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# Substrate for Seedling Emergence of White Sapote in Brazil

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## ABSTRACT

Aiming to evaluate seedling emergence of white sapote (*Casimiroa edulis* - Rutaceae) an experiment was developed with seeds from mature fruits originating from the Active Germplasm Bank of the São Paulo State University, Jaboticabal, Brazil. A completely randomized design was adopted and four treatments (substrates) were performed, with five repetitions of 50 seeds each, reaching 250 seeds in total. Substrates studied were: Plantmax® (processed and enriched barks + expanded vermiculite + processed and enriched peat); Coconut fiber; Washed sand and soil mixture commonly used for seedling formation [soil (red Oxisol) + sieved sand + bovine manure (3:3:1)]. The percentage seedling emergence and emergence rate were calculated at the end of the experiment, i.e., at 40 days after sowing. Seedling emergence and the speed of this process were not influenced by the substrate and all substrates studied could be used for seedling establishment of white sapote.

**Keywords:** *Casimiroa edulis*, exotic fruitful, growing media, seed propagation

## INTRODUCTION

White sapote (*Casimiroa edulis*, Rutaceae) also known as matasano and zapote celanco is a fruit native to Central America and Mexico (Yahia 2005). This species presents the following characteristics: the plant can reach 15 m whether propagated by seeds; fruit 4 to 10 cm in diameter; each with white seeds, 2.5-5.0 cm long and 1.25-2.5 cm thick; the harvest time is indicated by changes in colour from green to yellow (Donadio *et al*. 1998). White sapote is commonly propagated by seed but vegetative methods are used as grafting in California and Florida in midsummer and air-layering in New Zealand (Morton 1987).

Some studies on white sapote exist, such as structural characterization of the fruit (Soni and Singh 1992), floral induction (Okuda *et al*. 2005) and harvesting index (Yonemoto *et al*. 2006). However, no references exist on seedling emergence of this species or substrate recommendation for seedling production. It is of significant importance to note that white sapote is resistant to *Phytophthora* and *Armilaria* (Yahia 2005), and is thus a potential species to be used as scion in inter-specific grafting studies, specially with economically important species of the same botanical family, such as orange (*Citrus* sp).

Despite the expansion of exotic fruit cultivation in Brazil, including white sapote, basic information about propagation, plant physiology and nutrition is needed. In addition, research on seedling emergence in a range of substrates is important to the expansion of exotic fruiticulture in Brazil.

Increased awareness of the environment as well as the need to dispose of or re-use ever-increasing amounts of waste and reduce the consumption of non-renewable resources like peat, have encouraged studies about alternative composts (Grigatti *et al*. 2007), biosolids (Papafotiou *et al*. 2004) and the use of products such as coconut fiber as substrate for seedling formation. Coconut fiber is abundant in Brazil where coconut is used by cosmetic, automobilist, chemist and food industries, so is a waste that could be used as substrate for seedling production.

The choice of substrate affects the root system and nutritional status of seedlings (Leinfelder *et al*. 1991). Accordingly, a good substrate is absent of pathogens, has a high level of soluble nutrients and adequate pH, bulk density, pore space, electrical conductivity and C/N-ratio (Horn 1996; Di Benedetto 2007), specially for fruits (Meletti 2000) and maintains an adequate proportion between water availability and air (Villagomez *et al*. 1979).

Nowadays, there are many cultivation systems for seedling formation of fruit species that use mineral, organic, natural and/or synthetic substrates, whose characteristics drastically differ from soil, nevertheless seedling emergence be controlled by species-specific requirements and the availability of favourable seedbed conditions (Leinfelder *et al*. 1991). Additionally, the substrate must have the necessary conditions for seedling emergence and development of each species.

In this sense, the objective of this research was to study the effect of substrate on seedling emergence of white sapote.

## MATERIALS AND METHODS

### Plant material

Seeds of white sapote (*Cassimiroa edulis* Llave & Lex), an exotic fruit species, were used in this study. Seeds were manually extracted from mature fruits released from one 9-year-old white sapote plant, formed by seed propagation, belonged to Germplasm Active Bank of São Paulo State University (Brazil) cultivated without specific management for fertilization, pruning, irrigation and control of pests and diseases.

### Growth conditions and experimental design

The experiment was carried out in a canvassed shelter under 50% of luminosity of the Faculty of Agrarian and Veterinary Sciences, São Paulo State University (Jaboticabal, Brazil). The local climate was classified as Cwa with an average precipitation of 1400 mm·year$^{-1}$ and temperatures between 18.5 and 25.0°C.

A completed randomized design with four treatments (substrates) was adopted, with five repetitions of 50 seeds each, with a total of 250 seeds. Substrates studied were: Plantmax® [Eucatex, Brazil (processed and enriched bark + expanded vermiculite +

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**Table 1** Some physical and chemical characteristics of the substrates used in the experiment.

| Characteristic | Plantmax® | Red Oxisol | Coconut fiber | Washed sand |
|---|---|---|---|---|
| Dry density (kg·m$^{-3}$) | 450 | 1380 | 92 | 1335 |
| Organic matter (%) | ** | 27 | 89 | 0.2 |
| Total porosity (%) | ** | 50 | 94 | 42 |
| Aeration capacity (%) | ** | 14 | 20 | 12 |
| WRC (%) | 47.5 | 28.5 | 51 | 26 |
| pH | 5.5 | 5.6 | 5.8 | 6.3 |

pH in water; WRC = Water retention capacity; **not informed by manufacturer.

processed and enriched peat)]; Coconut fiber; Washed sand and a soil mixture used commonly for fruit seedling formation in Brazil under soil (red Oxisol):sieved sand:bovine manure at a 3:3:1 ratio. Some physical and chemical characteristics of the substrates studied are in **Table 1**.

After extraction, the seeds were washed with running water air-dried under shade conditions for 24 hours, cleaned by rubbing on a sieve until being naked, treated with Captan® (Milenia, Brazil) fungicide and sown at a depth of 4 cm in polyethylene trays measuring 80 mm in height, 310 mm in width and 500 mm in depth filled with each substrate. Fifty seeds were sown in each tray and irrigated once daily with ~0.5 L each of good quality water (pH = 6.8 and Electrical conductivity = 0.3 dS.m$^{-1}$) according to Ayers and Westcot (1999) by a microsprinkler irrigation system (Amanco, São Paulo, Brazil). No tests of viability were conducted.

Two batches of 25 seeds were dried at 105 ± 3°C for 24 hours to determine the initial humidity of seeds (Anonymous 1992), which was 49%.

When seedlings appeared visibly from the soil surface they were considered as emerged, and counted. After sowing seedling emergence was monitored daily during peak and at 2-day intervals during non-peak periods. The beginning of emergence was considered to be at 18 days after sowing (DAS) and stabilization at 32 DAS.

## Statistical analyses

At the end of the experiment, the emergence percentage (%) and emergence rate were calculated by a speed emergence method proposed by Maguirre (1962). The experiment was conducted in duplicate, so all results refer to the average of two assays.

Statistical analyses included analysis of variance (ANOVA) and mean separation of seedling emergence percentage and emergence rate data using Tukey's test (Ferreira 2000) and correlation analysis between dependent variables and substrate characteristics. Emergence rate data were $arc\ sin\sqrt{x/100}$ transformed, since it did not have a normal distribution by the Kolmogorov-Smirnov test. SAS program was used and terms were considered significant at P≤0.01.

## RESULTS AND DISCUSSION

Seedling emergence ranged from 70.142% to 78.102% of viable seeds (**Table 2**). The substrates studied had no statistical influence on seedling emergence percentage of white sapote, but Plantmax®, which promoted nearly 8% more seedling emergence than washed sand, was quantitatively (i.e. statistically) superior. The lack of significance is in agreement with other studies about fruit species found in the scientific literature such as Iossi *et al* (2003) who studied sphagnum, sawdust, sand and vermiculite as substrates for seedling production of date (*Phoenix reebelenii* O'Brien) and observed no difference among these substrates; Santos and Nascimento (1999) that tested sand, vermiculite and black land as substrates for *Hancornia speciosa* Gomes and verified no statistical difference among them. On the other hand, Yang *et al* (2008) reported that soil type and depth had significant effects on seed germination and seedling emergence of *Camellia nitidissima*, a recalcitrant seed species and concluded that the emergence percentage of seeds in clayed soil was significantly lower than that in mixed (50% of sandy and 50% of clayed) or sandy soil. These authors explain that sandy and mixed soils have larger particle size than clayey soil, so more air would penetrate deeper into sand than into clay, what may have resulted in the better seed germination and emergence in sandy and mixed soil than in clayed soil at the same soil depth.

**Table 2** Average seedling emergence of white sapote as a function of substrate.

| Substrate | Seedling emergence (%) |
|---|---|
| Plantmax® | 78.102 a |
| Soil mixture | 74.102 a |
| Coconut fiber | 70.100 a |
| Washed sand | 70.142 a |
| "F" value | 1.15 ns |
| SMD | 14.75 |
| SD (%) | 8.14 |

Averages followed by the same letter do not differ by Tukey's test at P ≤ 0.01; SMD = significant minimum difference; SD = standard deviation.

White sapote has been poorly studied, especially in Brazil, making it difficult to find references to compare with the data of the present work. Additionally, the absence of statistical differences among substrates studied could be caused by endogenous seed factors because sand is the heaviest substrate used in horticulture with a cubic foot of dry sand weighing about 45 kg (Horn 1996) and contains virtually no mineral nutrients, but promoted seedling emergence as for the other substrates studied. Accordingly, coconut fiber, a substrate readily available in Brazil and considered a waste in some regions, also promoted seedling emergence compatible to commercial substrate Plantmax® and Soil mix, a substrate commonly used for fruit seedling production in Brazil, thus confirming a previous report of Hartmann *et al.* (2002) who argued that coconut fiber is an economical peat substitute that can be mixed with mineral component as propagation media. Organic soil, or vermiculite, or both (1:1), or both combined with sand, *xaxim*, sphagnum, pine cone, carbonized rice hull, turf, but not sawdust (1:1:1), were found to be suitable for the ex vitro acclimatization of *Anthurium andreanum* (Viégas *et al.* 2007).

White sapote seeds seem to be recalcitrant (Klein and Moore Jr 1985) i.e., seeds are unable to withstand maturation drying. Accordingly, Hartmann *et al.* (2002) argue that these seeds must not dry below 30% or they lose their ability to germinate, thus, species whose fruits ripen early in summer drop to the ground, and contain seeds that germinate immediately. As during the experiment, seeds were irrigated daily, and perhaps the appropriate environmental conditions of available water, proper temperature regime, oxygen supply and light (Baskin and Baskin 1998) for this species were satisfied, independently of the substrate studied. This hypothesis finds resonance with Horn (1996) who verified that emergence of recalcitrant seeds cannot take place at low levels of dryness what is an important basis for maintaining viability and controlling emergence.

It is important to detach that shoot tip of white sapote emerges first that characterizes a hypogeous germination, i.e., this species presents a section between the cotyledons and the first true leaves called epicotyl (Hartmann *et al.* 2002). In this way, Smith *et al* (2001) reported that cells from the epicotyl are relatively more exposed during dehydration than those of the hypocotyls, thus failure (or a marked delay) to produce shoots in all axes dried could have occurred because of greater dehydration of these cells, increased sensitivity to desiccation, rehydration, or a combination of these factor. This could be occurred with white

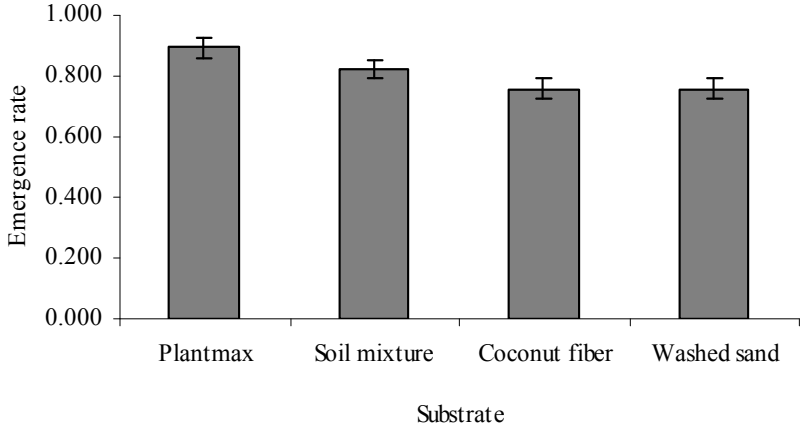


**Fig. 1 Emergence rate of white sapote seedlings as a function of substrate.** Vertical bars indicate the standard error. "F" value = 2.85 ns; SMD (Significant Minimum Difference) = 0.145; SD (Standard Deviation) = 9.89.

sapote because, even though the emergence rate was statistically not influenced by substrate, Plantmax® showed the highest rate (0.90, see **Fig. 1**), i.e., seedlings emerged in less time, or faster, than in other substrates. According to Walters *et al* (2001), recalcitrant seeds present a low limit to desiccation tolerance and exposure to low water contents can result in desiccation damage and make seedling emergence need more time that can explain the lower emergence rate of washed sand, which presented the lower water retention capacity (**Table 2**).

Despite environmental conditions directly surrounding a seed (water retention capacity, light, pH and soil density) determining the germination success and subsequent seedling emergence and establishment (Horn 1996), no significant differences among substrates were registered for emergence percentage and emergence rate. This can be explained by the high emergence potential of the seed, previously reported by Morton (1987).

Only pH was significantly correlated with seedling emergence and emergence rate: substrates with a higher pH (**Table 1**) promoted less seedling emergence (**Table 2**) and this effect was more pronounced over time (**Fig. 1**), with a significant ($P \leq 0.01$) correlation coefficient (r) of -0.78 and -0.77, respectively for seedling emergence and emergence rate. This result indicates that substrate pH directly influenced seedling emergence of white sapote, but, that it was not a crucial factor.

Despite white sapote seeds having a high emergence index under adequate conditions of climate (temperature and light) and soil (pH, electrical conductivity, water retention potential and aeration) according to Nerd *et al.* (1990), there are no comparative parameters in the scientific literature to define a satisfactory seedling emergence rate for this species or the adequate condition for seedling emergence, showing the importance of research aimed at defining agronomic substrate standards for this fruit species. In addition, Horn (1996) evidences that temperature and water potential of the substrate are the major driving factors for seed germination and seedling emergence under controlled and natural environments.

In relation to other species of Rutaceae genus, citrus and black sapote (*Diospyros ebenaster*) have an adequate substrate for seedling production. Nowadays, a commercial substrate is used for rootstock citrus production in Brazil while black sapote seedlings are grown in a soil mixture (red Oxisol):sieved sand:bovine manure at a 3:3:1 ratio predicted by Oliveira *et al* (2006) that tested the same substrates studied in this experiment and registered the higher seedling emergence (87.03%) and emergence rate (0.35) soil mixture.

Seedling emergence percentages presented in **Table 2** are above 42% reported by Ricker *et al.* (2000) for black sapote using as substrate soil from surface (10 cm depth), without bovine manure.

Many studies on fruitful species have indicated the best substrate for each, important for economic seedling production. In this sense, Costa *et al* (2005) studied the effect of different mixes of vermiculite, carbonized rice rusks, humus, coconut fiber and commercial substrates for seedling formation of soursop (*Annona muricata*) and concluded that any mix studied can be used; Andrade *et al.* (2000) tested paper, vermiculite and soil for seedling formation of *Genipa americana* and observed that both soil and vermiculite presented satisfactory results of seed germination; Silva *et al* (2001) submitted yellow passion fruit (*Passiflora edulis*) seeds to combinations of two commercial substrates (Plantmax® and Nutriplanta®) with vermiculite, pine barks and organic manures (humus and bovine manure) and recommend Plantmax® and Nutriplanta®; Andrade *et al.* (2004) for lychee studied vermiculite, washed sand, filter paper, carbonized rice husks and sphagnum as substrate and concluded that carbonized rice rusks is the best substrate for lichee. These reports show the importance of determining a specific substrate for the propagation and establishment of each species.

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