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Spanish cedar: Relationbetween Fruit and Seed Characteristics with Germination

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Abstract

For Spanish cedar (*Cedrelaodorata* L., Meliaceae) is unknown if big fruits contain a greater number of seeds and if those seeds have better physic and biological quality than those coming from small fruits. Therefore, the relation between physic characteristics of fruit and seed with Spanish cedar germination was determined. One hundred seeds per tree from three groups of 12 trees were sown in individual nursery bags and 100 in a seedbed. Fruit weight and number of seeds per fruit differed significantly among the three groups of trees. Fruit size was directly related to seed weight and inversely related to number of seeds per fruit.Germination in nursery bags did not correlate neither to fruit nor seed variables, but correlated in seedbed ($r = 0.99$, $P = 0.03$) to both fruit weight and number of seeds per fruit. The conclusion is that weightier fruits correspond to higher number of seed per fruit, smaller seeds and higher germination in Spanish cedar which would be useful to sure the plant production in nurseries.

Keywords: *Cedrelaodorata*, fruit-seed-characteristics, germination

1. Introduction

Spanish cedar (*Cedrelaodorata* L.) is native to the neotropical region (Pennington *et al*., 1986). This speciesis important for the diversity of uses of its wood, which is considered one of the major tropical woods in the forest market. For this reason this species has been overexploited (Navarro *et al*., 2002). In 1997 the species was classified in the Red List Category and Criteria as vulnerable by selective logging (Hilton – Taylor, 2000). In 2010, CITES considered *C. odorata* as an endangered species. Therefore, research on morphological, genetic and physiological aspects as well as physical, biotic and ecological aspects of *C. odorata*is useful to eliminate factors that limit its development and prevent the establishment of plantations (Alvarez, 1999).

For the successful establishment of forest plantations both the biological quality of the used seed and the conditions of germination are important since they determine the quality of the produced seedlings (Bewley, 1997).Betancourt (1983) quoted that larger seeds tend to germinate quickly and produce vigorous and larger seedlings, compared to the small seeds which germinate more slowly, resulting in weak and poorly formed seedlings.This is true for cultivated species such as cacao (*Theobroma cacao* L.) (Frazáo*et al*., 1984) andpapaya (*Carica papaya* L.) (Neves-Martins *et al*., 2005) or for some forest species such as Black lapacho (*Tabebuiaheptaphylla*) (Damsceno-Riveiro*et al*., 2012),Drumstick tree (*Moringaoleifera* Lam) (Bezerra*et al*., 2004), *Acacia catechu*Willd.,*Acacia nilotica*Willd., *Albizzialebbek* and *Dalbergiasissoo*Roxb. (Khera*et al*., 2004). However, this relation has not been proven for most tropical forest species.

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Since studies of seed germination are essential for the improvement of techniques that enable appropriate management (Trindade-Lessa *et al.*, 2015) and the interest to know, characterize and manage the tropical forest resources has increased in recent years, the objective of this study was to determine the relationship among fruit and seed characteristics with seed germination of Spanish cedar (*C. odorata* L.).

2. Material and methods

2.1 Study site and experimental design

In the state of Tabasco Mexico, 45 Spanish cedar trees were selected in the municipalities of Cardenas (site 1, 17° 56' - 18° 25´ NL and 93° 17' - 94° 07´ WL, 13 trees), Huimanguillo (site 2, 17° 19' - 18° 13´ NL and 93° 18' - 94° 07 'WL, 18 trees) and Cunduacan (site 3, 17° 57´ - 18° 15' NL and 93° 01´ - 93° 24' WL, 14 trees). Trees were selected from April to May 2011 based on dasometric variables such as total and commercial height (≥ 10 m and ≥ 6m), maturity (on reproductive stage) and healthiness (without visible damage per fire or pests). On Februaryand March 2012,the mature fruits on the middle part of each tree cup were harvested. The indicator of a mature fruit was their brown-dark color (Rodríguez *et al.*, 2001). Then 100 fruits were taken at randomfrom each tree to determine their weight, length and thickness. Later, fruits were dried at open sky until their dehiscence to collect the seeds. The number of seeds per fruit and its characteristics were determined on 40 fruits per tree. Later 40 seeds were randomly chosen to register the weight and length with and without wing, and the width and thickness of seed, and the weight, width and length of the wing. Weight and length were determined by using an analytical balance (accuracy 0.0001 g) and a digital vernier (0.1 cm Graduation), respectively. The bulk of seed was stored on sealed plastic bags at 2 °C along 4 months until the rainy season when the germination test was carried out.
To relate the fruit and seed characteristics with the capacity to seed germination, 100 seeds from each one of 12 trees for each site were sow both in a germination bed 1.25 x 8.5 x 0.30 m and in nursery bags of 0.5 L capacity. The 12 trees were selected at random among the trees in each site. A mixture of forest soil and cocoa husks ratio of 1:2 was used as substrate. The experimental design was completely random, with four replications of 25 seeds each one per tree. The experiment was established in the nursery of the Graduate College – Campus Tabasco in Cardenas, Tabasco Mexico. The germination percent was evaluated five weeks after sown since germination usually requires from 2 to 4 weeks (Rosero, 1976).

2.2 Statistical Analysis

Data were examined to determine normal distribution and homogeneity of variances required for analysis of variance (ANOVA). Data were transformed by the formula Y = arcsin (sqrt (Y)) previous to ANOVA (Steel *et al.*, 1997). When the ANOVA was performed (Proc GLM, SAS, 2013) and the F test indicated a statistically significant difference, a comparison of means was performed based on the Tukey test ($P \leq 0.05$). Finally, the Pearson correlation coefficient (ProcCorr, SAS, 2013) between germination and fruit and seed variables was calculated.

3. Results and Discussion

The highest values of weight, length, and thickness of fruit were found in sites 1, 2 and 3 respectively, while the minimum values were found in site 1. Also, a lower coefficient of variation was observed for weight fruit in site 1 and for length and thickness of fruit in site 3. The highest number of seeds per fruit was 38 for sites 1 and 2, and 37 for site 3, but with a greater variation in site 2 and lower in site 3 (Table 1). Regarding to seed weight with and without wing, the maximum and minimum values were found at site 2. In this site, the entire seed weight (*i.e.* seed with wing), had less variation than seed weight without wing. The wing weight had highest value at site 3 and the lowest was equal in all three sites, but there was more variation in site 1. For seed length with and without wing, and the wing length and width the maximum values were found at site 2. The minimum values for the length of seed with and without wing were found at site 1 and 2, respectively, while the minimum for both wing length and width were found at site 3. Seed length without wing had more variation at site 2, the other three variables had more variation at site 3. The maximum and minimum values for seed width were found at site 3, but with more variation at site 1, while in seed thickness these values were observed at site 1 but with more variation in site 3 (Table 1).

The fruit characteristics reported here are partially consistent with the results obtained by Alvarez (1999) who reported variations between and within two provenances of *Cedrelaodorata* in Costa Rica; in the provenance 1, the fruit weight was from 2.41 to 11.65 g, and seed number varied from 6 to 35.

In provenance 2, the fruit weight was from 1.98 g to 12.27 g and seed number varied from 29 to 62. However, the coefficients of variation for fruit weight and seed weight were twice greater (CV = 47 and 30, respectively) than those found in the present study (Table 1). Alderete & Márquez (2004) found *C. odorata* fruit weight, length and thickness of 5.87 g, 3.42 cm and 1.97 cm, respectively. Based on these values, our fruits are lighter (2.16 g), larger (3.58 cm) and equal on thickness (1.96 cm) (Table 2). The different weight could be attributed to the water content since we wait almost at the moment of dehiscence determined by the minimum water content on fruits.

Table 1. Descriptive statistics for four variables of fruit and nine of seed from 45 Spanish cedar trees (*Cedrelaodorata* L.), from three locations in Tabasco State, Mexico.

Variable	Maximum			Minimum			Coefficient of Variation		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
FW	7.4	6.5	6.9	0.2	0.7	0.8	15.6	19.7	19.2
FL	47.8	52.4	46.5	10.4	16.8	18.2	8.4	7.7	6.7
FT	35.7	32.3	36.4	9.3	13.0	12.8	8.0	8.5	6.4
SF	38.0	38.0	37.0	0	0	0	29.5	55.1	24.8
SWw	280.0	340.0	280.0	33.0	20.0	42.0	16.5	14.4	14.8
SW	267.0	300.0	257.0	17.0	4.0	9.0	18.3	16.1	15.7
Ww	63.0	120.0	165.0	1.0	1.0	1.0	59.0	50.2	54.5
SLw	30.3	34.0	30.4	7.1	16.2	13.2	8.0	6.9	9.78
SL	12.8	15.0	11.2	2.5	1.9	3.7	11.3	11.5	10.6
WL	22.3	24.7	21.3	7.8	5.8	2.7	11.1	11.6	14.3
WW	10.3	10.8	10.5	4.6	4.1	0.9	9.0	8.9	9.76
Sw	5.9	6.3	9.3	1.9	1.0	0.8	19.2	12.9	14.5
ST	2.4	2.0	2.0	0.1	0.2	0.4	23.1	22.0	25.2

S1: Cardenas, S2: Huimanguillo, S3: Cunduacan, FW: Fruit weight (g), FL: Fruit lenght(mm), FT: Fruit thickness (mm), SF: Seeds per fruit, SWw: Seed weight with wing (mg), SW: Seed weight without wing (mg), Ww: Wing weight (mg), SLw: Seed length with wing (mm), SL: Seed length without wing (mm), WL: Wing length (mm), WW: Wing width (mm), Sw: Seed width (mm), ST: Seed thickness (mm).

The fruit weight did not correlate to fruit size, but correlate to the number of seeds per fruit. Niembro (1996)quoted that the greater weight and size of *Swieteniamacrophylla* (Meliaceae) fruit, the greater number of seeds and seeds of better quality. In our study, the fruits with reduced weight were those which had fewer seeds but heavier. In contrast, for Penagalilin (*Messuaferrea*, Calophyllaceae) Kham *et al.* (1999) cited that the seeds from small fruits weight less than the seeds from large fruits. Moreover, Alderete & Márquez (2004) cited that weight, length and thickness of *C. odorata* fruits are not related to the number of seeds per fruit.

Based on the mean squares and the mean comparison test, statistical differences for fruit weight, number of seeds per fruit and wing weight were observed among the three sites. Although the site 3 was statistically superior in the cited variables, in site 2 were found larger fruits. These fruits had fewer, heavier and larger seeds (Table 2).

Table 2: Mean comparison of four variables for 100 fruits and nine variables for 40 seeds per Spanish cedar tree (*Cedrelaodorata* L.) from three locations in Tabasco State, Mexico.

	Fruit*				Seed**								
Site	FW	FL	FT	SF	SWw	SW	Ww	SLw	SL	WL	WW	Sw	ST
S1	2.0ab	34.8a	18.7a	19.2b	175a	158a	173b	23.4a	8.9a	14.7a	7.2a	3.8a	1.1a
S2	1.9b	38.3a	21.8a	18.7c	187a	160a	205ab	24.1a	9.0a	15.1a	7.5a	3.7a	0.9a
S3	2.6a	34.5a	18.5a	22.4a	170a	146a	245a	23.7a	8.4a	14.5a	8.1a	3.8a	1.0a

* n=1200, ** n=480. S1: Cardenas, S2: Huimanguillo, S3: Cunduacan. FW: Fruit weight (g), FL: Fruit lenght (mm), FT: Fruit thickness (mm), SF: Seeds per fruit, SWw: Seed weight with wing (mg), SW: Seed weight without wing (mg), Ww: Wing weight (mg), SLw: Seed length with wing (mm), SL: Seed length without wing (mm), WL: Wing

length (mm), WW: Wing width (mm), Sw: Seed width (mm), ST: Seed thickness (mm). Different letters in each column indicate significant difference (Tukey, $P \leq 0.05$).

Although there was no statistical significant difference for total seed weight (seed weight with wing), there wasstatistical differences for the weight of the wing. This result could be important in seed dispersion, assuming that the greater weight wing the larger area of wind exposure and the greater possibility of dispersion. However, the weight of the wing can also be an impediment to seed dispersion when there is not wind.

The fact that the heavier and smaller fruits have had more seeds but lighter and with heavier wings (site 3), agrees with Alvarez (1999) who reported that in *C. odorata* smaller fruits had a higher number of seeds per fruit. In contrast,Betancourt (1983) pointed that *S. macrophylla* seeds are heavier on heavier and bigger fruits, since they have successfully completed their growth and could have better biological quality.

Regarding to seed germination, the mean squares showed significant statistical differences among sites, but not within sites (data not tabulated). The mean comparison showed that seed germination of the site 3 was statistically superior to the other two sites both in the germination bed and in nursery bags (Table 3). This contrasts with expectations at site 2 which has a larger percent of germination by having larger seeds. Table 3 shows the overall means for reference.

Table 3. Mean comparison for percent of Spanish cedar (*Cedrelaodorata* L.) seed germination percent in three locations in Tabasco State, Mexico under two nursery conditions.

Location	Germination (%)		
	Seed bed	Nursery bag	Mean
Cardenas	50.0 b	21.0 b	35.5
Huimanguillo	48.0 b	29.0 a	38.5
Cunduacan	71.0 a	30.0 a	50.5
Mean	56.3	26.7	41.5

The difference between the germination percent obtained in seedbed and nursery bag can be attributed to substrate conditions through the influence of moisture, as was noted by Hartmann *et al.* (2011). This is because the research was carried out in natural environmental conditions. In such conditions, moisture uptake by nursery bags is low comparedwith the seedbed. Also, light and temperature conditions of germination in the field are more similar to the conditions in a seedbed than in a bag nursery. Hartmann *et al.* (2011) indicates that the ideal temperature for germination tends to be equal or similar to that prevailing in the natural habitat of the species.

The germination percent in seedbed for site 3, was 9% less than that obtained in routine analysis of germination of Spanish cedar at the Forest Seed Bank at the Tropical Agricultural Research and Higher Education Center in Costa Rica (Mesen&Hersome, 1999). However, such analysis were carried out in controlled conditions while in our research germination was carried out on natural conditions in a nursery at 25 to 32 °C.

The overall percent of germination recorded in this study is 15% lower than that recorded for Mesen&Hersome (1999) using the same type of substrate, but under controlled conditions of humidity, light, and temperature. However, there had not difference between the germination of our study and that reported (56%) by these authors. The low germination obtained in nursery bags, could be attributed both,to microclimatic characteristics of the bag and the features of the seed (e.g. seed moisture content and viability). Specifically, seed viability in Spanish cedar, and in other forest seeds,is affected by time and storage conditions (García, 1998; Jara, 1996). The seed used in this study have been stored in sealed bags for 4 months at 2 °C. Rosero (1976) notes that a dry atmosphere and temperature of 3-5 °C ensure higher germination of Spanish cedar seeds. In puerto Rico, it was showed that when Spanish cedar seeds were stored between 2 and 4 °C in open bags and sealed containers by 4 months of storageand then sown, the germination was 31 to 34%. Instead,seed stored at 25 °C by the same time dropped to 0 or 2% germination when they were sown. Therefore, the germination percent obtained from the seed of the three sites in this study can be considered within normal limits.

However, our germination rates were low compared to germination of fresh seeds of *Toonaciliata* (Meliaceae) where it has been recorded up to 84% germination in laboratory conditions (Grijpma & Ramalho, 1969), or up to 80% in natural conditions (Pérez-Flores J., Pers.Obs.). Even thought, the seed of site 3 germinated faster and at a higher rate than seed of the other two sites.

This result could be explained by the higher biological quality of seeds given by the morphological characteristics of fruits and seeds (small and heavy fruits, small seeds), which agrees with Oberbauer (1990). This author noted that small seeds germinate faster than large due to increased contact with the substrate germination and faster hydration of the embryo. Therefore, we would expect a positive correlation between the size and weight of the fruit and seed germination capacity of the seed, but it was not quite like that.

The percent of germination in bag did not correlate with any of the characteristics of the fruit or seed (Tables 4 and 5). However, the average percent only showed a positive and significant correlation with the wing width of the seed (Table 5). In contrast, the germination percent seedbed showed high positive and significant correlation with fruit weight and number of seeds per fruit. For weight and length of the seed without wing, the correlation was highly significant, but negative (Table 5).

Table 4. **Correlation coefficients and probability for four variables of fruit with the germination percent of Spanish cedar (*Cedrelaodorata* L.).**

Germination (%)	Fruit Variables			
	Weight (g)	Length (mm)	Thickness (mm)	Seeds number
Seedling	0.9985	-0.6235	-0.6101	0.9989
	0.0343	0.5714	0.5822	0.0295
Bag	0.4729	0.3438	0.3597	0.4796
	0.6864	0.7765	0.7657	0.6815
Mean	0.9484	-0.3935	-0.3779	0.9508
	0.2054	0.7425	0.7533	0.2005

Table 5. **Correlation coefficients and probability for nine variables of seed with the germination percent of Spanish cedar (*Cedrelaodorata* L.).**

Germination	Seed variables								
(%)	SWw	SW	Ww	SLw	SL	WL	WW	Sw	ST
Seedbed	-0.7785	-0.999	0.8586	-0.1602	-0.997	-0.8050	0.9163	0.5664	0.0785
	0.4319	0.0343	0.3426	0.8976	0.0494	0.4044	0.2623	0.6166	0.95
Bag	0.1314	-0.473	0.8841	0.7600	-0.452	0.0885	0.8184	-0.4096	-0.8109
	0.9161	0.6864	0.3095	0.4504	0.7015	0.9436	0.3897	0.7313	0.398
Mean	-0.5840	-0.948	0.9639	0.1076	-0.941	-0.6186	0.9897	0.3273	-0.189
	0.603	0.2054	0.1715	0.9314	0.2205	0.5754	0.0913	0.7877	0.879

SWw: Seed weight with wing (mg), SW: Seed weight without wing (mg), Ww: Wing weight (mg), SLw: Seed length with wing (mm), SL: Seed length without wing (mm), WL: Wing length (mm), WW: Wing width (mm), Sw: Seed width (mm), ST: Seed thickness (mm).

The correlation between the percent of germination in seedbed with fruit weight and number of seeds per fruit coincides with the results found by Grijpma and Rhamalo (1969) in *T. Ciliata*. These authors pointed out that there is seemingly a positive correlation between seed weight and the percent of germination. Besidesthe correlation among the germination with weight and number of seeds per fruit allows to induce that the weightier the fruit or the greater the number of seeds per fruit, the lesser germination in seedbed. Likewise, a weightier or bigger seed (without wing) related to a lesser germination (also in seedbed). Latter correlation could be explained by some influence that the wing characteristics have on germination (maybe in moisture uptake). It because a high positive and significant correlation between wing width and the mean percent of germination was observed, and the imbibition at the end of germination process is essential for embryo emergence (Bewley, 1997).

A high positive and significant correlation between the weight and number of seeds per fruit, as well as between the thickness and length of fruit was observed. Regarding to the characteristics of the seed, a high positive and significant correlation between length and weight of the seed with or without wing, and between the width and weight of the wing was registered. The correlation between seed length without wing with the fruit weight and number of seeds per fruit was highly significant, but negative. Similarly, the correlation of seed width and the length and thickness of the fruit, and between the seed thickness and seed length with wing was highly significant, but negative (Table 6).

Table 6.Correlation coefficients for four variables of fruit and nine variables of Spanish cedar seed (*Cedrelaodorata* L.).

Variable	FW	FL	FT	SF	SWw	SW	Ww	SLw	SL	WL	WW	Sw
FL	-0.6647											
	0.5371											
FT	-0.6519	0.9998										
	0.5479	0.0108										
SF	0.9999	-0.6590	-0.6461									
	0.0048	0.5419	0.5528									
SWw	-0.8112	0.9761	0.9722	-0.8068								
	0.3976	0.1395	0.1503	0.4024								
SW	-1	0.6647	0.6519	-1	0.8112							
	<.0001	0.5371	0.5479	0.0048	0.3976							
Ww	0.8298	-0.1347	-0.1179	0.8340	-0.3469	-0.83						
	0.3769	0.914	0.9248	0.372	0.7745	0.3769						
SLw	-0.2131	0.8716	0.8797	-0.2057	0.7442	0.2131	0.3684					
	0.8633	0.3262	0.3154	0.8681	0.4657	0.8633	0.7598					
SL	-0.9997	0.6822	0.6697	-0.9995	0.8249	0.9997	-0.8164	0.2362				
	0.0151	0.522	0.5328	0.0199	0.3825	0.0151	0.392	0.8482				
WL	-0.8358	0.9658	0.9612	-0.8316	0.9991	0.8358	-0.3872	0.7146	0.8486			
	0.37	0.1671	0.1779	0.3749	0.0275	0.37	0.7469	0.4932	0.3549			
WW	0.8934	-0.2582	-0.2418	0.89679	-0.4621	-0.893	0.9921	0.2485	-0.883	-0.5		
	0.2966	0.8337	0.8445	0.2918	0.6942	0.2966	0.0803	0.8401	0.3117	0.6667		
Sw	0.6100	-0.9975	-0.9985	0.6040	-0.9582	-0.61	0.0640	-0.9042	-0.629	-0.9459	0.189	
	0.5823	0.0452	0.0344	0.5872	0.1847	0.5823	0.9592	0.2809	0.5672	0.2123	0.879	
ST	0.1321	-0.8283	-0.8377	0.1245	-0.6867	-0.132	-0.4435	0.9966	-0.156	-0.6547	0.3273	0.8660
	0.9157	0.3786	0.3678	0.9205	0.5181	0.9157	0.7074	0.0524	0.9006	0.5456	0.7877	0.3333

FW: Fruit weight (g), FL: Fruit lenght (mm), FT: Fruit thickness (mm), SF: Seeds per fruit, SWw: Seed weight with wing (mg), SW: Seed weight without wing (mg), Ww: Wing weight (mg), SLw: Seed length with wing (mm), SL: Seed length without wing (mm), WL: Wing length (mm), WW: Wing width (mm), Sw: Seed width (mm), ST: Seed thickness (mm)

The correlation between weight and number of seeds per fruit, and between the thickness and length of fruit agrees to Niembro (1996) results. This author evaluated the yield and biological quality of seeds onripen and unripe Mahogany (*Swieteniamacrophylla* King) fruits and recorded significant positive correlations between the weight and size of the fruits with the quantity and quality of seeds. Furthermore, despite having less stored reserves, seedlings of small-seeded species, can extend their roots as deep as the large-seeded species because of the rapid growth and the fine roots (Oberbauer, 1990).

In summary, in the characteristics of the seed, a high positive and significant correlation between length and weight without wing, between wing length and weight of the seed with wing, and between the width and weight of the wing was observed. The correlation was high and significant, but negative, among the seed length without wing with both fruit weight and number of seeds per fruit. Such result is logic, otherwise one would expect that heavier fruits or fruits with a higher number of seeds having smaller seeds). Likewise, the correlation was high and significant, but negative, amongseed widthwith both the length and the thickness of the fruit, and between seed thickness and seed length with wing. Therefore, larger fruits have more narrow and small seeds, which does not imply a higher number of seeds, since the wing characteristics (length, width and weight) correlated highly, positively and significantly with seed weight with wing, and among them.

4. Conclusions

In Spanish cedar (*Cedrelaodorata* L.): a) the smallest fruits are heavier and they have a higher number of seeds; b) larger seeds are found in the biggest fruits, although in a small amount; c) the seed weight and length without wing show a higher negative correlation to germination; d) the seed wing widthcould serve not only as a mean of dispersal of the species but also as a mean of propagation and survival as it correlated positively and significantly to the meanpercent of germination.

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