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Article

Características morfométricas, reproductivas y germinativas del germoplasma de *Swietenia humilis* Zucc. en Guerrero

Morphometric, reproductive and germination characteristics of *Swietenia humilis* Zucc. germplasm from *Guerrero* State

Celi Gloria Calixto Valencia[1], Víctor Manuel Cetina Alcalá[1]*, Carlos Ramírez Herrera[1], Miguel Ángel López López[1], Gregorio Ángeles-Pérez[1], Armando Equihua Martínez[2], Erickson Basave Villalobos[3]

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[1]Posgrado en Ciencias Forestales. Colegio de Postgraduados. Campus Montecillo. México.
[2]Posgrado en Fitosanidad. Colegio de Postgraduados. Campus Montecillo. México.
[3]Campo Experimental Valle del Guadiana, CIRNOC. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. México.

*Autor de correspondencia; correo-e: vicmac@colpos.mx
*Correspoding author; e-mail: vicmac@colpos.mx

Abstract

Swietenia humilis is a tropical hardwood species threatened by habitat fragmentation due to deforestation and changes in land use. Therefore, it is essential to characterize the germplasm of natural populations to define conservation, propagation and reforestation strategies. The morphometric and reproductive characteristics of fruits, as well as the viability and germination capacity of seeds of *S. humilis* trees from natural stands from several sites in the state of *Guerrero*, Mexico: *Zacapalco*, *Buenavista*, *Santa Fe Tepetlapa*, *Cieneguillas*, *Tuxpan* and *Arcelia* were analyzed. At each site, fruit and seed size and weight, number and proportion of developed and undeveloped seeds, viability and germination capacity were characterized. A Kruskal-Wallis nonparametric analysis of variance and a comparison of means were performed at a significance level α=0.05. Fruit and seed characteristics varied among the sites evaluated. *Cieneguillas* presented fruits of smaller size, weight and number of seeds. *Tuxpan* had the highest number of seeds but a high proportion of undeveloped seeds. *Santa Fe* presented the highest number of developed seeds, although the heaviest, most viable, vigorous and with the highest germination capacity were those of *Zacapalco*. *S. humilis* trees in the natural stands analyzed in the state of *Guerrero* produce fruits and seeds that differ in their morphometric and reproductive characteristics, as well as in their germination capacity.

Key words: *Caobilla*, germination, Meliaceae, seed production, fruit size, seed viability.

Resumen

Swietenia humilis es una especie tropical de madera preciosa amenazada por la fragmentación de su hábitat debido a deforestación y cambios en el uso del suelo. Por lo tanto, es esencial caracterizar el germoplasma de las poblaciones naturales para definir estrategias de conservación, propagación y reforestación. Se analizaron características morfométricas y reproductivas de frutos, así como la viabilidad y capacidad germinativa de semillas de *S. humilis* de seis sitios en el estado de Guerrero, México: Zacapalco, Buenavista, Santa Fe Tepetlapa, Cieneguillas, Tuxpan y Arcelia. En cada sitio, se caracterizó el tamaño y peso de frutos y semillas, el número y proporción de semillas desarrolladas y no desarrolladas, la viabilidad y la capacidad germinativa. Para cada variable se hizo un análisis de varianza no paramétrico *Kruskal-Wallis* y una comparación de medias. Las características de los frutos y semillas variaron entre los sitios evaluados. Cieneguillas presentó menor tamaño de frutos, peso y número de semillas. Tuxpan registró la mayor cantidad de semillas, pero una proporción alta fueron no desarrolladas. Para Santa Fe se obtuvo la mayor cantidad de semillas desarrolladas, aunque las más pesadas, viables, vigorosas y con mayor capacidad germinativa fueron las de Zacapalco. Los árboles de *S. humilis* de los rodales naturales analizados en el estado de Guerrero producen frutos y semillas que difieren en sus características morfométricas y reproductivas, así como en su capacidad germinativa.

Palabras clave: Caobilla, germinación, Meliaceae, producción de semillas, tamaño de frutos, viabilidad de semillas.

Introduction

Swietenia humilis Zucc. it is a forest species of high ecological and socioeconomic importance that is distributed along the Pacific coast of Mexico and Central America (Rosas *et al*., 2011). Its wood is of excellent quality and medicinal properties have been attributed to it, for which it has been the subject of pharmacological studies (Flores *et al.*, 2019). However, the distribution area and size of the natural populations of S. humilis have decreased rapidly (Rosas *et al*., 2011). Due to the fragmentation of its habitat, it is common to find isolated trees or small remnant stands, which has affected its natural regeneration and genetic diversity (Rosas *et al*., 2011). For example, in the *Chamela-Cuixmala* Biosphere Reserve, allelic richness in seeds from isolated populations was lower (6.1 alleles per *locus*) compared to that from continuous forests (8.3) (Rosas *et al.*, 2011). Consequently, *S. humilis* is included in the red list of threatened species of the International Union for Conservation of Nature (Barstow, 2019).

The reduction and loss of natural populations of *S. humilis* justifies the need to implement actions for its conservation and sustainable use. For species under similar conditions, seed storage in germplasm banks, reforestation and restoration projects are the main *ex situ* and *in situ* conservation and preservation strategies that have contributed to the sustainability of such forest genetic resources (Ledig, 2004). However, the limited availability of good quality germplasm, mainly from the genetic and physiological components, limits the success of these conservation, preservation and management strategies (Núñez-Cruz *et al*., 2018).

The collection of germplasm of native species does not guarantee fruits and seeds with the sufficiency and quality required (Aitken, 2004); the latter in terms of viability, vigor and germination capacity. This is explained because, for the most part, the material comes from small and fragmented populations or from isolated individuals, in which the parental sources suffered impacts at the genetic level (Pereira *et al.*, 2020). On the one hand, it is argued that these sources of germplasm are not the most appropriate due to the adverse effects caused by habitat fragmentation on the productivity and reproductive success of forest species (Broadhurst and Boshier, 2014; Melo *et al.,* 2021); on the other, it is emphasized that the use of local seed sources should be prioritized to meet the immediate demands for germplasm in reforestation and restoration programs on a small and medium scale (Melo *et al.*, 2021), since this genetic material is better adapted to the conditions of the plantation sites (Broadhurst and Boshier, 2014).

A relevant guideline is to collect germplasm in local populations, specifically when it comes to native species whose biological and forestry knowledge is limited (Luna-Nieves *et al*., 2019). However, it is important to know the characteristics such as the size of the fruits and the number of seeds they produce, as well as their weight and germination capacity, which is necessary information for good germplasm management and to better plan future breeding projects, conservation or propagation (Valverde-Rodríguez *et al.*, 2019).

There are few studies aimed at describing the fruits and seeds of *S. humilis*. Some antecedents are the works carried out by Patiño (1996) and González-Vélez *et al.* (2020). It is necessary to generate information to identify local sources of good quality germplasm to meet the demands of germplasm banks and forest nurseries. The objective of this study was to analyze the morphometric and reproductive characteristics of *S. humilis* fruits in six sites in the state of *Guerrero*, as well as the viability and germination capacity of its seeds. It is hypothesized that there are differences in the morphometric and reproductive characteristics of *S. humilis* fruits, as well as the viability and germination capacity of its seeds among the six study sites.

Materials and Methods

The germplasm of *S. humilis* was collected in February 2020 in natural stands of six populations in the state of *Guerrero*, Mexico (Table 1).

Table 1. Geographical location and environmental conditions of the *Swietenia humilis* Zucc. germplasm collection sites in *Guerrero*, Mexico.

Site	Longitude	Latitude	Altitude (masl)	Average temperature (°C)	Annual mean precipitation (mm)
Zacapalco	18°32´22″	99°26´26″	1 204	23.7	1 091
Buenavista	18°29´12″	99°25´09″	1 190	24.1	1 048
Santa Fe Tepetlapa	18°33´21″	99°25´43″	1 117	24.2	1 052
Cieneguillas	18°25´05″	99° 28´24″	1 053	24.7	988

Tuxpan	18°21´25˝	99° 30´17˝	755	25.3	1 010
Arcelia	18°19´32˝	100°16´29˝	413	27.8	1 142

Based upon the criteria for the identification and establishment of forest germplasm production units (Conafor, 2016), five trees with outstanding phenotypic characteristics were selected at each site: clean stem (≥3 m) with straightness, larger crown according to visual inspection, abundant presence of fruit, visible absence of pests and diseases. The normal diameter of all individuals was recorded, this ranged between 38 and 60 cm. From each tree, at least two fruits were collected from the middle part of the crown, to avoid the dehiscence of the capsules (Sol *et al.*, 2016) and these were transported in jute sacks to later reduce their moisture content by exposing them to the sun inside paper bags for five days.

Morphometric characterization and reproductive capacity of the fruits

The following morphometric variables were measured in the fruits of each site: diameter, measured at the widest part of the capsule with a Mitutoyo® Digimatic CD-4” AX caliper; length of the capsule, measured with a vinyl tape measure from the base of the peduncle to its apex, that is, along the carpelar suture; fresh weight of the fruit, recorded with a digital scale with 0.01 g a precision (iBalance® i2001, Myweigh®). Likewise, with the methodology adapted from Willan (1991), the reproductive variables were evaluated for each fruit: number of developed seeds (full seeds that present all the essential tissues for germination), number of undeveloped seeds (flat seeds lacking embryo and endosperm), and total number of seeds (developed and undeveloped seeds). Then the proportion of undeveloped and developed seeds was determined.

The developed seeds were weighed to estimate their average weight, the weight of a thousand seeds using the complete sample method (seed weight/seed number*1 000) and the number of seeds per kilogram (1 000 g/seed weight). The sample size per site was: *Zacapalco* n=22, *Buenavista* n=29, *Santa Fe* n=13, *Cieneguillas* n=44, *Tuxpan* n=29, *Arcelia* n=28. Each fruit was a replication under a completely randomized design.

Viability test

The viability of the seeds was evaluated with a 2,3,5-triphenyl tetrazolium chloride solution at 0.1 %, as described by Barone *et al.* (2016). For each site, four replicates of 25 seeds were analyzed. The viability percentage was determined based on the ratio of the number of viable seeds according to the staining pattern and the number of seeds sampled.

Emergence test

In April 2020, an essay was established to assess the emergence of seedlings at the nursery. This was made at a facility located in the community of *La Bajada*, *Coyuca de Catalán*, *Guerrero* (18°19´11.81” N, 100°40´18.10” W, 254 masl), where the temperature ranged between 20 and 40 °C. and relative humidity between 40 and 90 % (data recorded with a Hobo® MX2304 environmental thermometer). 3 000 seeds were used for this trial. A completely randomized experimental design was used, with five replications (blocks) of 100 seeds per site (treatment). The wing was removed from each seed and the seeds were held against the light to delimit the area of the embryo and avoid damaging it; subsequently, they were soaked in artesian well water for 12 h before sowing. Sowing was carried out at 2 cm deep in plastic trays with 50 cavities (180 mL volume per cavity), filled with a substrate mixture 70 % peat moss, 15 % perlite and 15 % vermiculite. Daily irrigations were applied to keep constant moisture of the substrate.

Based on the methodology of Carvalho *et al.* (2020), an emerged seedling was considered when the hypocotyl appeared on the surface of the substrate. Daily counts were made from the time the first seedling emerged until there was no more emergence in consecutive monitoring (stabilization stage). The maximum emergence value or peak value (*VM*), emergence percentage (*PE*), mean daily emergence (*EMD*) and Czabator germination value (*VG*) were determined based on equation (1) (Willan, 1991):

$$VG = EMD\ (final) \times\ VM$$

Where

VG = Germination value

EMD (final) = Average daily emergence, that is calculated as the accumulated percentage of seeds emerged at the end of the trial, divided by the number of days

VM = Maximum emergence value that is the sum of the emergence percentage divided by the number of days

Finally, the emergence percentage (*PE*) was calculated with the proportion of the number of seeds emerged in relation to the number of seeds sown.

Statístical analysis

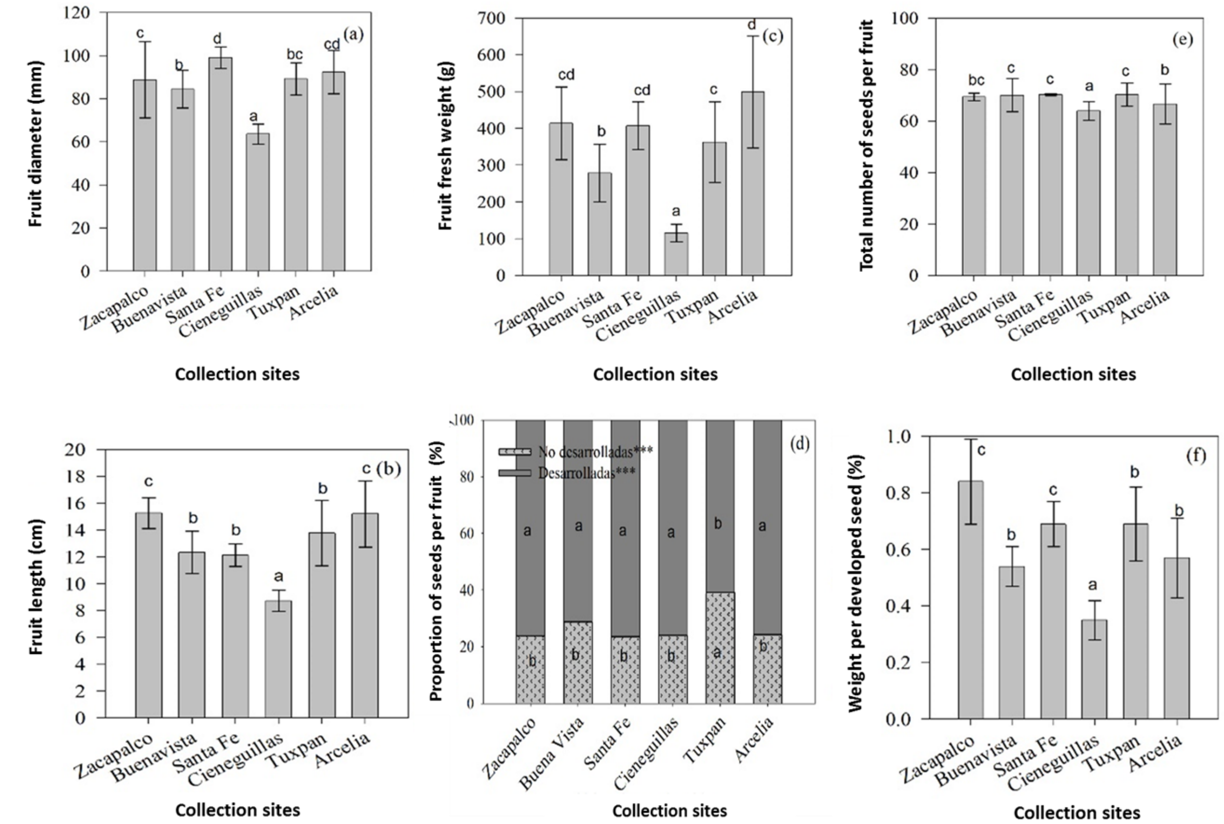
The statistical differences between the sites for the morphometric, reproductive, viability and emergence variables, were determined with a non-parametric analysis of variance (Kruskal-Wallis test) due to non-compliance with the assumptions of either normality or homogeneity of variances ($P<0.05$) or both, as was the case for the count and percentage variables. Normality was validated with the Shapiro-Wilk test and homogeneity with the Levene test (Balzarini *et al.*, 2008).

In the variables with differences, a multiple comparison of means was made from the means of the ranges (comparison of the minimum significant difference with the absolute value of the difference between both mean ranges) according to the procedure described by Balzarini *et al.* (2008). Statistical analyzes were performed with the InfoStat program (Balzarini *et al.*, 2008).

Results

Characteristics of the fruits

The morphometric and reproductive characteristics of the fruits of *S. humilis* differed between sites ($P<0.0001$; Figure 1). The fruits with the largest diameter were those from *Santa Fe*, with an average value of 99.03 mm, which was 56 % greater than the diameter of the fruits from *Cieneguillas*, which were the smallest (Figure 1a). For its part, *Zacapalco* presented the fruits with the longest length (15.27 cm), almost double the length of those from *Cieneguillas* (8.73 cm) (Figure 1b). The heaviest fruits were those of *Arcelia* with 498.96 g, whose weight was four times greater than that of the *Cieneguillas* fruits, which, as in the previous variables, had the lowest values (Figure 1c). *Tuxpan* had the fruits with the highest proportion of undeveloped seeds (39.23 %) (Figure 1d) although on average, it recorded the highest total number of seeds with around 70 per fruit (Figure 1e). In contrast, *Cieneguillas* presented a higher proportion of developed seeds compared to *Tuxpan* (Figure 1d), but presented the lowest number of seeds (64) per fruit (Figure 1e).



Bars represent means ± standard deviation. Means with a letter in common are not significantly different (*P*>0.05).

Figure 1. Morphometric and reproductive characteristics of *Swietenia humilis* fruits at six collection sites in *Gurrero*, Mexico.

Unlike both sites, *Santa Fe* recorded the highest proportion of developed seeds (76.34 % of a total of 70) followed by *Zacapalco*, which also registered high values (76.06 % of a total of 70) in this variable (Figure 1d). The seeds developed from *Zacapalco* were the heaviest with an average weight per seed of 0.84 g, which is 140 % higher than the weight of 0.35 g recorded in *Cieneguillas* (Figure 1f). These differences in weight are also shown by the values for the weight of a thousand seeds and the number of seeds per kilogram, so that a thousand seeds from *Zacapalco* weigh almost 500 g more than those from *Cieneguillas* and less than half is required for one kilogram (Table 2).

Table 2. Weight of a thousand seeds and number of seeds per kilogram of *Swietenia humilis* Zucc. in each of the study sites.

Collection site	Weight of 1 000 seeds (g)	Number of seeds per Kg
Zacapalco	839.7±154.9d	1 260.5±436.3a
Buenavista	537.5±67.8b	1 897.4±315.0c
Santa Fe	686.0±84.6cd	1 484.2±237.9ab
Cieneguillas	354.5±65.5a	2 926.3±606.3d
Tuxpan	688.9±130.4c	1 496.6±254.0b
Arcelia	572.3±137.1b	1 875.9±583.8c

Means ± standard deviation. Means with a letter in common are not significantly different ($P>0.05$).

Viability

Seed viability did not differ between sites (P=0.8020) and most exceeded 95 %. *Zacapalco* had 98 % viability, followed by *Cieneguillas*, *Arcelia* and *Tuxpa*n which had 97 %, while *Buenavista* and *Santa Fe*, 96 % and 95 % viability, respectively.

Emergence

Emergence began 10 days after planting and the maximum accumulated emergence was obtained at 22 days (Figure 2). In the seeds of *Zacapalco*, *Buenavista* and *Santa Fe*, there was an emergency from day 10 and one day later an emergency was registered in the seeds of *Cieneguillas*, *Arcelia* and *Tuxpan. Zacapalco* registered the maximum accumulated emergency with 86 % and those of *Arcelia* the minimum, with only 17 % (Figure 2). Likewise, there were significant differences in the emergence parameters for all sites (P<0.0001; Figure 3).

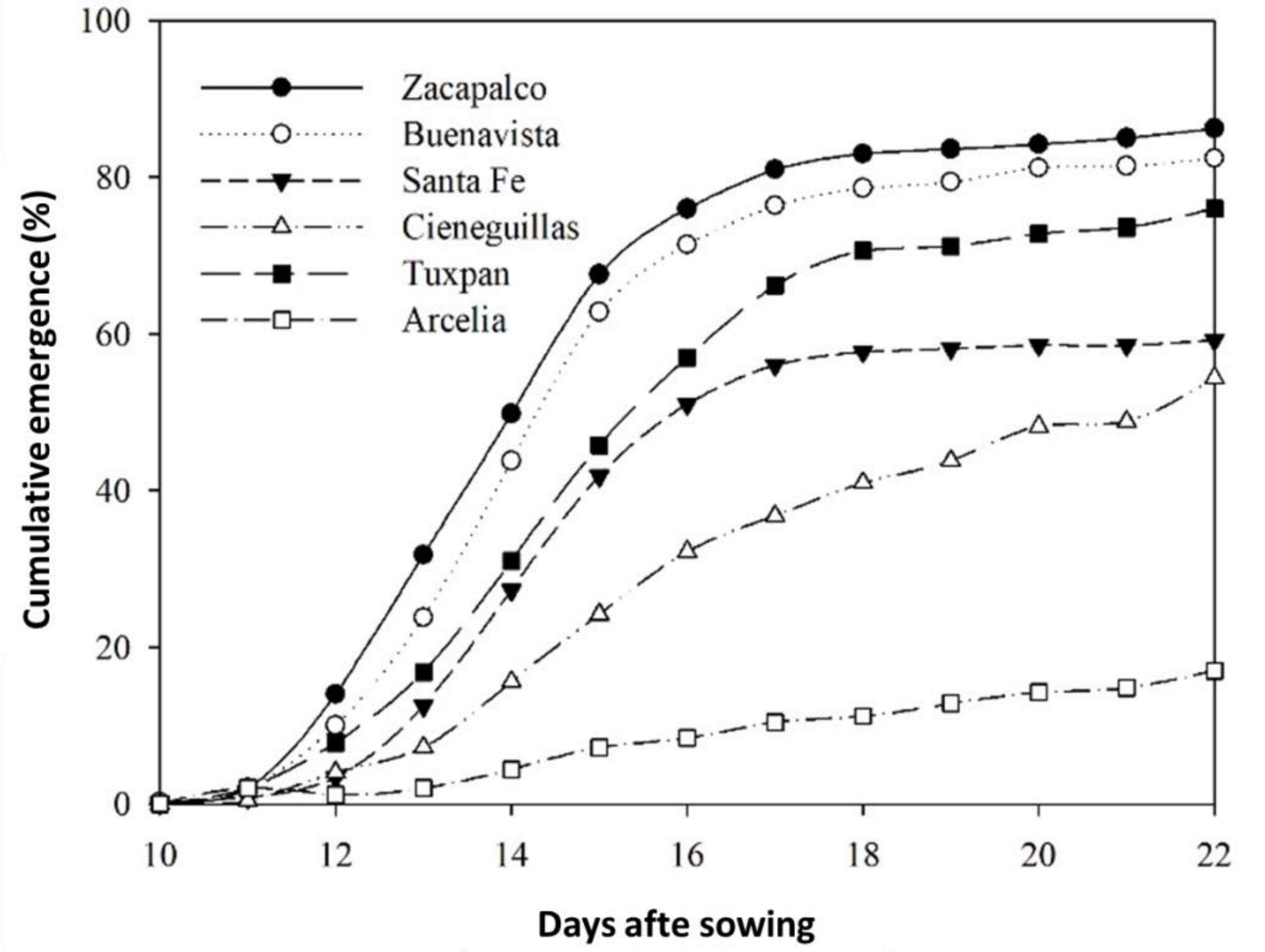
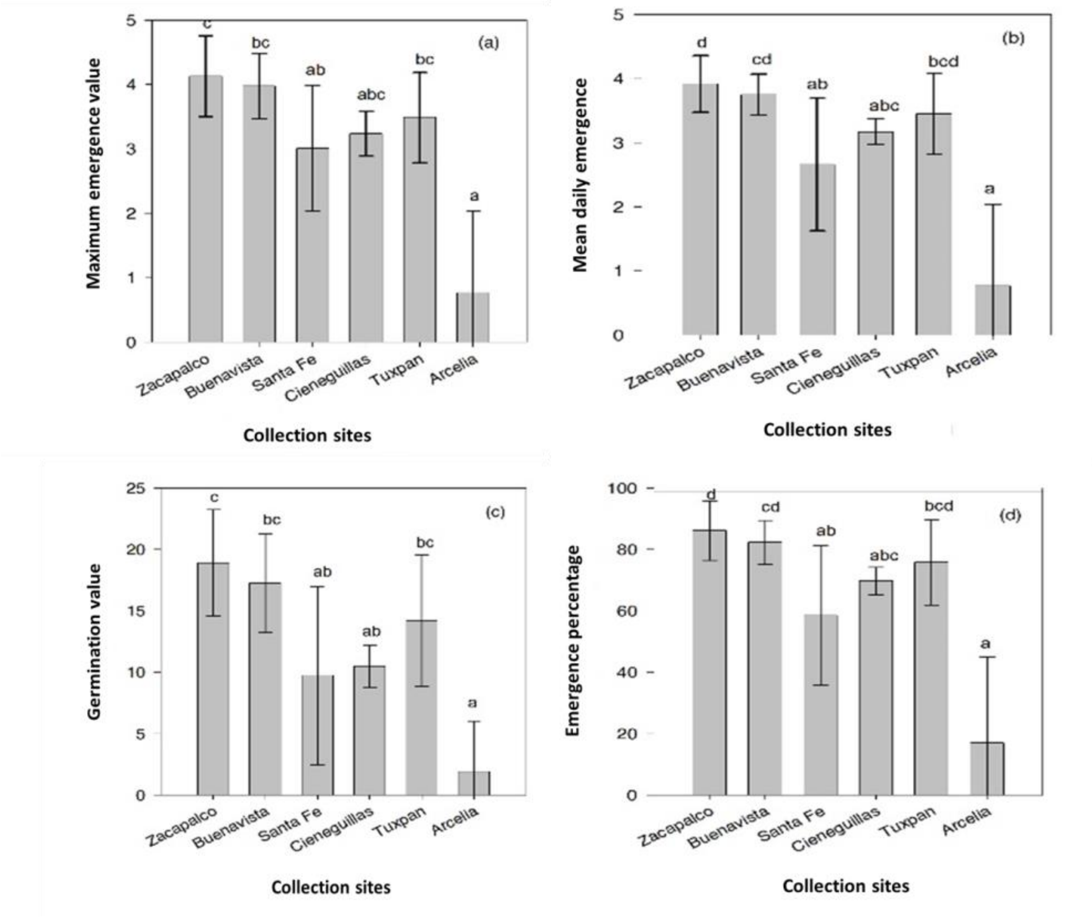


Figure 2. Cumulative emergence curve of *Swietenia humilis* Zucc. seeds from six collection sites in the state of *Guerrero*, Mexico.



Bars represent means ± standard deviation. Means with a letter in common are not significantly different (*P*>0.05).

Figure 3. Maximum emergence value (a), average daily emergence (b), germination value (c) and emergence percentage (d) in *Swietenia humilis* seeds from six collection sites in *Guerrero*, Mexico.

According to the cumulative emergence trend, *Zacapalco* and *Buenavista* had the seed with the highest percentage of emergence, which was higher than 80 % (Figure 2). In turn, *Zacapalco* stood out with the highest observations in peak value, average daily emergence and germination value (3a, b and c). In contrast, the percentage of emergence of the seeds of the others was lower. *Tuxpan*, *Cieneguillas* and *Santa Fe* registered an emergence percentage that ranged between 80 % and 60 %, together with the respective reduction in the values of the germination parameters (Figure 3a, b, c and d). The *Arcelia* seed presented a drastic reduction in the values of all the emergency parameters (Figure 3), for example, when comparing their emergence values against those of *Zacapalco*, there is a difference of almost 70 % (Figure 3d).

Discussion

The variation in the size and weight of the fruit and the amount of seed produced recorded by *S. humilis* is a condition that has also been recorded in *S. macrophylla* King at the stand level (Pramono *et al.*, 2019) and families (Niembro and Ramírez -Garcia, 2006); however, the average values in the size and weight of the fruits, as well as the amount of seeds contained in them, are similar to the values for *S. humilis* (Patiño, 1996).

In *Zacapalco*, *Santa Fe* and *Arcelia*, the largest fruits (size and weight) and the heaviest seeds were found, a response that can be explained hypothetically with the reproductive effort of the trees (Turner, 2001), which usually varies and is influenced by internal factors such as the reproductive success of each parental source as well as the availability of nutriments that affect seed development (Pramono *et al*., 2019).

There is a close relationship between the size or weight of the seeds and the amount of reserves they contain (Kitajima, 2007). These characteristics affect seedling growth rates and their competitive ability under natural conditions (Khurana and Singh, 2001; Kitajima, 2007). In practice, the size of the seed is an important characteristic to produce plants in the nursery, since in some species such as *Enterolobium contorsiliquum* (Vell.) Morong (Trindade-Lessa *et al*., 2015) and *S. macrophylla* (Pramono *et al*., 2019), larger or heavier seeds tend to germinate faster and in a more uniform way.

The magnitude of the variation in the weight of the seeds recorded between the studied sites, based on the difference between the seeds of *Zacapalco* and those of *Cieneguillas*, can be attributed to the fact that the amount of reserves in the seeds that each tree produces depends, in turn, the number of fruits that it is able of producing, since a plant can invest both in a reduced number of larger or heavier seeds, as well as in more but smaller seeds (Kitajima, 2007). However, to verify this assumption it is necessary that, in subsequent studies, the total production of fruits is also counted because in this study it was not planned to assess that aspect.

On the other hand, although it has been reported that in the morphology of the fruits there are traits that remain constant within the same species, such as the size of the fruit and its number of seeds (Sol *et al*., 2016), the actual data indicate differences in these characteristics between the evaluated sites. Additionally, this last author also mentioned that there are other traits that are not kept constant and that exhibit variations between and within the parental sources, such as germination capacity and seed vigor. This is corroborated by the results of the germinative parameters obtained in this study. This behavior is explained by the wide variation in the germination response recorded by some forest species, such as *Enterolobium cyclocarpum* (Jacq.) Griseb., depending on the origin of the material due to the influence of environmental conditions such as temperature, humidity, light and soil fertility (Viveros-Viveros *et al*., 2017).

In the germinative response, the seed from *Zacapalco* and *Buenavista* are outstanding, so that these sites may have potential as local sources of germplasm in the study region. This condition is supported by the data reported with *S. humilis* seeds collected in *Huehuetlán el Chico* municipality, *Puebla* State (González-Vélez *et al.*, 2020), because the figures recorded in germination coincide with the values determined in recently collected seeds in that area. It should be noted that for the differences in emergence, the influence of the genetic quality of each parental source is not excluded, mainly because *S. humilis* is a threatened species by the fragmentation of its habitat, and a consequence of the fragmentation of the natural populations so documented, is precisely the affectation of the germinative capacity (Pereira *et al*., 2020).

In addition, the high proportion of undeveloped seeds recorded in *Tuxpan* or the limited germination capacity of the sites with germination percentages below 70 %, may be evidence of a decrease in the reproductive success of *S. humilis* due to negative impacts on the genetic diversity as argued by Broadhurst and Boshier (2014), and experimentally demonstrated by Rosas *et al.* (2011) with populations of *S. humilis* from the Pacific coast in the state of *Jalisco*.

However, given that the other sites, mainly *Zacapalco* and *Santa Fe*, had fruits with high production of developed seeds and high germination capacity, it is recommended that the hypothesis of the effect on reproductive capacity be verified with studies at the genetic level with an experimental approach similar to that of the studies by White *et al.* (2002), Moraes *et al*. (2018), Garcia *et al*. (2019) and Manoel *et al*. (2021) because, in addition, in several tropical species a reduction in their genetic diversity has not been observed despite suffering fragmentation in their natural populations.

This is particularly true for *S. humilis*. A study carried out in fragmented natural populations in regions of Central America, confirms that the remnant patches of trees and isolated individuals have an important role in maintaining genetic connectivity and improving the variability of populations because they buffer the deleterious genetic effects of habitat destruction (White *et al*., 2002). These implications are supported by the fact that *S. humilis* and its congener *S. macrophylla* are predominantly allogamous species that preserve high levels of diversity and genetic connectivity, thanks to their cross-mating system due to a certain degree of self-incompatibility and dichogamy, as well as their generalist pollination system and long-distance dispersal (White *et al*., 2002; Lemes *et al*., 2007).

Finally, the results of this study also suggest how decisive the collection date is in the quality of the germplasm, based on the notoriety of the germinative responses of the *Arcelia* seeds, which in this case were the worst. Although the trees at this site produced large fruits with enough seeds of an intermediate weight, it is possible that the date of collection there was not appropriate, because the seeds of some fruits did not have the characteristic brown hue with which they are grown in the fieldand that it can visually define its maturity (Salazar *et al*., 2000). Several studies with forest species from the tropics have emphasized the importance of defining adequate harvesting times due to the asynchrony in maturity that the species have at the tree or population level according to their distribution interval (Núñez-Cruz *et al*., 2018; Luna-Nieves *et al.*, 2019). This is particularly important for native tropical species such as *S. humilis*, because information on reproductive phenology is lacking for several local populations, such as that already generated for other species of the dry tropics (Núñez-Cruz *et al.*, 2018).

Conclusions

The *S. humilis* trees from the natural stands analyzed in the state of *Guerrero*, produce fruits and seeds that differ in their morphometric and reproductive characteristics, as well as in their germination capacity. This information has important implications because it serves as the basis for defining their use as local sources of germplasm to meet the demands for this small or medium-scale material for conservation, propagation and reforestation initiatives that are promoted with the species.

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Conflict of interests

The authors declare no conflict of interest.

Contribution by author

Celi Gloria Calixto-Valencia: conducting the study and writing the manuscript; Víctor Manuel Cetina-Alcalá: coordination and administration of the study; Carlos Ramírez-Herrera: experimental approach, estimation of variables and review of the manuscript; Miguel Ángel López-López, Gregorio Ángeles-Pérez and Armando Equihua-Martínez: experimental approach and review of the manuscript; Erickson Basave-Villalobos: obtaining and processing the experimental material and statistical analysis.

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