Ultra-weak delayed luminescence in coffee seeds (*Coffea arabica* and *C. canephora*) and their germination potential: some indications for a photonic approach in seed viability

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***Abstract*— The delayed luminescence of six groups of coffee seeds were measured and analyzed by the decay behavior and statistics parameters. All groups exhibited high germination rate with normal aging behavior with exception of an unripe group. Possible correlations with the photon counting data are discussed, since all measurements exhibited huge total counting ($\Sigma(x)>1.10^4$). The best correlation was observed within the total counting data over the entire period (500 points in 25s), analyzed together with the luminescence decay behavior quantified by hyperbolic decay fitting and statistical parameters**.

***Index Terms*— Biophoton, coffee germination, delayed luminescence, photonic and life sciences.**

I. INTRODUCTION

THE Coffee seed normally presents high germination potential, just after appropriate harvest and desiccation. However, it loses its physiological quality very rapidly under usual storing conditions. Therefore, it is not possible to have feasible seeds, i.e. able to germinate, for more than some months. Many research groups are working for techniques to improve the seed's viability, based on data of storing conditions [1,2], methods of controlled re-hydration [3] and also low-temperature induced hibernation [4]. Although some progress has been achieved, the usual way for checking the seeds' viability and vigor is to allow them to germinate, losing so the hibernation condition. In order to distinguish between feasible and not feasible seeds, enabling an optimization of seed's storage conditions, a quick and non-destructive method is demanded, as well for other types of sensitive seeds. In this article, preliminary results for an investigation for a biophotonic approach for viability of coffee seeds are presented. The delayed luminescence (DL) of seeds separated in six different groups were measured using a special photon-counting apparatus [5]. Thereafter, the seeds were induced to germinate under usual conditions and the germination rate was established after 15 days and 30 days. The photon counting experimental data was analyzed on basis of DL-fitting parameters, statistics analysis and counting distribution. The possible correlations between the photon-counting behavior and seed viability are discussed, with some indications for further investigation.

A. *The delayed luminescence: photonic approach of vitality*

The biophotonic phenomena, i.e. the ultra-weak delayed luminescence and spontaneous emission found in living organisms, with detected intensity of 10-1000 photons/cm^2.s, has been studied by many multi-disciplinary groups all over the world, in a broad variety of themes. (For a good review in experimental and theoretical work, see ref. [6,7] and also the recent edition of the *Indian Journal of Experimental Biology* - May 2003, R. Bajpai ed.).

This peculiar luminescence holds much longer than the usual bio-fluorescence, and is found far from normal thermal emission, covering the entire visible spectrum and the near IR and UV [5]. This phenomenon is still not well understood, and many arguments are displayed trying to correlate its sources, reservoirs and range of activity [5-7]. Many groups, joined within the *International Institute of Biophysics* – IIB [8], point to some experimental indications indicating regulatory aspects of that biophotonic field, which should integrate tissues through the organism [9]. Based on statistical properties of photon detection, recent works propose that this weak bioluminescence could be emitted from coherent or even squeezed quantum states created inside living organisms, exhibiting properties of non-classical light [10,11]. Research groups in which the possible regulatory aspects of a quantum-macroscopic field are neglected, including one headed by Inaba, one of the fathers of the Optical Coherence Tomography Technique, have also reported consistent results of correlation between the photon counting data and the subject's physiological conditions [12,13].

Recently, possible correlation between the DL behavior and the germination capacity in barley seeds was presented [14] (international patent PCT/CH00/00180 [15]). Other useful reports can be found in ref. [6,7,16].

II. MATERIAL AND METHODS

The DL of six groups of coffee seeds, given by the *Instituto Agronômico de Campinas* (IAC – Faz. Sta. Elisa), were measured after a five-second exposure to white-light (halogen lamp). All

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facilities were supported by the IIB [8], during the *Summer School on Biophysics*, August-2003. The experimental apparatus, constituted by an automatically controlled dark chamber with a specially cooled photon-counting set-up, is well described in ref.[5]. The seeds (*Coffea arabica*, designated here *Arabica*, and *Coffea canephora,* designated here *Robusta* (commercial identifications)) were grouped, named and measured in subgroups, as presented in Table I in reverse chronological order. The size of each sub-group was determined by the seed’s size, since a constant volume was filled each time (an aluminum cuvette with front quartz window, 2 *x* 3.5 *x* 0.8 cm), in each measurement. The freshest groups (g1-g3) were also measured within their original papyrus cover protection, and the comparison with the nude measurements is discussed at the end of this work.

TABLE I - SEED’S GROUPS DIVISION
harvest date, type/Cultivar, number of measurements/group and average number of seeds/measurement

Group	**Harvest date**	**Type**	**Cultivar**	**Measur. /group**	**Seeds/ measurement**
g1	Aug/20/03	*C. arabica*	IAC 4721	9 (14*)	15 (10*)
g2	Aug/20/03	*C. canephora*	IAC 133/c327/1653-4	9 (11*)	18 (15*)
g3	Aug/08/03	*C. canephora*	IAC 133/c298/1647-2	5 (11*)	21 (10*)
g4	Jul/15/03	*C. canephora*	IAC 133/c377/1650-6	4	25
g5	May/03/03	*C. arabica*	IAC 99	12	25
g6	Apr/20/03	*C. arabica*	IAC 144	9	20

* *seeds still with natural papyrus cover*

The measurements were performed under controlled temperature (22°C). In each measurement, the cuvette was filled randomly with seeds of a specific group and put inside the dark chamber. A computer software was used to control the shutters and the electronics involved in the measurement procedure, with the excitation light exposure time set to 5s, and the photon-counting started immediately after (30 ms for shutter opening/closing, light off and electronic starting), doing photon counting for the next twenty-five seconds, within 0.05-second discrete integration interval, for a total of 500 counting points. After the automatic turning-off, the cuvette was removed out of the chamber, and the procedure was repeated for all seeds. The long term DL, after more than five minutes in the dark, was also observed for each group.

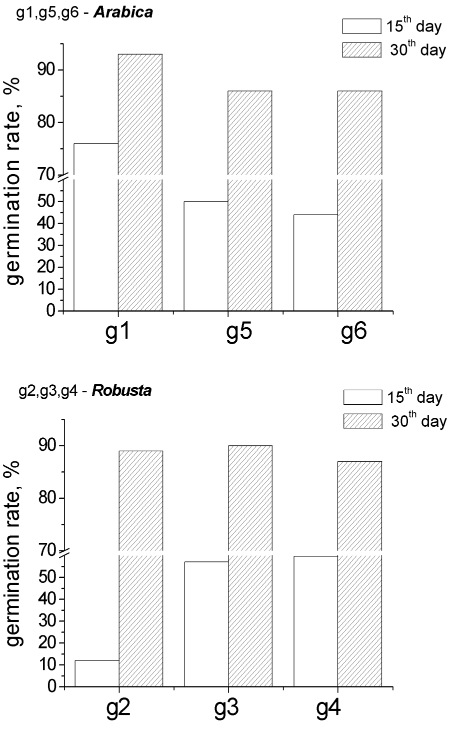
In order to distinguish numerically the different decaying curves, three different approaches were used for the entire 25s period (500 points *x* 0.05s): 1) main statistic parameters (total number of photon counting - Σ(x), mean, median, variance and kurtosis); 2) the best fitting parameters for a curve based on a hyperbolic-like decay, formulated by:

$$\frac{a}{(1+b)^{\alpha}} + y_0 \qquad (1)$$

where $a >> y_0$, since the photon counting is initiated just after turned off the excitation light (“a” is related to the initial values as “y_0” is related to the final ones; “b” rules the short term decay velocity (~ until t =1), and “α” rules mainly from 1< t <10); and finally, 3) the counting incidence distribution, performed in order to give a graphical quantitative tool for the decaying behavior investigation.

After the dark chamber measurements, the seeds returned to Brazil and were induced to germinate under usual conditions, in the IAC facilities. In the 15th and 30th days, the germination rates were observed and registered. The germination rates, for short (15th day) and long-term (30th day) periods, are presented in Figure 1, for the three *Arabica* and the three *Robusta* groups.

The *Arabica* groups (Fig.1a) present, in both initial and final germination rates, the aging regular behavior, as well the last two *Robusta* groups (Fig.1b). But the freshest *Robusta* group (*g2*, Fig.1b) presented a very low initial rate, probably due to its non-complete ripening condition.



(a)

(b)

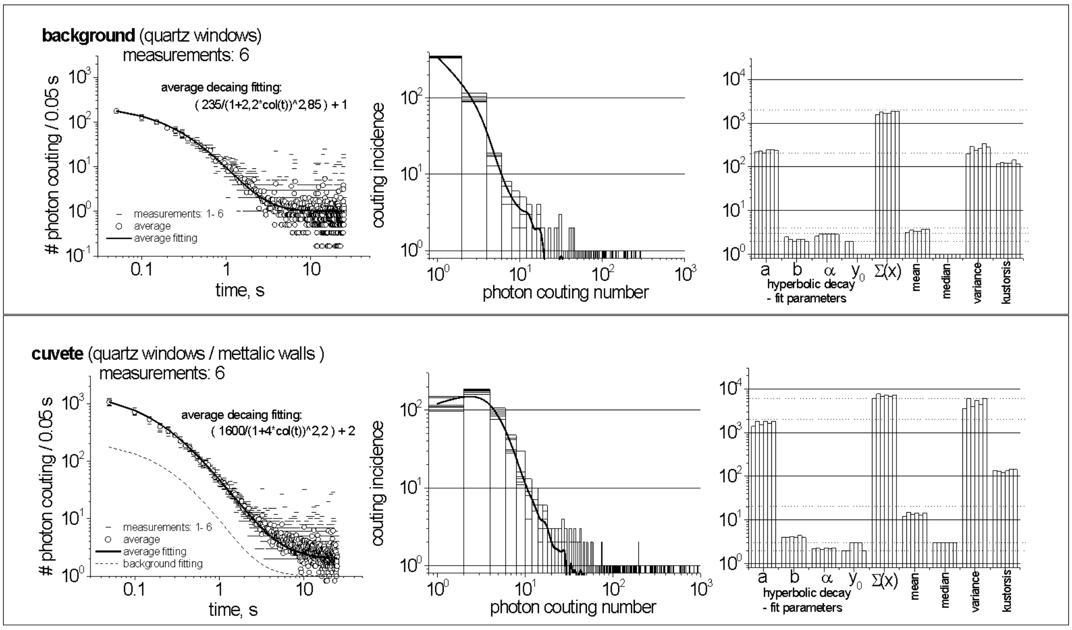
Fig.1 – germination rate for the 15th and the 30th days, *arabica* (a) and *robusta* (b) groups.

III. RESULTS AND DISCUSSION

A – Dark-counts and background

In order to compare the results and attest the set-up stability and sensitivity, the DL for the empty chamber (background) and the cuvette itself were also measured, between each seed’s group measurements. The DL curves, their incidence distribution, fitting and statistic parameters are presented in Figure 2.

As shown in Fig.2, the background measurements present very low photon counting, starting little above 100 and quickly going to the final value around 1. This behavior is indicated, besides the time curves themselves, by their parameters: low initial value (a~200) and total counts ($\Sigma(x)<2.10^3$), median = 1, α = 3 and y_0 ~1.



(a) (b) (c)

Fig.2 – DL for the background (empty chamber) and the cuvette itself: time decay and average with hyperbolic fitting (a); photon counting distribution (b); fitting and statistical parameters (c) for each measurement.

The ultra-low DL counting can be so related to light stored in the quartz windows and inside the chamber volume itself, since just noise can be found after t = 5 seconds (last 380 time-points).

The other case also presented in Fig.2, the empty cuvette, shows the same behavior but with a gain equally distributed in time (a~10^3, α~2.8, y_0 ~ 2, Σ(x)~6.10^3, median = 3), what can be related to the additional power stored in between the cuvette metallic walls and its quartz windows and within other chamber's windows. The cuvette was than chosen as the equivalent background in this experimental situation.

In both these two cases, the kurtosis is high (~100), as they have a pronounced distribution peak, and the variance goes with the '*a'* factor (in eq.1).

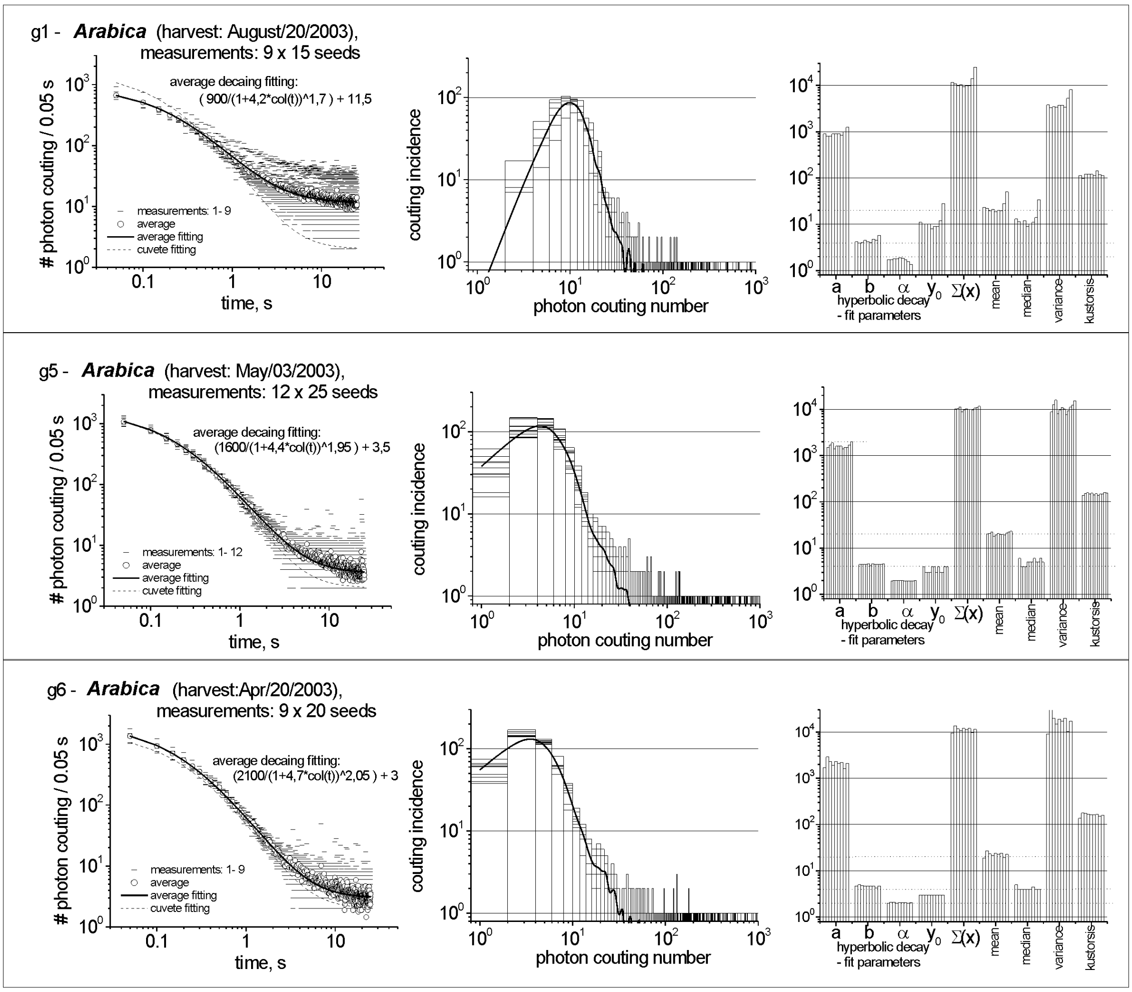
B – The Coffea arabica and Coffea robusta groups

The photo-counting data for the three *Arabica* groups are presented in Figure 3, as done in Fig.2 for background and cuvete. The *g1* data shows a pronounced difference from de cuvette data, with lower initial counting values and much more high final values, reached after a slower decay ($\alpha<2$, y_0 ~ 10, median ~ 10). This behavior is nicely illustrated in the counting distribution (Fig.3b(*g1*)) and by the total counting (Σ(x)~1.10^4). For the other two groups, *g5* and *g6,* the main difference from the cuvette data is noted mainly after t = 5s, and so they also present high Σ(x), but with lower final value than *g1*, (median ~ 4 and y_0 between 3 and 4).

All three *Arabica* groups exhibit distinguishable data from the cuvette, mainly for t>5s, with similar Σ(x) but distinguishable medians/y_0. No usual short-term photoluminescence as observed, for example, in leaves and other tissues with chlorophyll, was measured [6,7,11], indicating that seeds could be in the desired dormant condition, with the predominant long-term photon-counting decay (t>5s).

The photon-counting data for the three *Robusta* groups are presented in Figure 4. A new behavior is found in the *g2* group: a very slow decay (α ~1.3) was detected, as observed in experiments with leaves [6,7,11]. The *g2* sub-groups present the highest initial values (a~2.8 .10^3) found in this work, but tending to a low final one (y_0 ~ 4). They also present the highest total count (Σ(x)~ 5.10^4, 20<median<30), and the photon counting distribution is broader, giving the smallest kurtosis (~6). All these data indicate that the evaluated seeds could be yet unripe and have not entered into the dormant state. The other two groups, *g3* and *g4*, present decays similar to the *Arabica* ones, with noted high final values (for *g3*: y_0 ~ 16, Σ(x)~2.10^4, median ~ 18, and for *g4*: y_0 ~ 7, Σ(x)~10^4, median~9). These behavior could indicate that these groups are well ripen and with high viability. As found for the three previous groups, all *Robusta* groups also showed distinguishable data in long-time photo-counting terms (t >5s).

The longer term DL, checked for all groups, holds for minutes (measured from 5 to 10 min. in dark), with very low (10%-20%) decreasing. It indicates that longer term DL could be a more efficient approach to check seed viability, at least for the coffee case. Looking back at Fig.1, simultaneously with Fig.3 and Fig.4, it is found that the Σ(x) could be tested as a qualitative indicator for germination capacity, and the final DL value (quantified by the median and y_0) could be tested as a quantitative indicator. The exception to this simple approach, group *g2*, needs a more elaborated treatment, involving the α parameters to quantify the non-usual (for seeds) slower DL. As mentioned in the section II, the first three groups were measured also with seeds within their



(a) (b) (c)

Fig.3 – DL for the three Arabica groups (*g1*, *g5* and *g6*): time decay and average with hyperbolic fitting (a); photon counting distribution (b); fitting and statistical parameters (c) for each measurement. The average cuvette fitting is also plotted in (a).

natural clad, formed by dead tissues and looking like a fine paper coating (*papirus*). For all the three cases (not shown here), the DL fitting tends to the same behavior as the "nude" ones after 5 seconds, differing from it by a factor lower than 2. This data indicates that is not necessary to remove the seed's protection to execute this type of measurement, enabling seed testing without any damage.

IV – CONCLUSION

The ultra-weak Delayed Luminescence measurements and analysis data of coffee seeds from three *C. arabica* and the three *C. canephora* different groups, all with high final germination capacity, presented sometimes small but always detectable differences when comparing to the empty cuvette data. All DLs exhibited high total counting ($\Sigma(x)>1.10^4$), as usually found for plants in the specific literature. A leaf-like behavior was found in one freshest group, indicating an unripe condition, which was corroborated by the group's low germination capacity in short-term regime (15 days).

The authors present with this work some parameters related to the DL in coffee seeds, which could be studied further as possible routes for a photonic approach of viability. The best correlation with the germination capacity was found within the total counting $\Sigma(x)$ over the entire period of 500 points (25s), together with the DL decay behavior, illustrated by the DL time decay itself and by the photon counting distribution over time. These characteristics were numerically quantified by hyperbolic decay fitting and by statistics parameters. Future work should consider broader groups of coffee seeds, by varying their age, harvest condition and even trying artificial stress. The technical viability of a practical photonic method in this field needs to be carefully analyzed, since it can promote advances in storing methods and related agricultural research.

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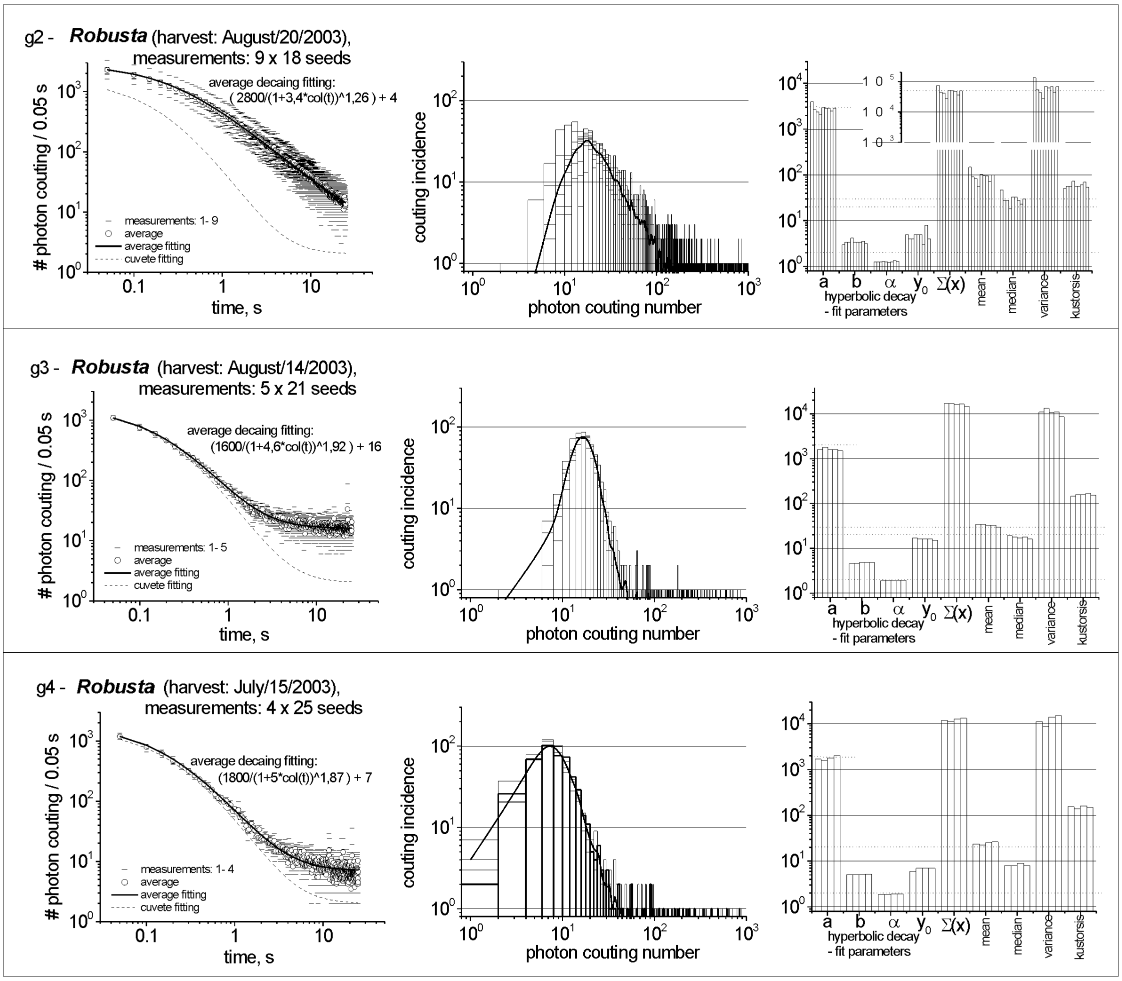

(a) (b) (c)

Fig.4 – DL for the three Robusta groups (*g2*, *g3* and *g4*): time decay and average with hyperbolic fitting (a); photon counting distribution (b); fitting and statistical parameters (c) for each measurement(c). The average cuvette fitting is also plotted in (a).

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