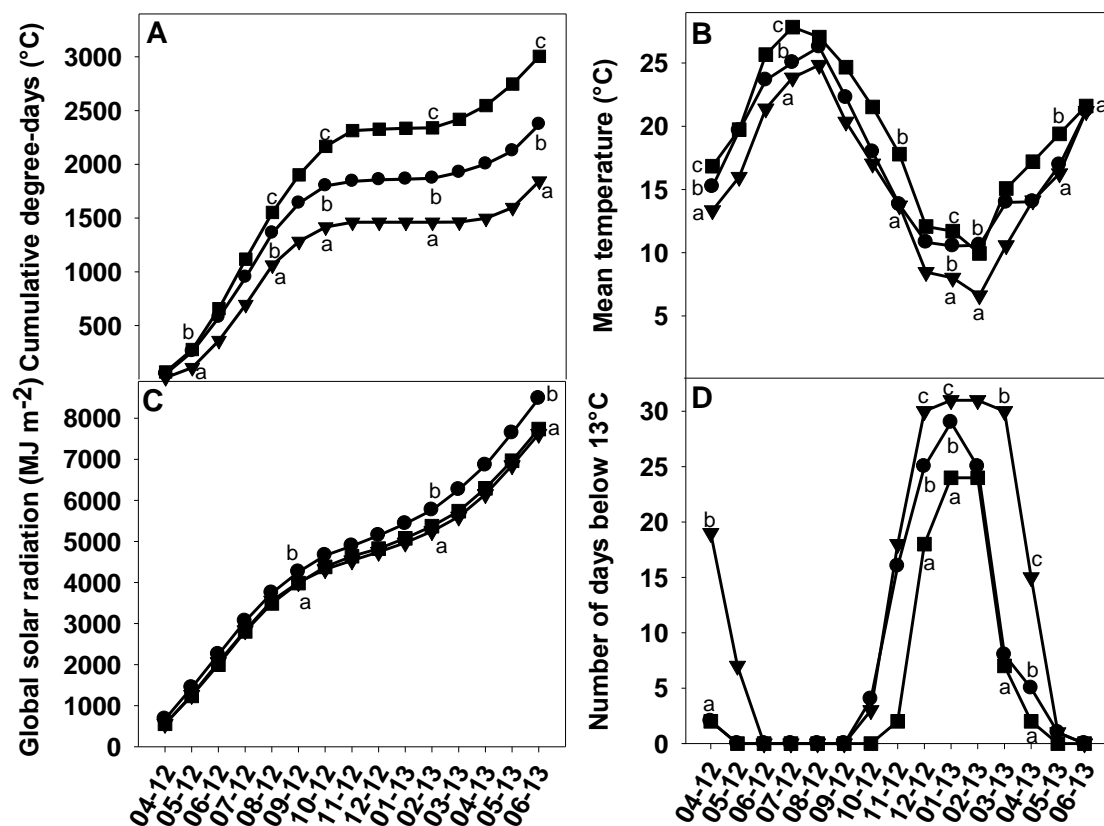


Figure 2. Climatic data recorded during the 2012/2013 season in Corsica (▼), Spain (●) and Tunisia (■). (A) Monthly variation in cumulative degree-days (°C). (B) Mean temperature (°C). (C) Global solar radiation (MJ m^{-2}). (D) Number of days with temperatures below 13 °C. The different letters indicate statistically significant differences ($p \leq 0.05$).

From April to the end of summer, trees were subjected to the same sum of global solar radiation (Figure 2C). Later on, at the beginning of fruit fall and until the fruit drop period, about 500 MJ m^{-2} more was recorded in Spain than in Corsica and Tunisia (period from September 2012 to March 2013).

From December to March, there were more cold days, i.e., with temperatures below 13 °C, in Corsica than in Spain and in Tunisia (Figure 2D). From February, the number of cold days decreased rapidly in Tunisia and Spain, whereas in Corsica, the number of cold days only decreased in April.

Rainfall was very irregular especially in Corsica, ranging from 0 to 169 mm, with a maximum in September. In the period from January to March, a second rainfall event was recorded, and rainfall increased from 1 to 143 mm. In Spain and Tunisia, rainfall was similar and did not exceed 60 mm per month from April to August 2012 (Figure S2A). From January to March, which corresponded to the fruit abscission period in Spain and Tunisia, rainfall was quite low (≤ 40 mm at both sites). Regarding wind, data showed that wind speeds in Tunisia were never higher than 19 km/h in the 2012/2013 season, whereas wind speeds in Corsica and Spain were much higher in the same period. In Spain, maximum wind speed ranged from 31 to 44 km/h and in Corsica, wind speed reached more than 61 km/h in October and February (Figure S2B).

2.3. Comparison of Fruit Maturity Attributes and Their Evolution during the Fruit Maturation and Fruit Drop Period

2.3.1. Fruit Mass, Firmness and Color Index

Increase in mass was monitored in ‘Navelina’, ‘Washington navel’, ‘Maltaise demi-sanguine’ and ‘Lane late’ at the three sites (Figure 3A–D). Fruit enlargement did not change from day 200 to day 350 after anthesis, whatever the site and the orange cultivar, except ‘Lane late’, which increased slightly in Corsica. Fruit mass tended towards a maximum value (plateau) that differed for each cultivar. The maximum value of this trait differed between the three sites, particularly for ‘Washington navel’ and ‘Lane Late’, but did not affect the general trend in the evolution of this character.

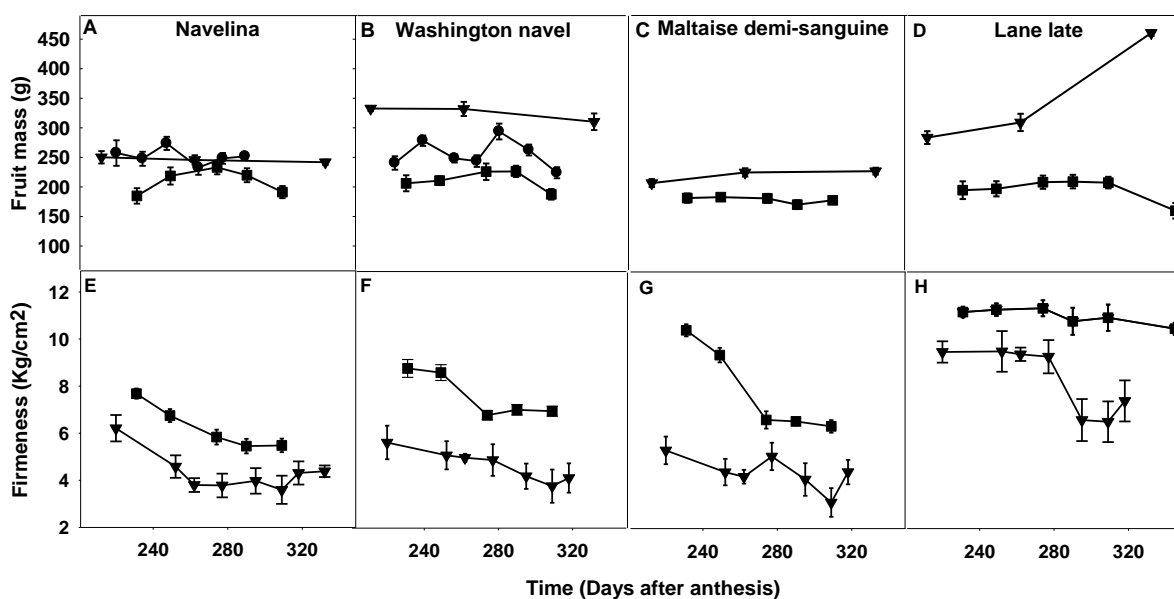


Figure 3. Fruit mass and firmness measured in different orange cultivars during maturation in Corsica (▼), Spain (●) and Tunisia (■). Fruit mass was measured in (A) ‘Navelina’, (B) ‘Washington navel’, (C) ‘Maltaise demi-sanguine’ and (D) ‘Lane late’. Firmness was measured in (E) ‘Navelina’, (F) ‘Washington navel’, (G) ‘Maltaise demi-sanguine’ and (H) ‘Lane late’. Values are means \pm SE ($n = 12$). Anthesis occurred in the first, second and fourth week of April, 2012 in Tunisia, Spain and Corsica respectively. In the graphs, day 150 after anthesis thus corresponds to 09/26/2012 in Corsica, 09/12/2012 in Spain, and 09/07/2012 in Tunisia.

Firmness was monitored in four orange cultivars in Corsica and in Tunisia during the period of fruit maturation (Figure 3E–H). At these two sites, fruit firmness decreased during maturation. Oranges cultivated in Corsica were always less firm than oranges grown in Tunisia. The color index of cultivars cultivated in Corsica and Spain increased until 250–280 days after anthesis and then remained constant (Figure 4). In Corsica, whatever the cultivar, the CCI values of the pericarp were always slightly higher than in Spain, indicating a more orange-colored rind. In Spain, the color of the fruit rind did not change during fruit abscission (from 270 to 300 days after anthesis).

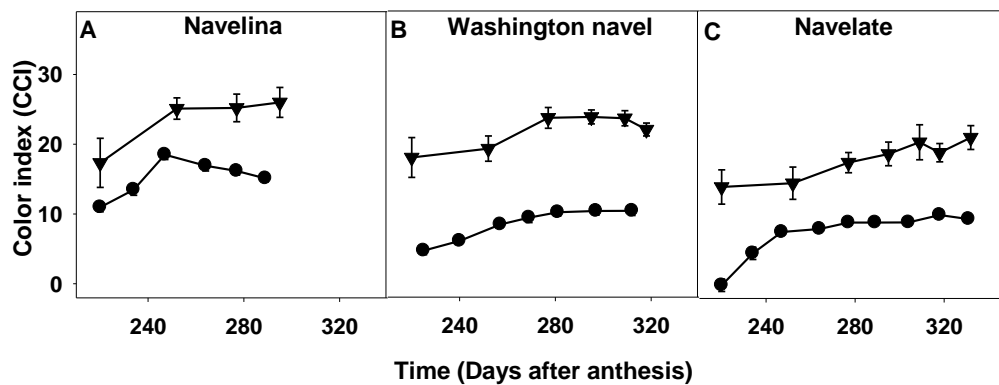


Figure 4. Color index measured in different oranges cultivars during maturation in Corsica (▼), Spain (●) and Tunisia (■). The color index was measured in (A) ‘Navelina’, (B) ‘Washington navel’, (C) ‘Navelate’. Values are means \pm SE ($n = 12$). Anthesis occurred the first, second and fourth week of April 2012 in Tunisia, Spain and Corsica respectively. Day 150 after anthesis thus corresponds to 09/26/2012 in Corsica, 09/12/2012 in Spain, and 09/07/2012 in Tunisia.

2.3.2. Juiciness, TSS, Acidity and Fruit Maturity Index

Changes in the juice percentage of ‘Navelina’ and ‘Lane late’ orange cultivars were monitored (Figure 5A,D). Juice percentage was similar at the three sites and remained constant throughout the maturation period.

Changes in TSS values were similar at the three sites: a slight increase was observed in all the cultivars (Figure 5E–H). In Corsica and Tunisia, TSS increased during maturation in ‘Navelina’, ‘Washington navel’ and ‘Lane late’ (Table 2). In Spain, TSS increased in ‘Navelina’ and remained constant in ‘Washington navel’ (Table 2).

Acidity decreased during maturation at all three sites but the range of values was bigger in Corsica than in Tunisia and Spain (Table 2). Indeed, acidity values higher than 2 g/100 g of juice were still recorded in ‘Navelina’ in Corsica on day 250 after anthesis. With the exception of ‘Washington navel’, which had the same rate of acidity in Tunisia and in Corsica at the end of maturation, acidity values in oranges cultivated in Tunisia were lower throughout the maturation period (Figure 5I–L). The maturity index increased during maturation at all three sites (Table 2), but changed much more rapidly in Tunisia than in Corsica and in Spain in all cultivars (Figure 5M–P). Indeed, on day 250 after anthesis, the maturity index of ‘Navelina’ was about 20 in Tunisia and continued to increase, while in Corsica and Spain, it remained below 5 and 10 respectively, over the same period. In Corsica, changes in the maturity index in ‘Washington navel’ and ‘Maltaise demi-sanguine’ were limited up to day 250 after anthesis, after which it increased rapidly from 8 to 19 for ‘Washington navel’ and from 5 to 10 for ‘Maltaise demi-sanguine’. The maturity index continued to increase in Corsica during the period of fruit abscission, whereas in Spain and Tunisia, it remained constant.

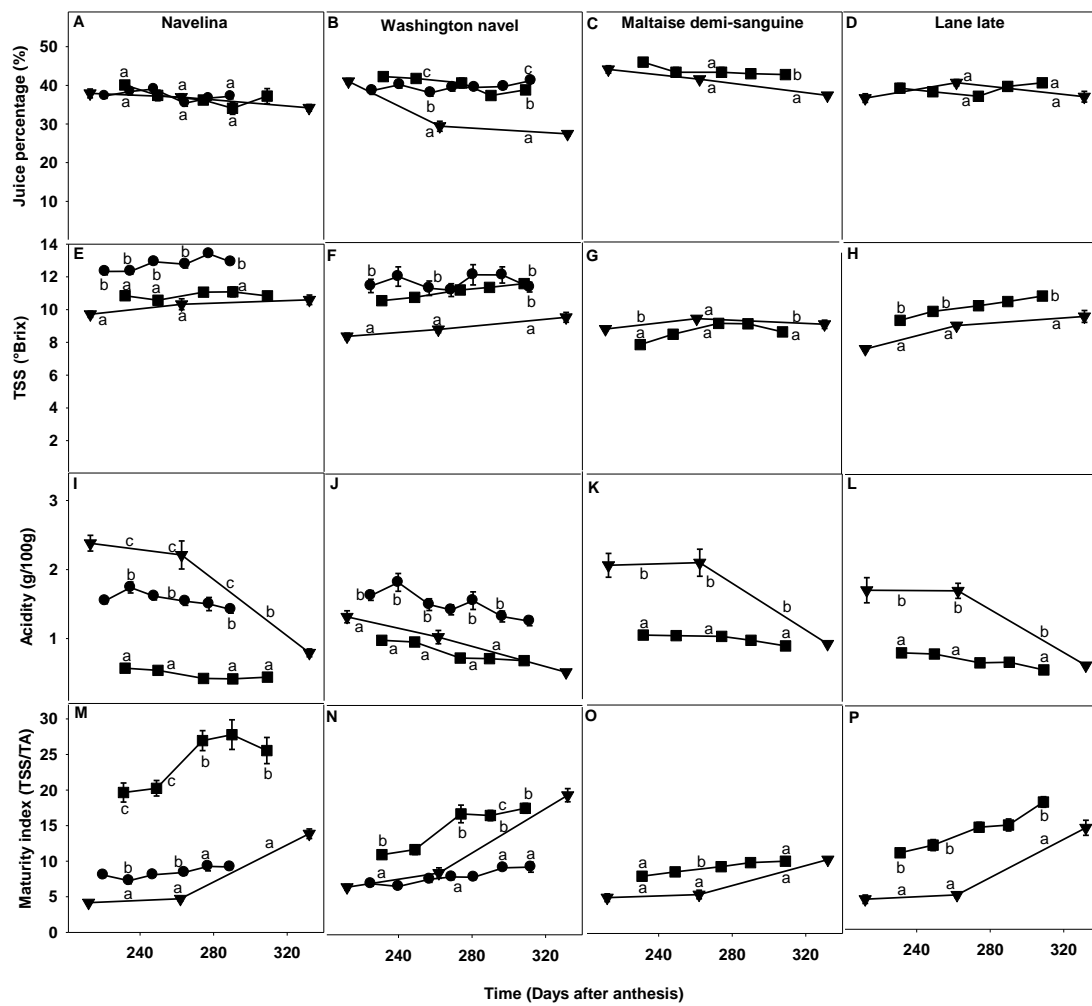


Figure 5. Fruit quality criteria measured in different orange cultivars during maturation in Corsica (▼), Spain (●) and Tunisia (■). Juice percentage measured in (A) ‘Navelina’, (B) ‘Washington navel’, (C) ‘Maltaise demi-sanguine’ and (D) ‘Lane late’. TSS measured in (E) ‘Navelina’, (F) ‘Washington navel’, (G) ‘Maltaise demi-sanguine’ and (H) ‘Lane late’. Acidity measured in (I) ‘Navelina’, (J) ‘Washington navel’, (K) ‘Maltaise demi-sanguine’ and (L) ‘Lane late’. The maturity index calculated for (M) ‘Navelina’, (N) ‘Washington navel’, (O) ‘Maltaise demi-sanguine’, (P) ‘Lane late’. Values are means ± SE ($n = 12$). Anthesis occurred in the first, second and fourth week of April, 2012 in Tunisia, Spain and Corsica, respectively. Day 150 after anthesis thus corresponds to 09/26/2012 in Corsica, 09/12/2012 in Spain, and 09/07/2012 in Tunisia. The different letters indicate statistically significant differences ($p \leq 0.05$).

2.3.3. Correlations between Maturation, Environment and Fruit Abscission

Spearman rank correlation coefficients were calculated to evaluate whether fruit maturation impacts fruit abscission. The Spearman rank correlation coefficients were very low in all the cultivars studied in Spain, Tunisia and Corsica (Table 3). Comparison of the TSS and changes in acidity during maturation as well as of changes in FDF showed that TSS and acidity parameters evolved in the same way in the cultivars that had a low (respectively high) FDF by the end of maturation (Table 2).

Table 3. Spearman rank correlation coefficients between FDF and fruit parameters measured in February in orange cultivars cultivated in Corsica, Spain and Tunisia (values in bold are statistically significant).

Site	Fruit Mass (g)	Juice Percentage	TSS (°Brix)	Acidity (g/100 g)	Maturity Index	Firmness (Kg/cm ²)	Color Index
Corsica	0.05	−0.13	−0.12	0.03	−0.06	0.37	−0.41
Spain	−0.09	−0.03	0.13	−0.29	0.25	-	−0.31
Tunisia	0	0.09	−0.35	−0.04	−0.03	0.38	-

3. Discussion

3.1. FDF Measurement and Fruit Shedding

The fruit detachment force fluctuates greatly from one site to another, for the same variety but also during the ripening period, particularly in Corsica. For example, in Spain, the FDF value is 30 to 50% higher than on both sites, whatever the variety. Several previous researches have been carried out on the fluctuation of FDF in oranges [25,26]. Pozo et al. [27] observed variations in FDF during the day of up to 23% between morning and afternoon. They showed that these FDF fluctuations were also correlated with relative humidity, temperature, fruit weight and fruit juice content. FDF also fluctuates according to the position of the fruit in the tree, with higher values for fruits located in the upper part of the canopy compared to fruits located at 1.5 m from the ground as well as those at the periphery compared to fruits inside the tree [28]. This work demonstrates the sensitivity of FDF to the environmental conditions of citrus growing, but also to the physiological state of the trees and the tree position of the harvested fruit for observation. We have no certain explanation for the observed fluctuations in FDF, but we may hypothesize that crop conditions or soil composition could be factors associated with its variation.

Nevertheless, these high values of FDF recorded in Spain decreased largely during maturation leading to an abscission wave. Indeed, in many studies, fruit drop followed a decrease in FDF and began when the FDF threshold was reached [29]. In Spain and Tunisia, FDF threshold to massive fruit drop was estimated to 55 and 20 N respectively, which is in the range of the thresholds estimated by Hartmond et al. [25]: 40 N or by Wilson [30]: 55–25 N.

In our hands, these two stages were observed in Tunisia and Spain but not in Corsica. In fact, FDF did not decrease during the measurement period. Therefore, although that FDF was below 40 N (FDF threshold) at day 438 after anthesis (July), fruit remained on the trees. These results confirm the complexity of abscission phenomena, which is under multifactorial control [31]. Indeed, fruit shedding is influenced by factors that can act individually or be linked, and may be sequential or simultaneous [9]. These factors may have a genetic or a molecular regulation basis [32], may involve the metabolism of the plant through the TSS content [24], concentrations of hormones, mainly ethylene [31], and could involve environment condition(s) [33].

3.2. Impact of the Environment on Abscission Evaluated via Changes in Fruit Traits and in Fruit Maturation

Environment-generated variability was observed among the sites in terms of orange fruit mass, firmness, fruit rind color, acidity and TSS. These differences are explained by the influence of environmental factors on fruit quality attributes, as previously documented for fruit size [34], rind color break and color intensity [6], percentage of fruit juice [16], juice acidity, sugar content and TA/TSS ratio [35–37]. However, in Corsica, differences in fruit maturation did not appear to be associated with the constant FDF. Indeed, in cultivars with a similar mass at the end of the experiment (e.g., ‘Navelina’ and ‘Navelate’) in Corsica and in Spain, the FDF decreased only in Spain, even though in Corsica, quality attributes including firmness and the color index did change during maturation. Moreover, the similarity of the juice percentage in the majority of oranges at all three sites suggests no correlation

between the accumulation of juice and changes in FDF during maturation. In our study, differences in acidity and in the maturity index did not correlate with the observed differences in FDF. Therefore, FDF was not influenced by the physical fruit traits we investigated (fruit mass, firmness and color index) nor by primary metabolites (TSS and TA and maturity stage TSS/TA). In the oranges we studied at the three sites in the years 2011/2012 and 2012/2013, correlations between FDF and phenotypic traits such as fruit mass, color index, firmness, juice percentage, acidity, TSS and maturity index were mostly not consistent, as evidenced by the mainly non-significant or low Spearman rank correlation coefficients. The low correlation suggested that the effect of these parameters on fruit abscission is unlikely.

3.3. Impact of the Environment on Abscission

Rootstock can affect the physiology of the whole tree (e.g., tree vigor) or fruit quality. Grafting can also promote holding fruit by the tree [38,39]. In Spain and Corsica, the cultivars were grafted on the same rootstock ('Carrizo' citrange) and FDF decreased in Spain, whereas it remained constant in Corsica. This suggests that on its own, 'Carrizo' citrange rootstock, cannot explain the difference in the evolution of FDF we observed in Spain and in Corsica. A study by Wirch et al. [40] showed that in sweet cherry, the type of rootstock could affect fruit quality but not FDF.

The soil water reserves were sufficient for the needs of the orange trees with the rainfalls of the period from January to April 2013 (40–50 mm/month), in Spain and Tunisia, and compensated for the evapotranspiration losses. Later, during the dry period (May to September), irrigation was necessary to maintain a sufficient useful water reserve in the soil and avoid stress caused by water scarcity. Water stress induces the increase of abscisic acid (ABA) content that modulates the levels of ethylene, the hormonal abscission activator of leaves and possibly of fruits [9,41,42]. During the period of the sudden orange fall in Spain and Tunisia, the orange trees did not suffer of a lack of water. A water limitation can therefore be ruled out from the factors that may explain the sudden and massive fall of the oranges. Furthermore, no water logging, which could have triggered fruit abscission [9], was observed during the period before fruit shedding. Despite the fact the orchards are surrounded by tree windbreaks to limit the impact of wind on fruit drop, a strong gust of wind can still give rise to extensive fruit drop [15,43]. However, this was not observed during the fruit shedding period in Tunisia. It is important to note that fruit in Corsica were subjected to stronger winds during the abscission period than at the two other sites, and that the fruit remained attached in the tree until July. Thus, rainfall events and wind were ignored as possible factors influencing the citrus fruit drop observed during our experiment.

Addicott [43] reported that fruit shedding can be affected by light and temperature. Moreover, it has been shown that the effectiveness of growth regulator treatment on reducing preharvest drop of Valencia orange depended on climatic conditions, stressing the influence of climatic conditions on preharvest drop [44].

Ultraviolet B radiation (UV-B) has significant effects on plant development and metabolism, which rely on the control of hormonal pathways. It stimulates the biosynthesis or signaling of ABA, jasmonate (JA), and salicylic acid (SA), and inhibits the biosynthesis or signaling of gibberellic acid (GA) and auxin hormones [45]. It was also shown that ethylene biosynthesis was stimulated under various UV-B radiation intensities in different plant species, such as Arabidopsis [46] and pear [47]. Therefore, UV-B radiation could have an indirect effect on abscission by increasing ethylene production. However, in our study, there was no difference in global solar radiation between Tunisia and Corsica all year round, i.e., including the fruit maturation period. Moreover, the season when solar radiation was the highest do not correspond to the period when the massive fall of orange fruit occurred. Therefore, at two sites with similar global solar radiation, we observed different trends of FDF suggesting that abscission is not affected by the solar radiation.

Temperature and plant development are related by the thermal summation or cumulated degree days the plant needs to reach maturity [6,17]. Thus, degree days are quite a good parameter to evaluate fruit maturity. As heat unit influences fruit maturation, we expected cumulated degree

days to influence fruit abscission, as suggested by the observations we made in the present study. However, data analysis failed to confirm this hypothesis. In fact in November, trees grown in Corsica, Spain and Tunisia cumulated 1400, 1800 and 2300 degree days, respectively. Degree day curves then remained constant until February-March and increased thereafter. Thus, one possible hypothesis is that abscission occurs beyond a threshold of 1800 degree days. Under this hypothesis, fruit would be shed much sooner in Spain and Tunisia. In Corsica, based on the remaining fruit on trees, fruit had been exposed to 1800 degree days in June, i.e., 13 months after anthesis, and no massive fruit drop or change in FDF was observed. Moreover, in tropical climates with strong light irradiance, such as in Reunion Island (at the CIRAD research station in St Pierre) or in the French West Indies (at the CIRAD research station in Capesterre, (Guadeloupe), severe orange fruit drop has never been reported. Taken together, these results suggest that the accumulation of heat units cannot be considered to have a direct impact on fruit shedding.

Temperature is known to be a limiting factor for the growth and development of citrus trees. The growth of all citrus tree organs has been shown to slow down below 13 °C [6] and cold hardening starts in citrus at about this temperature [48]. In Corsica, citrus trees exposed to a long period with mean temperatures below 13 °C stopped their vegetative development. In Spain and especially in Tunisia, there were fewer days below 13 °C during winter than in Corsica. Concomitantly with fruit shedding associated with decreasing FDF, which, in Spain and Tunisia, occurred in February/March, the number of cold days suddenly decreased while temperatures increased and became favorable for vegetative growth. Such a change can explain the preharvest drop phenomenon. Yuan and Burns [49] reported that temperature shifts can induce ethylene production, which is the key factor of abscission. This transition from dormancy to vegetative growth is characterized by high carbohydrate demands in trees that may not be satisfied by current photosynthesis [50]. By causing defoliation of the tree, Gómez-Cadenas et al. [42] showed that nutrient shortage led to fruit abscission, which was promoted by an increase in ABA and ethylene contents. An indirect relation between the increase in temperature and the decrease in FDF can thus be hypothesized. This relationship was demonstrated by Yuan and Burns [49] who showed that the application of abscission compound as Ethephon and CMNP (5-chloro-3-methyl-4-nitro-1H-pyrazole) had no effect on either FDF or fruit abscission, when air temperature was maintained at 10 °C or 15 °C. However, an increase of temperature caused an increase of ethylene evolution and a drastic decrease of FDF, and then a marked increase of fruit abscission. Under our hypothesis, a rapid change from a low temperature (13 °C) to a higher temperature at the end of winter would indirectly favor abscission by causing a carbon shortage. Conversely, a longer period of lower temperatures would delay carbon shortage and the decrease in FDF and then fruit drop. However, it is important to note that in Corsica, although the change from days below 13 °C to days above 13 °C occurred in April (i.e., two months later), no massive fruit abscission was triggered. Consequently, other factors are required to explain this phenomenon.

The flowering and fruit set period is characterized by an increase in endogenous plant hormones, including auxin and gibberellin [51–55], and in carbohydrates [9,56]. In Corsica, tree flowering and fruit set occur from April to May and coincide with a return to favorable vegetative conditions. In Tunisia and in Spain, the drop in FDF occurs before flowering and fruit set and thus before the associated changes in the hormone balance. This hypothesis is in agreement with the work of Yuan et al. [57], who showed that a period when mature ‘Valencia’ oranges were resistant to abscission occurred in late April and early May during the harvest season in Florida. These young growing tissues are characterized by a high level of endogenous plant hormones [53,58]. Yuan et al. [57,59] proposed that the lower responsiveness of mature fruit to ethylene application was due to the increase in the concentration of auxin and to the decrease in the concentration of ABA in the abscission zones.

4. Materials and Methods

4.1. Plant Material

The first experiment was performed in the INRA-CIRAD citrus Biological Resource Center (BRC) at San Giuliano in Corsica (42°18'55" N, 9°29'29" E; 51 m above sea level) during fruit set in the 2011–2012 season on different orange cultivars (*C. sinensis* (L.) Osb.). Three early orange cultivars ('Salustiana', 'Navelina', 'Hamlin'), three mid-season cultivars ('Washington navel', 'Shamouti', 'Moro') and three late cultivars ('Navelate', 'Valencia late', 'Maltaise blonde') were used. Trees were about 18 to 24 years old, 3 to 4 m tall and spaced at 4 × 6 m. All the cultivars were grafted onto Carrizo citrange (*C. sinensis* (L.) Osb. × *Poncirus trifoliata* Raf.), cultivated in the same orchard with an homogeneous soil composition (alluvial deposits and classified as fersalitic, pH range 6.0–6.6). Water was supplied to replace 100% of actual evapotranspiration using the Penman–Monteith equation [60] and the cultural coefficient usually used for citrus orchards in Corsica [4]. Fertilizers were supplied to meet standard requirements [4], and insects and diseases were controlled according to local agriculture department recommendations. The annual fruit production exceeds 150 kg for each tree. Differences among cultivars were thus not influenced by soil and climatic factors or cropping techniques. Measurements began in December (around 200 days after anthesis).

The second experiment was carried out in the following fruit set season (December 2012 to June 2013) in three different environments, in Corsica, Spain and Tunisia. Two early ('Navelina', 'Newhall Navel'), two mid-season ('Washington Navel', 'Maltaise demi-sanguine') and two late ('Navelate' and 'Lane late') maturing orange cultivars were investigated. The experiments were conducted in irrigated orchards at the INRA-CIRAD citrus Germplasm Bank in Corsica (France, 42.1° N, 9° E), at the *Instituto Valenciano de Investigaciones Agrarias* (IVIA) in Valencia (Spain, 39.4° N, 0.3° W) and in the "*Société Tunisienne de l'Agriculture Moderne*" (SOTAM) in Tunis (Tunisia, 37° N, 11° W).

In Tunisia, the cultivars were grafted onto sour orange (*C. aurantium* (L.)) rootstock and in Spain and Corsica cultivars were grafted onto 'Carrizo' citrange.

Samples were regularly harvested from December 2012 to July 2013. Ten to twelve fruits were collected from three trees of each cultivar. Fruit were randomly selected all around the canopy approximately 1.5 m from the ground.

Anthesis occurred during the first, second and fourth week of April in Tunisia, Spain and Corsica, respectively. Because anthesis occurred at different dates, the same number of days after anthesis at the three sites correspond to different calendar dates. The date of anthesis was used as the reference date to plot abscission kinetics and changes in fruit parameters.

4.2. Climatic Data

Daily climatic data (mean temperatures, global solar radiation, total rainfall and wind speed) were recorded by an agro meteorological weather station located at each site.

In citrus, root, shoot and fruit growth and development slow down considerably below 13 °C [61] and above 36 °C [62]. In our study, these temperatures were thus used as thresholds, and temperatures below 13 °C and above 36 °C were discounted when calculating degree days. To calculate growing degree days, we used the formula proposed by [37]:

Growing degree days = $[(T_m + T_n)/2] - 13$ °C; where T_m is maximum air temperature and T_n is minimum air temperature. In addition to growing degree days, the numbers of days below 13 °C per month were calculated.

4.3. Soil Conditions

Soil conditions differed among the three sites. Soils in Tunisia and in Spain are calcareous, sandy loam and the pH is about 8.5 and 8 respectively. However in Corsica, the soil is alluvial and fersalitic, and acidic with a pH varying from 6 to 6.5.

4.4. Evaluation of External and Internal Fruit Maturity

Fruit samples were periodically analyzed for weight, color, firmness, juice percentage, total soluble solids (TSS), acidity (TA) and maturity index (TSS/TA). The equatorial diameter and the peduncle thickness were measured using a digital caliper (Mitutoya, Absolute digimatic). In Tunisia and Corsica, firmness was recorded with a digital penetrometer (Agrosta[®] 14) and in Spain with a texturometer (INSTRON texturometer 3343). The color of the fruit rind was measured with a CIE L*a*b under Hunter Lab colorimetric system using a Minolta CR-200 colorimeter (Minolta, Ramsey, NJ USA) at four points around the equatorial plane of the fruit. Using these color parameters, a citrus color index (CCI = $1000 \times a/(L \times b)$) was calculated [63]. Negative values of CCI mean green, positive values close to zero yellow and high positive values mean reddish-orange. The total acidity of the juice, determined by titration, is expressed as grams of citric acid per 100 g of juice. The soluble solid content was determined using a digital refractometer (RFM710) (Bellingham + Stanley Ltd., Longfield Road, Tunbridge Wells, Kent TN2 3EY, United Kingdom) and is expressed as °Brix.

4.5. Measurement of the Fruit Detachment Force (FDF)

The fruit detachment force called FDF is defined as the force needed to separate the fruit from the calyx. It indicates how strongly the fruit is attached to the tree. In several experiments, this parameter was used to follow and then to evaluate the progress of the fruit abscission process [29,64,65]. Hartmond et al. [25] showed that decreasing FDF was always associated with fruit shedding. The FDF was measured every 15 days using a pull-force gauge (Compact Gauge, Mecmesin) on samples of 10 fruit with a 10 cm long pedicel collected from three different trees.

4.6. Statistical Analyses

Data were analyzed using two software packages: Statistica10 (StatSoft Inc., Tulsa, OK, USA) and R version 3.0.1 free software (R Foundation for Statistical Computing 2013). FDF and fruit phenotypical data were subject to analysis of variance using the Newman–Keuls test for means separation. This test was performed using 10 to 12 reps. In addition, simple correlation analysis was performed using Spearman rank correlation coefficient to investigate relationships between the FDF and fruit characteristics.

5. Conclusions

Our results suggest that fruit abscission is strongly influenced by environmental factors but not by the parameters of fruit maturity. Indeed, the correlations between FDF and fruit attributes were low, suggesting the two phenomena are not related. Other parameters such as carbohydrates and hormones could explain the observed results. Thus, other investigation would be required to determine whether carbohydrate shortage or changes in the hormone balance are likely associated with the sudden orange drop.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/4/591/s1>, Figure S1: FDF kinetics in Corsica during fruit maturation in two consecutive seasons. Figure S2: Climatic data recorded during the 2012/2013 season in Corsica, Spain and Tunisia.

Author Contributions: H.K. performed the experiments in Corsica, data analysis, and wrote the first draft. F.T. and R.S. scored data in Spain and in Tunisia respectively. F.L., M.B.M., F.T. and R.M. conceived and designed experiments and revised and wrote the final version of the manuscript. All the authors discussed the data and approved the final version of the manuscript.

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