

## TECHONOLGY OF *Qualea grandiflora* MART. (VOCHYSIACEAE) SEEDS

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**ABSTRACT:** *Qualea grandiflora* Mart. (Vochysiaceae), commonly known as “pau-terra”, is an arborous species native to the Brazilian savannah which possess commercial interests, as it can be used either as an ornamental or as a medicinal plant. “Pau-terra” can also be used in the heterogeneous reforestation of areas which are destined for restoration of permanent preservation degraded areas. Propagation studies with this species are scarce, being necessary then further clarification regarding the factors that influences the germination process. In this context, the objective of this work was to evaluate the influence of different temperatures, substrates and light conditions on seed germination. We selected light brown seeds which were subjected to different interactions between temperatures (15-25, 20-30, 25 and 30°C), substrate (paper, sand and vermiculite) and light (light and dark). All seeds were later dry-incubated at 32°C for 3, 6 and 12 hours. After treatments, seeds were kept in BOD at 58% RH and the following parameters were calculated: germination (%G) and germination speed index (GSI); the formation of normal and abnormal seedlings and the number dead seeds. Interaction was observed for all variables. In the optimum temperature range, the seeds behaved as photoblastic neutral or indifferent. Under alternating temperatures, darkness enhanced the germination, especially when combined with the lower temperatures. We noted that the sowing in sand, at 25°C, allowed the maintenance of suitable combinations of germination and seedling development. With respect to desiccation tolerance, “pau-terra” seeds presented an orthodox behavior, with a linear increase of the vigor as function of drying.

Key words: Light, temperature, substrate, drying, orthodox.

## TECNOLOGIA DE SEMENTES DE *Qualea grandiflora* MART. (VOCHYSIACEAE)

**RESUMO:** *Qualea grandiflora* Mart. (Vochysiaceae), conhecida como pau-terra, é uma espécie arbórea nativa do cerrado brasileiro que apresenta interesse comercial, como ornamental e medicinal, podendo ainda ser empregada em reflorestamentos heterogêneos, destinados à recomposição de áreas degradadas de preservação permanente. Estudos de propagação com essa espécie são escassos, sendo necessários maiores esclarecimentos quanto aos fatores que influenciam no processo germinativo. Nesse contexto, objetivou-se avaliar a influência de diferentes temperaturas, substratos e condições de luminosidade na germinação de sementes de pau-terra. Foram selecionadas sementes de coloração marrom clara e submetidas a interação entre temperaturas (15-25, 20-30, 25 e 30°C), substratos (sobre papel, entre areia e entre vermiculita) e luz (claro e escuro), e a secagem em estufa a 32 °C, por 3, 6 e 12 horas. Após o tratamento, as sementes foram mantidas em BOD com 58% UR e calculados os parâmetros germinabilidade (%G) e índice de velocidade de germinação (IVG), formação da plântula normal e anormais e de sementes mortas. Foi observada interação para todas as variáveis analisadas. Na faixa de temperatura ótima, as sementes apresentaram comportamento fotoblástico neutro ou indiferente. Sob temperaturas alternadas, o escuro favoreceu a germinação, principalmente quando em combinação com a temperatura mais baixa. A semeadura entre areia a 25 °C permite a manutenção de combinações adequadas para a germinação e desenvolvimento da plântula. Com relação à tolerância a dessecação, as sementes de pau terra apresentam comportamento ortodoxo, havendo um incremento linear do vigor em função da secagem.

Palavras-chave: Luz, temperatura, substrato, secagem, ortodoxa.

### 1 INTRODUCTION

*Qualea grandiflora* Mart. (Vochysiaceae) is popularly known as “pau-terra”, “pau-terra-do-campo”,

“pau-terra-do-cerrado”, “pau-terra-da-folha-larga”, “ariavá”, among others (CORRÊA, 1978). It is an arborous species native to the Brazilian savannah, which occurs in the gallery forests and in the savannah, widely distributed

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from the Legal Amazon to São Paulo, Minas Gerais, Goiás and Mato Grosso, being usually found on high, dry and well drained terrains (ALMEIDA et al., 1998; LORENZI, 2000). This species possess commercial interests, as it can be used either as an ornamental or as a medicinal plant, as well as a timber source and for environmental purposes.

It is a heliophytic and selective xerophytic, being pioneer species adapted to poor lands, which enables it to be exploited in heterogeneous reforestation of areas which are destined for restoration of permanent preservation degraded areas (LORENZI, 2000). It is honey and by cooking its green fruits, a dye can be extracted (ALMEIDA et al., 1998). The crude extract of its bark is antibacterial (ALVES et al., 2000) and the hydroalcoholic extract of the bark has antiulcer action (HIRUMA-LIMA et al., 2006). The hydroalcoholic extract of its leaves is an analgesic with anticonvulsant potential (GASPI et al., 2006). The ethanol extract of the leaves also possess antioxidant (SOUSA et al., 2007) and antibacterial effects (AYRES et al., 2008).

Silvicultural knowledge of that specie are scarce, most of which is summarized by Felipe (1990) e Melo et al. (1979). Both suggested the occurrence of seed dormancy, which was overcome with the complete withdrawal of the integument or by keeping the seeds in a storage cloth-bag at room temperature for a period of 6 months.

Seed germination may be defined as a sequence of physiological events that occur before radicle protrusion in non-dormant soaked seeds, being the step that involves the establishment of seedlings, called the post-germination event. The physiological state of the seeds differs considerably in the germination and post-germination processes in terms of gene expression profiles and desiccation tolerance (NONOGAKI, 2006). It is therefore possible that besides the differences in the germination and post-germination metabolism, that the responses to environmental conditions might also be different.

The germination process is regulated by many environmental factors such as moisture, oxygen, temperature, light and nutrients (SEO et al., 2009). Temperature, light of photoblastic positive seeds, and water are the most important environmental factors that promote seed germination (SOCOLOWSKI et al., 2008). The seeds of photoblastic positive behavior are dependent of the action of phytochromes, for its turn, changes according variation of temperature (FRANKLIN, 2009; HESCHEL et al., 2007; SIMÃO et al., 2007). The phytochromes modulate

the endogenous levels of gibberellin (GA) and abscisic acid (ABA) and also the responsiveness to GA (SEO et al., 2009). The temperature influences the growth potential of the embryo as well and hormonal levels, especially ABA and GA (NONOGAKI, 2006; PENFIELD, 2008; TOH et al., 2008; YAMAGUCHI, 2008) so, there is a relationship between light intensity and temperature (SEO et al., 2009).

The substrate is a complex factor that influences, in a variety of ways, the germination and post-germination processes. While choosing the material for the substrate, one should take into consideration then, the size of the diaspores and its requirements in relation to moisture and light; especially considering density, water absorbency and retention, aeration, absence of pathogens and toxic substances. The ideal substrate should also provide some sort of ease for seedling evaluation.

The seeds water content is another factor that decisively influences the germination process and longevity. With respect to drying tolerance, seeds are classified physiologically into tree groups, the orthodox, intermediate and recalcitrant. The orthodox seeds have a strong desiccation at the end of ripening on the mother plant and are able to maintain its germination potential for long periods of storage in dry state. On the other hand, intermediate and recalcitrant seeds have high water content at maturity, are intolerant to desiccation and begin its germination immediately after the ripening stage, without passing through the phases of drying and metabolic quiescence. Several studies have been undertaken to elucidate the possible need or sensitivity of the seeds to the desiccation process, being of great importance to quantify this behavior (ANGELOVICI et al., 2010; FISHER, 2008; MASETTO et al., 2008).

Therefore, due to the increasable demand for propagation methods of medicinal species of the Brazilian savannah (DOUSSEAU et al., 2007; ZAIDAN; CARREIRA, 2008), there is an urgent need of further elucidation of the factors that influences the germination of these species, including "pau-terra". The objective of this work was to evaluate the influence of different temperatures, substrates, light intensity and desiccation on germination of "pau-terra" seeds.

## 2 MATERIAL AND METHODS

The experiments were conducted at the Laboratory of Plant Growth and Development, a sector of Plant Physiology, Department of Biology, Federal University of Lavras, MG, Brazil. The fruits of pau-terra were collected

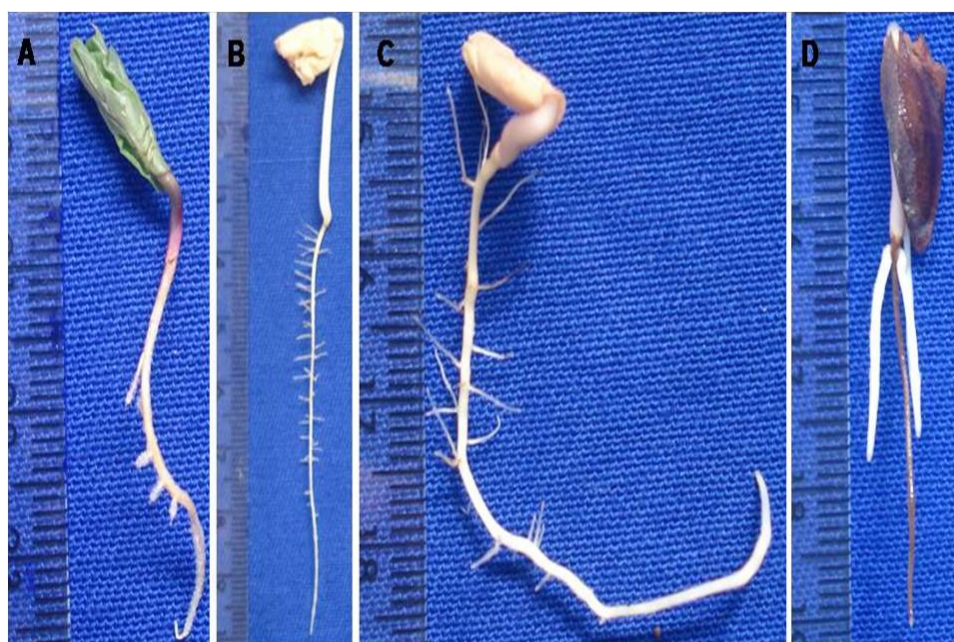
from 10 selected mother trees in a natural population located in the municipality of Ijaci (MG), from August 2007 to October 2009. The fruits were collected immediately before the beginning of dehiscence; i.e. when they exhibited a small opening at the distal portion to the petiole; and left on a concrete yard during the day and collected at night for three consecutive days to allow the seeds release. The seeds were selected and only those that had a light brown integument were used for all experiments (FELIPPE, 1990). The winged expansion of the seeds was removed manually, either for the biochemical analysis or for the physiological assessments. The moisture content was determined according prescriptions of Brasil (2009).

To evaluate the interaction between light intensity, temperature and substrate on seed germination and post-germination were tested differents substrates (Germtest® paper and sand in acrylic gerboxes and Germtest® paper roll) combined with periods of exposure the light (12 h photoperiod and dark) and temperatures (15-25, 20-30, 25 and 30°C). The dark condition was achieved by engaging the gerboxes in aluminum foil and black polyethylene bags, being the germinating behavior evaluated under green light.

The test was conducted in (B.O.D.) chamber, with approximately 58% RH. The evaluations were performed

daily for a period of 22 days, using as techological criteria a 0.9 cm protrusion, being 0.5 cm of primary root and of 0.4 of hypocotyls; and post-germination, normal seedlings (FERREIRA et al., 2001), as depicted in Figure 1. From these data we determined the germination (%G) and germination vigour by speed index (GSI) calculated according to Maguire (1962). At 40 days after sowing, we evaluated the overall percentage of normal seedlings (%NS) and abnormal (%AS). We used a completely randomized 3x2x4 factorial scheme with four replications of 25 seeds per treatment. Statistical analysis was performed by using the statistical program SISVAR (FERREIRA, 1999). The data did not suffer any kind of transformation. We performed an analysis of variance and means were compared by the Tukey test ( $p \leq 0.05$ ).

To evaluate the desiccation tolerance, seeds were dried in an oven at 32°C for 3, 6 and 12 hours. After each drying period, it was determined the moisture content; and the physiological quality of the seeds was evaluated by means of the emergency. The seeds were disinfected with 1% Cercobin for 5 minutes, sown in gerboxes in substratum autoclaved sand and kept at 25°C and 12h photoperiod (ideal conditions for germination and normal seedling formation identified in the previous experiment).



**Figure 1** – Appearance of seedlings of *Qualea grandiflora*. Normal A-, B-roll dark; C dark sand and paper, D-abnormal.

**Figura 1** – Aspecto das plântulas de *Qualea grandiflora*. A- Normal; B- Rolo no escuro; C areia e papel no escuro; D-anormal.

The criteria used for germination was 0.9 cm protrusion, being 0.5 cm of primary root and of 0.4 of hypocotyls. The germination was evaluated for 22 days and determined the germination (%G) and germination vigour by speed index (GSI) calculated according to Maguire (1962). At 40 days after sowing, we evaluated the overall percentage of normal seedlings (%NS) and abnormal (%AS).

The experiment was conducted in completely randomized design with four replications of 25 seeds per treatment. We also performed an analysis of variance and the means were compared by polynomial regression ( $p \leq 0.05$ ), using the statistical program Sisvar (FERREIRA, 1999).

### 3 RESULTS AND DISCUSSION

The Table 1 represent a summary of the analysis of variance (ANOVA) for seed germination characteristics. For the %G double interaction was observed between the studied factors, while for the GSI, %NS and %AS triple interaction was significant.

Temperatures of 25°C and 30°C provided the highest values of %G and GSI, regardless of the presence or absence of light (Table 2 and 3). In the range of optimum temperature, the seeds behaved as photoblastic neutral or indifferent.

However, alternating temperatures, even though it has not favored the germination process, as it caused a

reduction in the %G and GSI (Table 2 and 3), altered the photoblastic response. At 15-25°C, the seeds germinated in the dark had a higher %G and GSI, than those germinated in the presence of light. At 20-30°C the %G did not differ regarding the presence and absence of light, however, the germination rate was higher in the dark. It seems that under less suitable conditions, darkness enhanced germination, mainly in the alternately condition that contained the lower temperatures. Koefender et al. (2009) also found an increase in the germination of seeds of *Calendula officinalis* L, when germinated in the dark under thermal stress. According Zaidan and Carreira (2008), seeds of most of the arborous species, native to the savannah, germinate between 20°C and 30°C and are indifferent to light. Alternate temperatures not always favor the germination of seeds, however, often alter the light sensitivity in some species (GODOY; TAKAKI, 2004), while in others not (SIMÃO; TAKAKI, 2008).

Regarding the interaction of light with the substrate, we found that seeds that germinated on paper were indifferent to light, while darkness seems to have favored the root protrusion in seeds germinated on sand (Table 2 and 4). In the roll, only the %G differed in response to light, being also higher in the dark conditions. Both in light and in darkness, the highest values of %G and GSI were observed in seeds germinated on paper and on the roll, which did not differ, while that on sand the numbers were lower when compared to the other substrates.

**Table 1** – Summary of the analysis of variance (ANOVA) for germination (%G), speed index (GSI), percentage of normal seedlings (%NS) and abnormal (%AS) of seed *Qualea grandiflora*.

**Tabela 1** – Resumo da análise de variância (ANAVA) para a germinação (%G), índice de velocidade de germinação (GSI), porcentagem de plântulas normais (%NS) e anormais (%AS) de sementes de *Qualea grandiflora*.

Source of variation	GL	Mean square			
		%G	GSI	%NS	%AS
Light (L)	1	3420,09**	0,16	988,17**	1980,17**
Temperature (T)	3	4213,26**	4,63**	371,28**	4264,17**
Substrate (S)	2	10658,01**	9,64**	40737,50**	25588,50**
L*T*S	6	296,93	0,16**	905,50**	537,72**
L*T	3	1915,10**	0,83**	3297,50**	1159,72**
L*S	2	871,59**	0,26**	352,17**	918,17**
T*S	6	2690,01**	0,34**	1120,61**	1828,50**
Error	72	154,7	0,05	58,06	61,06
Coefficient of variation (%)		15,18	13,95	13,64	24,14

\*\* , \* Significant respectively, at 1% and 5% probability of the test F.

**Table 2** – Germination (G%) of seeds of *Qualea grandiflora* under different light intensity, temperature and substrate. Breakdown of interactions between light and temperature and between light and substrate.

**Tabela 2** – Germinabilidade (%G) de sementes de *Qualea grandiflora*, submetidas a diferentes condições de luminosidade, temperaturas e substratos. Desdobramento das interações entre luz e temperatura e entre luz e substrato.

Temperatures (°C)	Light intensity	
	Light	Dark
15-25	49 Bc	78 Ab
20-30	68 Ab	75 Ab
25	90 Aa	93 Aa
30	94 Aa	96 Aa
Substrates		
On sand	48 Bb	73 Ab
On paper	94 Aa	96 Aa
Paper roll	84 Ba	95 Aa

Means followed by same letter do not differ by Tukey test (p <0.05). Uppercase letters and lowercase compare the line in the column.

According to the RAS (BRASIL, 2009), on paper (OP) is recommended for small and light sensitive seeds, while that between paper roll (BPR) for large seeds, which prefer moist environments and are not sensitive to light. On the other hand, the sand is a substrate used alternatively to confirm the seedling evaluation in case of doubt, when phytotoxic symptoms appear or when the evaluation of a sample is impractical due to excess of infection (BRASIL, 2009).

Regarding the interaction between substrate and temperature, it can be seen that for %G, only in alternating temperatures response to the substrates differed, with lower values on sand (Table 4).

In this substrate, the %G and the GSI were higher at constant temperatures and lower in the alternating (Table 4 and 3). For seeds germinated on paper there were no differences for %G, regarding the thermal regimes (Table 4), however, to GSI (Table 3), the behavior was the same as that we found on the sand, i.e. higher values at constant temperatures and lower in the alternate. Unlike other substrates, for seeds germinated on the roll, the %G was higher above 30°C and 20-30°C and lower below 15-25°C;

**Table 3** – Germination vigor by speed index (GSI), percentage of normal seedlings (%NS) and abnormal (%AS) of *Qualea grandiflora* seeds under different conditions of temperature, light and substrate. Decomposition of the triple interaction.

**Tabela 3** – Índice de velocidade de germinação, porcentagem de plântulas normais e anormais de sementes de *Qualea grandiflora*, submetidas a diferentes condições de temperatura, luz e substrato. Desdobramento da interação tripla.

Temperatures (°C)	Substrates					
	Paper roll		On paper		On sand	
	Light	Dark	Light	Dark	Light	Dark
IVG						
15-25	0.93 aAb	0.92 aAb	0.66 cBc	0.87 abAbc	0.19 bBd	0.71 bAc
20-30	0.93 aAb	1.02 aAb	0.87 aAb	0.76 bAc	0.87 aAc	0.71 bBc
25	1.00 bAb	0.88 bAb	1.35 aAa	1.04 bBab	1.50 aAa	1.41 aAa
30	1.34 aAa	1.34 aAa	0.66 bBc	1.08 bAa	1.20 aAb	1.15 bAb
%PN						
15-25	8 Bcb	27 aCa	44 bBb	73 aBa	95 aAa	98 aAa
20-30	46 aBa	29 aB	57 abBa	41 bBb	97 aAa	94 aAa
25	51 aCa	4 bBb	66 aBa	4 cBb	95 aAa	94 aAa
30	40 aBa	32 aBa	11 cCb	36 Bba	99 aAa	100 aAa
%PA						
15-25	74 aAa	71 aAa	53 bBa	22 bBb	0 aCa	2 aCa
20-30	53 bAa	66 aAa	41 bcAa	48 aBa	0 aBa	1 aCa
25	48 bAb	64 aAa	32 cBa	0 cBb	0 aCa	0 aBa
30	56 bBa	0 aBb	86 aAa	60 aAb	0 aCa	0 aBa

Means followed by same letter do not differ by Tukey test (p <0.05). Capital letters compare light condition within each temperature and substrate. Lowercase letters compare temperature within each light condition and substrate. Italic lowercase letters compared substrate within each condition of light and temperature.

**Table 4** – Germination (G%) of seeds of *Qualea grandiflora* under different light intensity, temperature and substrate. Breakdown of interactions between temperature and substrate.

**Tabela 4** – Germinabilidade (%G) de sementes de *Qualea grandiflora*, submetidas a diferentes condições de luminosidade, temperaturas e substratos. Desdobramento das interações entre temperatura e substrato.

Temperatures (°C)	Substrates		
	On sand	On paper	Paper roll
15-25	39 Bb	91 Aa	75 Ab
20-30	23 Bb	95 Aa	98 Aa
25	88 Aa	98 Aa	90 Aa
30	91 Aa	97 Aa	97 Aa

Means followed by same letter do not differ by Tukey test ( $p < 0.05$ ). Uppercase letters and lowercase compare the line in the column.

however, at 25°C the values did not differ between the thermal regimes (Table 4). The GSI of the seeds on this substrate was higher at 30°C, followed by those at 25°C and 20-30°C that did not differ among them, and lower at 15-25°C (Table 3). Several other studies also reveal the interaction between substrate and temperature, with very distinct results between species (MARTINS et al., 2008; NOVENBRE et al., 2007; PACHECO et al., 2006; SOUZA et al., 2007). For the root protrusion, it can be concluded that the best vigor expression is found when seeds germinate at 25 and 30°C, on paper or on roll, in both lighting conditions.

In the post-germination process, as well as in the germination, we also observed a significant interaction for all factors studied (Table 3), however, with different response between the two processes. With regards to the relationship of the interaction for the percentage of normal seedlings, we found that the development of normal seedlings was affected differently in relation to temperature, light and substrate (Table 3).

At 25°C the percentage of normal seedlings was higher in light conditions, whereas at 15-25°C was higher in dark conditions (Table 3). As for the germination under heat stress, the dark favored the development of normal seedlings. On the other hand, at 30°C and 20-30°C, there was no influence of light. The temperature of 25°C under light conditions provided the highest values, followed by 30°C and 20-30°C, with lower values at 15-25°C. In the

dark, the values were higher at 30°C and 15-25°C and lower at 25°C and 20-30°C. Darkness favored the development of abnormal seedlings at 25°C and decreased the rate of abnormal seedlings at 30°C, while in alternating temperatures; there was no difference (Table 3). In the light, the greater percentage of abnormal seedlings was observed at 30°C, followed by the other temperatures, which did not differ. On the other hand, in the dark, the highest percentage occurred at 25°C followed by 30°C and 20-30°C and lowest at 15-25°C.

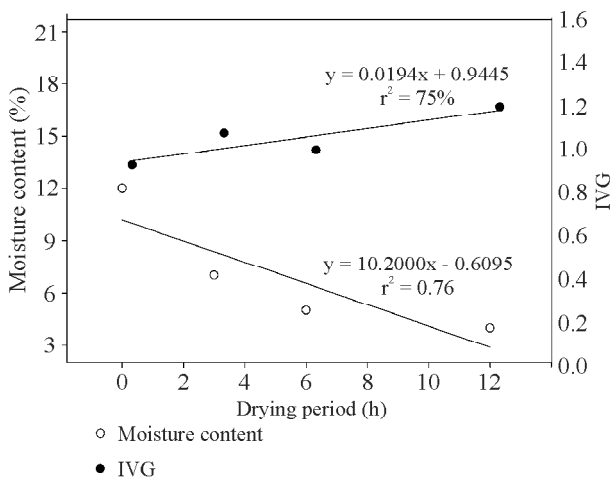
Regarding the interaction of light with the substrate, it can be seen that on sand the higher percentage of normal seedlings occurred in the light, while that on roll was in the dark (Table 3). On paper did not differ in response to light. In light, the highest percentage of normal seedlings was obtained for on sand and lower for on roll. However, paper does not differ from other substrates. On the other hand, in the dark, the values were higher for on sand, followed by on paper and by on roll (Table 3). We observed the appearance of abnormal seedlings, with malformation, in function of the type of substrate used (Table 3). Both on paper and on roll occurred intense formation of abnormal seedlings. In the dark, the highest percentage of abnormal seedlings was obtained on the roll, followed by on paper and on sand. In light, the highest values were obtained when we tested the seedling grown on roll and on paper, which did not differ. In the treatment on sand, practically no formation of abnormal seedlings was observed, independent of the luminosity.

That for root protrusion, most normal seedling occurred on sand, at constant temperatures and lower in the alternating (Table 3). On paper, the values were higher at 15-25°C and lower at 30°C, while the other temperatures, promoted intermediate values. On roll the percentage of normal seedlings showed the same pattern as that observed for germination, i.e. higher at 30°C and 20-30°C and lower at 15-25°C; however, at 25°C the values did not differ from other thermal regimes.

For normal seedlings, we observed that its formation on the sand was the lowest (Table 3). In alternating temperatures, the values of abnormal seedlings were higher on the roll, followed by on paper and on sand (Table 3). At 25°C, the percentage was higher on the roll and on paper, which did not differ. At 30°C, the percentage was higher on paper, followed by on the roll. On sand, the percentage of abnormal seedlings was lower on alternating temperatures in relation to the constant temperatures. On

paper, the highest percentage was observed at 30°C and lowest at 15-25°C, while the other temperatures, promoted intermediate values. On the roll the percentage was higher at 30°C and 20-30°C and lower at 15-25°C, however, at 25°C values did not differ from other thermal regimes. At sowing on paper, there was a higher occurrence of necrosis of main root, which often appear truncated and thicker, with thick hypocotyls with few secondary roots, being this effect intensified by the higher temperature (30°C) and by presence of light, probably due to root dryness due to exposure to air. On roll, the effect was probably due to high humidity of this system, making seedlings abnormal with a higher proportion of necrosis of the main root, being that only at 15-25°C, we observed a higher amount of truncated roots. It can be concluded then, that for normal seedling, the most favorable condition is the one that uses sand and that maintains temperature constant at 25°C.

The moisture content was higher in seeds recently benefited (12%) and lowered with drying, with values between 2 and 3%, which did not differ (Figure 2). It was observed that %G did not differ between the drying periods, with values varying between 90 and 100% and all developed into normal seedlings. Regarding the GSI, there was a linear increase in the vigor as seed desiccation proceeded.



**Figure 2** – Moisture content (%M) and germination speed index (GSI) of the seeds of *Qualea grandiflora*, submitted to desiccation.

**Figura 2** – Grau de umidade (%U) e índice de velocidade de germinação (IVG) de sementes de *Qualea grandiflora*, submetidas a dessecação.

In recalcitrant seeds, a diverse range of damages is observed with drying (FARIA et al., 2004; MASETTO et al., 2008), while in orthodox seeds various mechanisms are activated to prevent such harms (ANGELOVICI et al., 2010; FISHER, 2008). In orthodox seeds, drying acts as a signal for the passage of the state of development to germination (ANGELOVICI et al., 2010; CATUSSE et al., 2008). With the results of our experiment, we can conclude that the “pau-terra” seeds exhibit orthodox behavior in relation to desiccation.

#### 4 ACKNOWLEDGEMENTS

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