Arq.Biol.Tecnol.39(3):595-606,set.,1996

ON THE GERMINATION AND DORMANCY OF DISPERSAL UNITS OF *Brachiaria decumbens* STAPF

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Recebido para publicação em 08 de abril de 1996

ABSTRACT

The effect of light and temperature on germination of *Brachiaria decumbens* as well as the action of some dormancy breaking chemicals were tested. Two seed batches stored different times were used. The results show that seeds failed to respond to alternating temperature regimes and different light qualities. Seeds were indifferent to white light at 25 °C. KNO_3, ethanol and H_2SO_4 failed to break seed dormancy, whereas KCN and H_2O_2 partially reduced dormancy of two month stored seeds. The results suggest a metabolic character of dormancy in "new" (freshly collected) seeds and confirm the occurence of two types of dormancy in *B. decumbens* seeds.

Key Words: *Brachiaria decumbens*; germination; dormancy

INTRODUCTION

About 64 millions Ha of Braziliam perennial pastures are seeded with grass especies, some of them of considerable economic importance such as "braquiaria" grass (*Brachiaria decumbens*, *B. humidicola* and *B.brizantha*) (1). *B. decumbens* is a herbaceous, decumbent grass capable of covering all the ground due to its high propagation by rhizomes and adventicious roots from the basal nodes of the stem, forming a dense 60-70cm height turf. *B. decumbens* is native of Africa and well adapted to extensive areas of tropical and subtropical regions where the annual precipitations are 800 to 1500mm. The species is drought tolerant staying green throughout the dry season and is adapted both to cold whether and excess of humidity. *B. decumbens* is capable of growing on sandy or poor soils, and it has been used to retard soil erosion because of its rapid growth and intense ground covering (2). The dispersal units of *B. decumbens* are caryopsis (a dry fruit) surrounded by glume, lemma and palea, the latter two structures closely attached to the caryopsis (3, 4).

Although it has introduced as forage crop, *B. decumbens* can be considered a weed always that a pasture area is converted in cultivated soil. Its persistence can be ascribed to its ability to germinate after variable and relatively long periods of dormancy in the soil, what makes difficult its chemical control (5, 6).

Seed dormancy or non-germination of the seed though viable and put under suitable environmental conditions is often considered as falling into two types: a) embryo dormancy, where the control of dormancy is within the embryo itself and; b) coat-imposed dormancy in which dormancy is maintained by the structures enclosing the embryo, that is, the seed coat (7). Another

more functional classification distinguishes a primary and a secondary dormancy, the former being related to seed development and maturation whereas the latter can only occur after seed dispersal (8). The term "after-ripening" is referred to as the whole changes acting during the dormancy period, which may be accelerated or replaced by proper treatments (9). The mechanisms by which the covering structures may prevent embryo germination can be: a) interference with water uptake; b) interference with gaseous exchange and; c) impose a high mechanical resistance on embryo. In such a type of dormancy only the removal of the restraint may enable germination to proceed (8). Embryo dormancy may be ascribed to a requirement for a particular stimulus, for example, light and temperature (10). Very many seeds are now known to react favourably to fluctuations in temperature and shifts (7). The temperature dependence on seed germination is frequently described by the parameters: the minimum cardinal point (*Tm*); the maximal cardinal point (*TM*) and; the optimum range (*To*). The intervals *To-Tm* and *To-TM* are called infra-optimum and supra-optimum, respectively (9). Light influences the germination of many seeds, and seeds whose germination is affected by light are termed photoblastic. The photoreceptor of light in the embryo is phytochrome which exhibit a red light absorbing form (Fv) and a far-red light absorbing form (Fve), the former being responsible for the early events resulting in germination (11).

Metabolic regulation must be considered as being involved in embryo dormancy control (except for immature embryos). A requirement for reductants must be met in the imbibing seed to maintain oxidative respiration. Furthermore, inhibitors of electron transport, the Krebs cycle and glycolisis often relieve seed dormancy in numerous species, particularly grasses (10). Most of the metabolic activities in the embryo are directly related to the supply of oxygen to the embryo, that can be a function of the seed coat permeability to the gas and, in the case of rice, oxygen consumption by extra-embryonic tissues (7).

The main purposes of this work were to observe the seed response to light and temperature, as well as to add some contribution to the knowledge of seed dormancy of dispersal units of *Brachiaria decumbens* Stapf.. In order to achieve this, several treatments (including chemical and physicals) were tested in laboratory to promote the germination of dormant dispersal units.

MATERIAL AND METHODS

Plant Material

Dispersal units (henceforth referred to as seed) of *Brachiaria decumbens* were used. The seeds were coming from Instituto de Zootecnia of Nova Odessa, SP (IZ batch) and Sementes Naterra Ltda., Ribeirão Preto, SP (NA batch). The NA and IZ batches were collected respectively 2 and 24 months before use. The seeds were harvested by the "pile" method and stored in darkness at 6 °C. A tetrazolium test was performed in seeds cut longitudinally to assess the viability of batches. The seeds were sterilized in a 5% solution of commercial sodium hypochlorite ("Varek") prior to use.

Germination assays

The experiments were carried out in a Fanem model 347 CDG growth cabinet where the temperature was kept constant whitin ±0.8 °C of the set value. Germination assays were performed with five samples of 50 seeds each which were imbibed in glass Petri dishes, 9cm in diameter, lined with two qualitative filter paper layers soaked with destilled water or solution. The seed was considered germinated as soon as the radicle was visible through the seed coat. Germinability data were converted to the respective angular value and submitted to analysis of variance, the LSD Tukey being determined for the significative differences at 5% level (12).

White light source was provided by two fluorescent tubes ($3.2W.cm^{-2}$); red light was obtained from two fluorescent tubes "day light" Phillips and two red cellophane sheets ($0.012W.m^{-2}.nm^{-1}$ at 650nm); and far red was obtained from one white light incandescent bulb Phillips whose light was filtered through two red cellophane and three blue celophane sheets ($0.013W.m^{-2}.nm^{-1}$ at 750nm) (13, 14). The darkness treatment was obtained by inserting the Petri dishes in black poliethylene bags. Seed germination under red light, far-red light and darkness was scored under a dim green safelight.

Manual scarification were carried out by removing the glumes, leaving the caryopse surrounded only by lemma and palea (15).

The chemicals used in the dormancy breaking assays were: hydrogen peroxyde, H_2O_2 (Merck); potassium nitrate, KNO_3 (Reagen); potassium cyanide, KCN (Merck); ethanol (LabSynth) and; sulphuric acid, H_2SO_4 (Merck). The treatments with H_2O_2, KCN, ethanol and H_2SO_4 were carried out by immersion of non imbibed seeds in the respective solutions in beakers, after which the seeds were washed in running tap water, blotted in filter paper and put in the Petri dishes with distilled water. Seeds were moistened in the Petri dishes with KNO_3 solutions, within which they were kept throughout the assay.

RESULTS AND DISCUSSION

The IZ lot exhibited 66% of viability whereas the NA lot presented a relatively high percentage (94%) of stained embryos suggesting a high germination capacity (Table 1). *B. decumbens* responded with increased germination when stored for two years as the germinability of scarified seeds was relatively high in IZ lot as compared to NA lot that was stored for short time (Table 1). SMITH (16) pointed out that several genotypes of *Panicum maximum* had lost most of their dormancy after storage, although the genotypes responded differently to storage at various temperatures. The relatively dormancy of newly collected seeds of *Brachiaria decumbens* appears to depend on the method of harvesting as seeds collected by "pile" method tend to be more dormant than seeds collected by "sweep" method, which stayed a more prolonged time in soil (Francisco D. de Souza, personal communication).

Table 1. Tetrazolium test and germination of scarified seeds of *Brachiaria decumbens*. Germination performed under white light at 25 °C.

	% stained cuts	% germination
IZ lot	66	57.2
NA lot	94	8.2

Both scarified and intact seeds of *B.decumbens* (IZ lot) imbibed at 25 °C were indifferent to light (Figure 1), exhibiting a non-photoblastic reaction at this temperature. Similar results were obtained by FREITAS et al. (17) with low dormant dispersal units of *Brachiaria plantaginea*. On the other hand GOEDERT (3) reports a favourable effect of white light exposure on seed germination of *Brachiaria decumbens* under an alternating temperature regime. More detailed investigations are needed to prove the involvement of phytochrome on the induction of seed germination of *Brachiaria* spp., particularly *B. decumbens*.

Intact seeds (IZ lot) germinated equally in constant (15, 25 and 35 °C) and alternating temperatures (15/35 and 25/35 °C) showing that such thermal fluctuations failed to promote the germination of *B.decumbens* under the present conditions (Figure 2). Similar results were obtained for scarified seeds whose germinability was higher than intact ones in all the thermal treatments, showing that the influence of the seed coat was maintained throughout (Figure 2). FELIPPE et al.

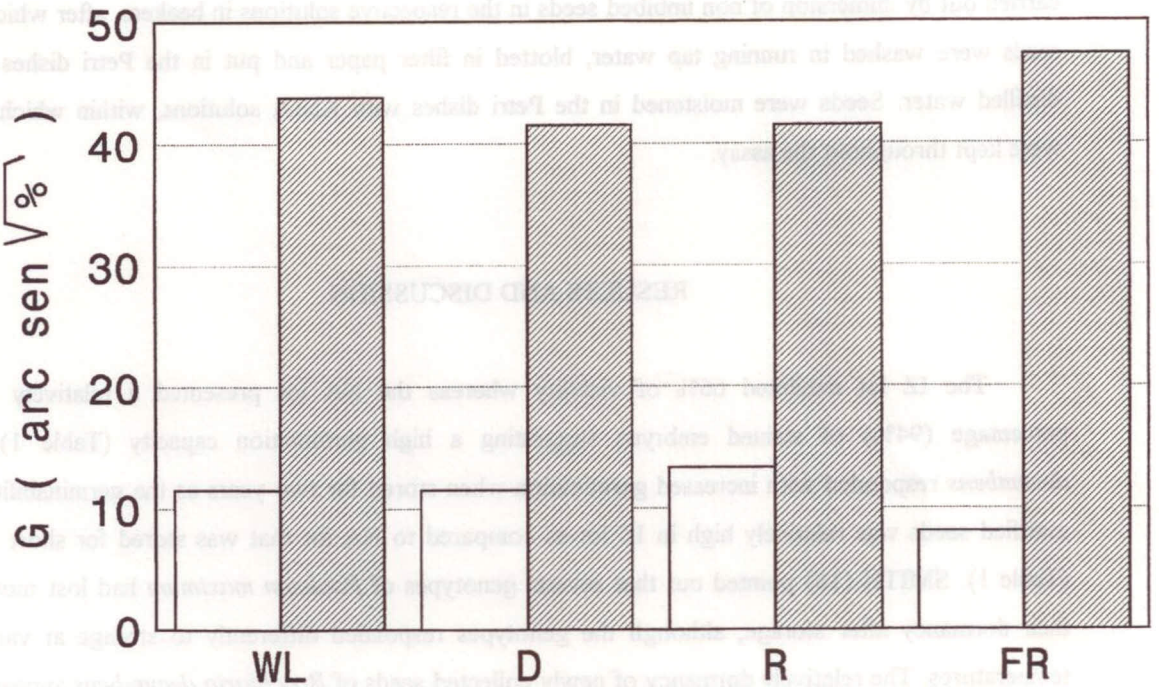

Figure 1. Action of white light (**WL**), red light (**R**), far-red light (**FR**) and darkness (**D**) treatments upon germination of intact (non hachured bars) and scarified (hachured bars) seeds of *Brachiaria decumbens* (IZ lot), germinated at 25 °C. **F** not significant for light treatments.

(18) didn't report any promotion of the seed germination of *Andropogon gayanus* by alternating temperatures as compared to the most effective isothermal treatments. On the other hand, GOEDERT (3) reported a stimulatory effect of temperature fluctuations on the germination of newly collected seeds of *B. decumbens*. The germination of scarified seeds of *B. decumbens* was slightly decreased at 15 °C and no difference was observed between 25 and 35 °C constant temperatures (Figure 2). As a reduction of the germinability at 15 °C was also observed both in *Andropogon gayanus* (18) and *Pennisetum pedicelattum* (19), one can conclude this temperature is sub-optimal for these tropical grasses. *B. decumbens* germinated well at 35 °C, that was shown to be supra-optimal for germinability of *Setaria pallidefusca* and *Pennisetum pedicellatum*, two widely distributed weeds in Nigeria (19).

A remark can be made from the germination assays with IZ lot is the solely effect of the surrounding structures inhibiting the germination of long time stored seeds of *B. decumbens*.

Since the seeds of the NA lot have shown no response to manual scarification we tried other dormancy breaking agents. Thus the following results were obtained only from intact seeds of NA batch.

Two months stored seeds of *B.decumbens* were indifferent to acid scarification as no significative differences were observed amongst the several scarification treatments and between them

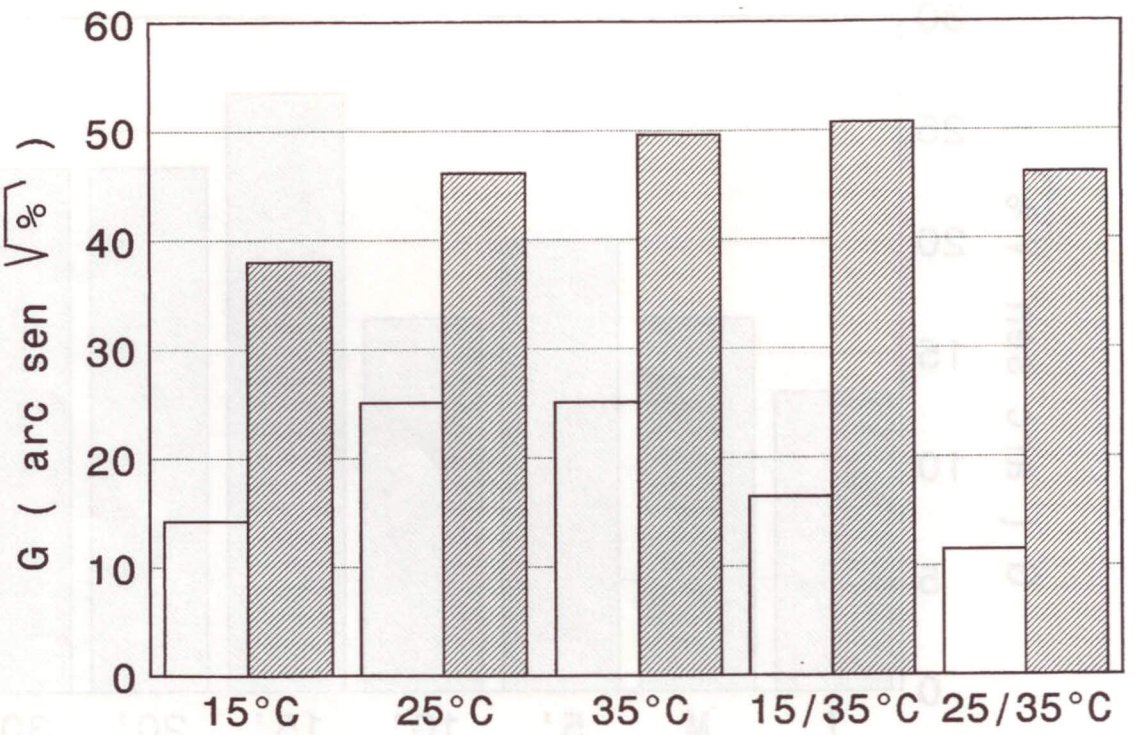

Figure 2. Effect of 15 °C, 25 °C and 35 °C constant temperatures and 15/35 °C (16/8h) and 25/35 °C (16/8h) alternating temperatures on germination of intact (non-hachured bars) and scarified (hachured bars) seeds of *B.decumbens* (IZ lot), imbibed under white light. **F** not significant for temperature treatments.

and control (Figure 3). WHITEMAN AND MENDRA (4) noticed the same with newly collected seeds of *B. decumbens* although their results were ambiguous in some extent. Grasses were shown to respond to immersion in H_2SO_4 by increasing its germination, although the effectiveness of the treatment may depend both on the storage time and species as well as on the time of immersion (3, 4, 17, 20). The treatments with sulphuric acid had no effect on further growth of seedlings of *B.decumbens* until two months after seeded (data not shown).

Potassium nitrate failed to promote the germination of *B.decumbens* (Figure 4), ratifying conclusions of ATALLA AND TOSELLO (21), WHITEMAN AND MENDRA (4) and JARK FILHO (15) on different lots with variable storage periods. Thus KNO_3 appears to be ineffective in breaking the dormancy of *B.decumbens* irrespective of its storage time, what lead us to object on role of hidrogen acceptors as a dormancy breaking agent in *B.decumbens*.

Cyanide, a respiratory inhibitor of terminal oxidases (22), can partially overcome the seed dormancy of *B. decumbens* as the germination of seeds after four days imbibed in 1 and 10mM KCN solutions have been promoted (Figure 5). Previous imbibition periods of 1 (data not shown) and 2 days failed to enhance the germinability suggesting a relatively extended period is required for action of the cyanide on intact seeds. This inhibitor has been reported to stimulate the germinability of several grasses such as *Avena fatua*, *Lolium perenne* and *Oriza sativa* (22) as well as *Brachiaria*

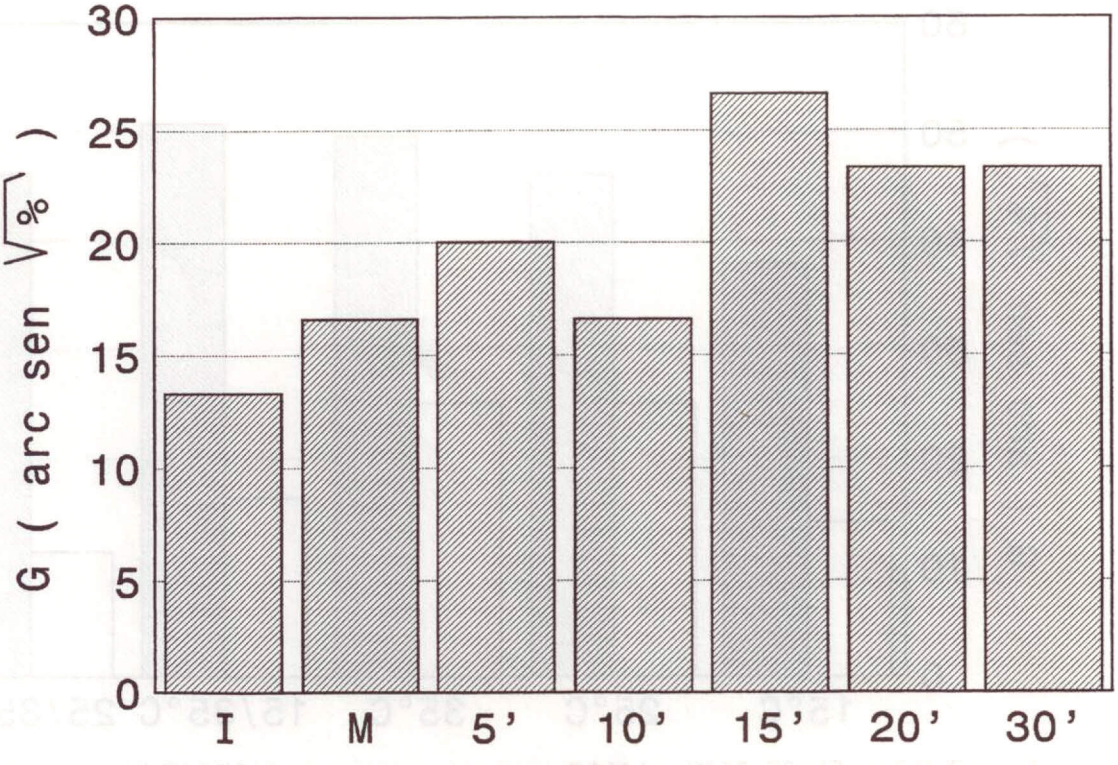

Figure 3. Effect of different times of immersion in H_2SO_4 of non-imbibed seeds on further germination of *B. decumbens* (NA lot), at 25 °C, under white light. **M**= manual scarification control; **I**= intact control . **F** not significant.

humidicola (3). Further studies are needed to check the role of alternative respiratory pathways (23) and/or pentose phosphate pathway (22) as related to the breaking of seed dormancy of *B. decumbens* and other grasses.

WHITEMAN AND MENDRA (4) report a stimulation by H_2O_2 of the germination of intact seeds of *Brachiaria decumbens* at alternating temperature regime (20/35 °C), such a promoting effect being confirmed in this work although it appears to be temperature dependent as one observed a increase of the germinability only at 25 °C as compared to 30 °C (Figure 6). Considering both the temperatures concerned to the optimal range of *B. decumbens*, such a response may be related to some reduction of the oxygen availability to embryo at higher temperatures. WHITEMAN AND MENDRA (4) postulated hydrogen peroxide acts improving oxygen tension at embryo level, thus overcoming the effects of the seed coat that limits oxygen diffusion in *B.decumbens* seeds. As temperature raises a reduction of stabability of the hydrogen peroxide molecule leading to an increase of oxygen escape from solution can account for the lack of H_2O_2 effect at 30 °C.

Ethanol is a end-product of fermentation and its effect was tested on germination of *B. decumbens* (Figure 7). Seeds soaked for 1, 2 and 4 days with aqueous solution of ethanol at concentrations of 100 and 500mM germinated as much as water imbibed seeds. GOEDERT (3) reported a little effect of ethanol on seeds of *Brachiaria humidicola* and CORBINEAU et al. (24) have noticed a strong stimulatory effect of this alcohol at low concentrations (50 and 200mM) on

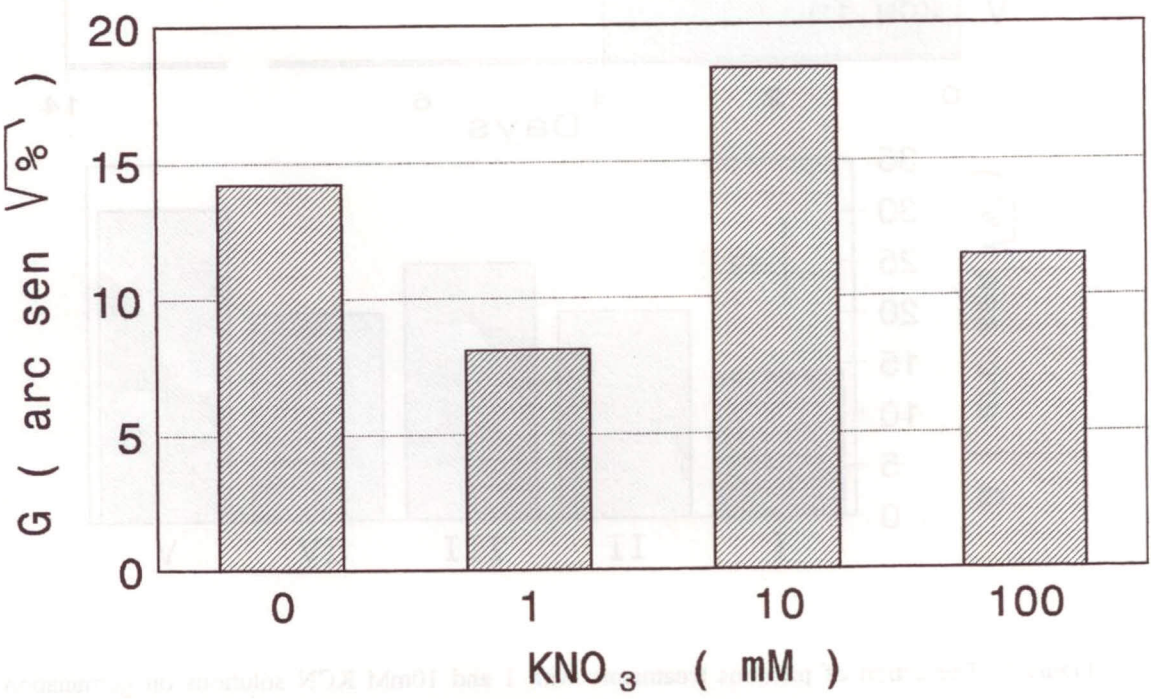

Figure 4. Effect of different nitrate potassium concentrations on germination of intact seeds of *B. decumbens* (NA lot) at 25 °C, under white light. **F** not significant.

dormant seeds of *Avena sativa*. CORBINEAU et al. (24) suggest the stimulatory action of ethanol on germination is related to its metabolism by alcohol desidrogenase and demonstrate that this action is always associated with an increase in oxygen uptake by seed. A possible explanation for the lack of action of ethanol on breaking seed dormancy of *B. decumbens* is the seed coat acting as a barrier to the diffusion of oxygen into the seed, thus limiting the action of ethanol.

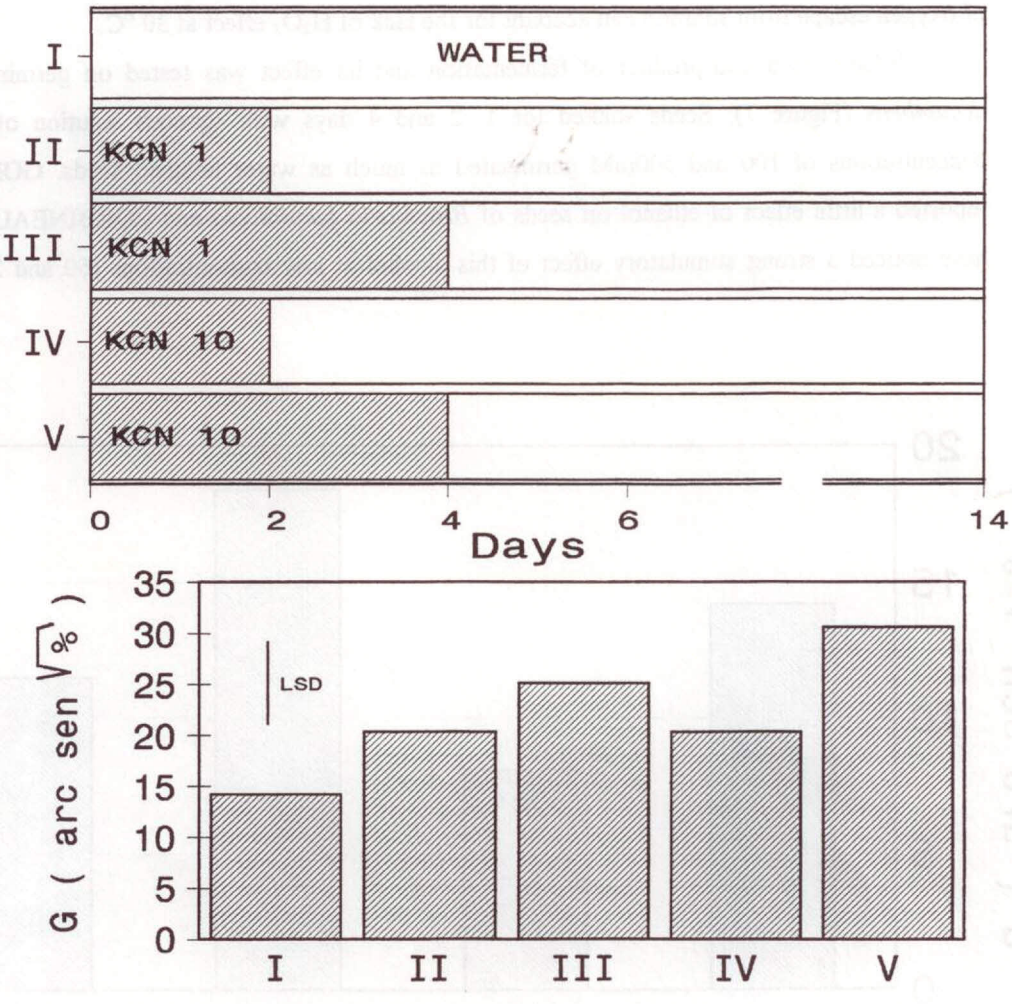

Figure 5. The action of previous treatments with 1 and 10mM KCN solutions on germination of intact seeds of *B. decumbens* (NA lot) at 25 °C, under white light. Treatments are showed above germination bars.

In conclusion, although some reports have been found concerned to the germination and dormancy of *B. decumbens*, a general conclusion is difficult to be made as the authors use seeds with variable storage times and harvesting methods. A observation can be made by analysing this and another reports on this species is that the response of *B. decumbens* to certain environmental factors (e.g. light and temperature) and treatments that can stop seed dormancy must be strongly influenced by factors such as storage time and seed lot. Neverthless it is reliable to state two forms of dormancy in *B. decumbens*, as WHITEMAN AND MENDRA (4) mention: a "primary dormancy" or a requirement for after ripening observed in freshly (up to six months) collected seeds and; a "long-term dormancy" related to the presence of the seed coat and predominant in stored seeds. Dormancy of "new" seeds of *B. decumbens* must be dependent in some extent on the seed metabolism as KCN (a respiratory inhibitor) and H_2O_2 (a oxidising agent) can partly overcome dormancy. Furthermore the called "long term" dormancy appears to be not exclusive of dry stored seeds but also occurs in two

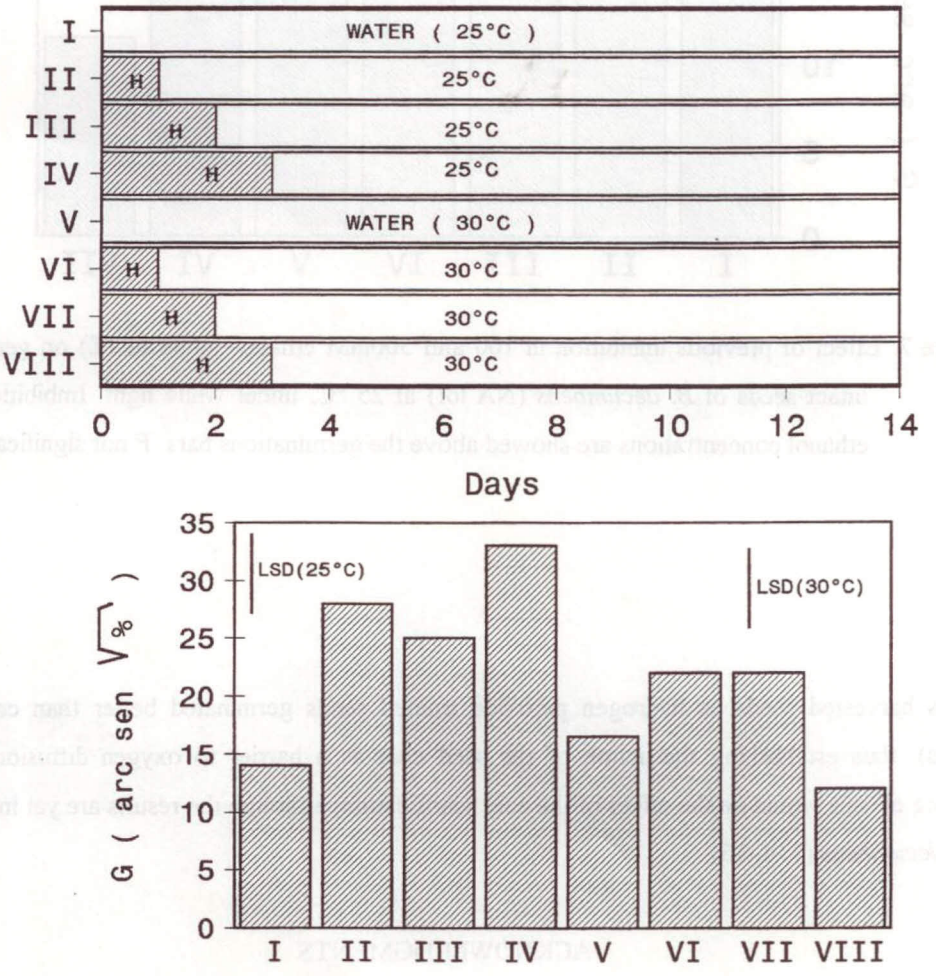

Figure 6. The action of previous treatments with 1M hydrogen peroxide solution (**H**) on germination of intact seeds of *B. decumbens* (NA lot) at 25 °C and 30 °C, under white light.

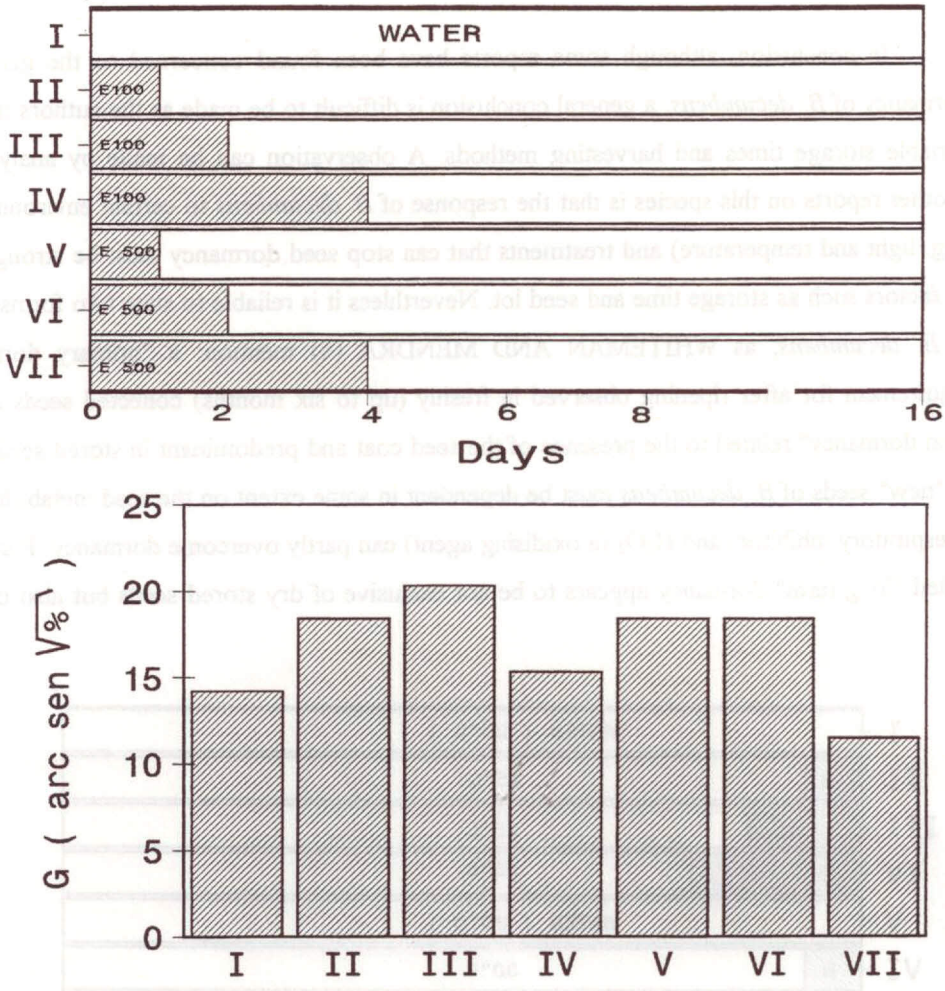

Figure 7. Effect of previous imbibition in 100 and 500mM ethanol solutions (**E**) on germination of intact seeds of *B. decumbens* (NA lot) at 25 °C, under white light. Imbibition time and ethanol concentrations are showed above the germinations bars. **F** not significant.

months harvested seeds as hydrogen peroxide treated seeds germinated better than control (see Results), thus establishing the action of the seed coat as a barrier to oxygen diffusion. Another evidence of this would be the effect of the acid scarification, although the results are yet inconclusive for *B.decumbens* (3, 4, 15).

ACKNOWLEDGMENTS

V. J.M.C. is CNPq Research Fellow. The authors tank Luiz R. Jordão for the making of graphs. This paper is dedicated to Vandré, *in memorian*.

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