

Effects of moisture content and temperature during storage on germination of the achenes of *Bidens gardneri* Baker

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ABSTRACT - (Effects of moisture content and temperature during storage on germination of the achenes of *Bidens gardneri* Baker). *Bidens gardneri* is a herbaceous species of the cerrados, whose seeds are light sensitive at 25 °C, but they become indifferent to light when stored in soil. In this work the effects of moisture content, temperature and light (during storage) upon light sensitivity during germination were studied. Ripe achenes were collected in the cerrados of Itirapina and Moji Guaçu, State of São Paulo, Brazil. The storage conditions of the achenes varied in each experiment. Achenes were stored in darkness or light, in closed bottles, at 4 °C, 20/30 °C or 25 °C. Achenes were imbibed for 24 h at 4 °C, 25 °C or 20/30 °C (in darkness) and then stored for 1, 10, 20, 30 and 40 days (40 days only for 4 °C and 25 °C). Germination tests were conducted at 25 °C and 20/30 °C. The achenes not previously imbibed showed sensitivity to light during germination. High moisture content did not affect light sensitivity of the achenes during germination but high moisture content together with storage temperatures of 25 °C and 20/30 °C had a deleterious effect upon the longevity of the achenes. Alternate temperatures during germination did not change the light sensitivity of newly collected achenes from Itirapina but changed the light sensitivity of the achenes stored imbibed at 4 °C in darkness. Alternate temperatures during storage of achenes with low moisture content did not change their photoblastism when germination was carried out at 25 °C. Alternate temperatures during storage of achenes with high moisture content followed by alternate temperatures during germination changed the light sensitivity of the achenes.

RESUMO - (Efeito do conteúdo de água e temperatura durante o armazenamento na germinação dos aquênios de *Bidens gardneri* Baker). *Bidens gardneri* é uma espécie herbácea dos cerrados, cujas sementes são fotoblásticas positivas a 25 °C, mas tornam-se indiferentes à luz quando armazenadas em solo. Neste trabalho, foram estudados os efeitos do conteúdo de água dos aquênios, da temperatura e da luz (durante o armazenamento) na sensibilidade à luz durante a germinação. Os aquênios foram coletados nos cerrados de Itirapina e de Moji Guaçu, estado de São Paulo, Brasil. As condições de armazenamento variaram em cada experimento. Os aquênios foram armazenados no escuro ou em luz, em vidros fechados a 4 °C, 25 °C e 20/30 °C. Os aquênios foram embebidos por 24 horas a 4 °C, 25 °C e 20/30 °C (no escuro) e então armazenados por 1, 10, 20, 30 e 40 dias (40 dias somente para 4 °C e 25 °C). Os testes de germinação foram conduzidos a 25 °C e 20/30 °C. Os aquênios que não foram embebidos apresentaram sensibilidade à luz durante a germinação. O conteúdo alto de água durante o armazenamento não afetou a resposta à luz dos aquênios durante a germinação, mas o conteúdo alto de água em temperaturas de armazenamento de 25 °C e 20/30 °C tiveram efeito deletério na longevidade dos mesmos. As temperaturas alternadas durante a germinação não alteraram a resposta à luz de aquênios recém coletados em Itirapina não embebidos, mas alteraram no caso de aquênios embebidos armazenados a 4 °C no escuro. Temperaturas alternadas durante o armazenamento de aquênios com baixo conteúdo de água não alteraram o fotoblastismo dos mesmos quando a germinação foi realizada a 25 °C. Temperaturas alternadas durante o armazenamento de aquênios com conteúdo de água alto seguidas de temperaturas alternadas durante a germinação alteraram a sensibilidade à luz dos aquênios de *Bidens gardneri*.

Key words - Cerrado, light sensitivity, storage, moisture content, temperature

Introduction

In recent decades 87% of the cerrado areas of the state of São Paulo, Brazil, had been destroyed due to an official encouraging policy towards sugar cane cultivation and timber and wood species plantation (Kronka 1998). Studies on the storage and viability of seeds of cerrado species are very important to

preserve representatives of this unique vegetation in the few remaining areas.

A common herb of the cerrados in the state of São Paulo which produces ornamental flowers is *Bidens gardneri* (Asteraceae). This species has been studied for nutrient growth requirements (Felippe & Dale 1990), flowering induction (Klein *et al.* 1992) and relations between controlled nutrient conditions and photoperiod for vegetative growth (Klein *et al.* 1996). Felippe (1990) showed that the species presents light sensitivity when germination is studied at 25 °C; with a long storage period in dry conditions at 4 °C (up to 14 months) the achenes lose their light sensitivity and readily germinate in darkness. Lon-

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gevity of achenes stored at 4 °C lasted for a long time; when stored for up to eight years 6% of the achenes did germinate (Sasaki *et al.* 1999b). *B. gardneri* presents heteroblastism, producing long and short achenes; germination in light was high when long achenes were used and very low with short achenes (Felippe 1990).

Sasaki *et al.* (1999a) showed that the achenes buried in the soil for one month could germinate in darkness. During this storage period the achenes were under the influence of alternating temperatures and showed a high moisture content. The objective of the present study is to relate moisture content and alternating temperatures to the loss of light sensitivity in achenes of *Bidens gardneri*, but several other aspects were considered here as for example the effect of light during storage, the size of achenes and temperature during storage in the germination in light.

Material and methods

Ripe achenes (seeds) were collected from several hundred plants of *Bidens gardneri* Baker in 1997 and 1998 (from now called 1998A) in the cerrado at Itirapina (22°51'S and 47°52'W), state of São Paulo, Brazil. One experiment was carried out with achenes collected in 1998 (from now called 1998B) in the cerrado of Moji Guaçu (22°18'S and 47°11'W), state of São Paulo, Brazil. *B. gardneri* population of Itirapina is located in a very disturbed area while the Moji Guaçu population in a preserved area of cerrado. In general, changes in viability and light sensitivity during storage were followed in achenes of median sizes (9 and 10 mm), but 5-6 mm and 12-13 mm long achenes were used in one experiment (see Sasaki *et al.* 1999a).

The newly-collected achenes were kept at 25 °C for three days in a bench in the laboratory and then stored in closed glass bottles. The storage conditions of the achenes varied in each experiment. Moisture content and imbibition were determined according to Sasaki *et al.* (1999a). Moisture content (%) of the achenes was measured in three replications of 20 achenes. Imbibition rate was calculated in achenes collected in Itirapina (1998A), using five replications of 20 achenes. The imbibition experiment was carried out with newly collected (1998A) achenes in three temperature regimes: 4 °C, 25 °C and 20/30 °C in constant white light. Germination tests were conducted in incubators (temperature varying ± 2 °C) at 25 °C and at 20/30 °C, depending on the experiment, in 9 cm diameter Petri dishes on moistened filter paper (three replications of 30 seeds) in darkness or in white fluorescent light at $437 \mu\text{W}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ at Petri dish level (Ruggiero & Zaidan 1997). Darkness was obtained by enclosing the Petri dishes in two black plastic bags and seeds were examined under a green safe light (Labouriau & Costa 1976). Final germination percentages were determined after 30 days, and the protrusion of the radicle was the criterion of germination.

In the first experiment, newly-collected achenes of 1997 harvest were imbibed at 25 °C for 1h (partially imbibed) and 24 h (totally imbibed) and after the excess water was removed they were stored at 4 °C in darkness for 10 and 35 days; dry achenes were stored at the same conditions. The moisture content was determined after 10 and 35 days storage. Germination was carried out in darkness and light at 25 °C. A second experiment was also carried out with achenes of 1997. The achenes were stored dry (not imbibed) in darkness at 4 °C, 20/30 °C (12/12 daily thermoperiods) or 25 °C. Achenes were imbibed for 24 h either at 4 °C and 25 °C (in darkness) and after the excess water was removed they were stored for 1, 10, 20, 30 and 40 days or at 20/30 °C (in darkness) and stored for 1, 10, 20 and 30 days. Germination was carried out at 25 °C in light.

The 1998A newly-collected achenes were used in the following experiments. The tetrazolium test (Delouche *et al.* 1962) was applied in the 1998A achenes stored dry and imbibed at 4 °C, 25 °C and 20/30 °C for 10 and 30 days. The test was carried out at 25 °C in darkness during 72 h using three replications of 30 achenes. The moisture content was determined in achenes stored dry and imbibed at 4 °C, 25 °C and 20/30 °C for 1, 10 and 30 days. The effect of light during storage was also studied. The achenes were stored dry in darkness and imbibed (50% moisture content) in darkness and white light for 10 and 20 days at 4 °C, 25 °C and 20/30 °C; germination was carried out at 25 °C in light. In one experiment with storage at 20/30 °C, very short (5-6 mm long) and very long (12-13 mm) dry achenes were stored for 10 days and imbibed achenes stored for 10, 20 and 30 days; germination was carried out at 25 °C in light and darkness. In the last experiment with this particular harvest achenes were stored dry or imbibed at 4 °C, 25 °C and 20/30 °C for 1, 10 and 30 days and the germination was carried out in light and in darkness at 25 °C and 20/30 °C.

The achenes from the 1998B harvest were used in just one experiment. In the experiment with this particular harvest achenes were stored dry or imbibed for one day and then stored for one day at 4 °C, 25 °C and 20/30 °C; the germination was carried out in light and in darkness at 25 °C and 20/30 °C.

The germination data were subjected to angular transformation before different treatments were compared by analysis of variance or Student's test (Snedecor 1962).

Results and Discussion

Storage period and temperature during germination are some of the factors that can change the light requirements of photoblastic seeds (Wareing & Phillips 1978). The behaviour of the achenes of *Bidens gardneri* to light was changed when stored in soil for one month (Sasaki *et al.* 1999a). These achenes buried in the soil presented a moisture content of *ca.* 50% while the ones stored dry in glass bottles in darkness at 4 °C had a moisture content of 10%; the achenes stored at 4 °C were light sensitive during germination while in those stored for one month in the soil the germination was the same both in light

and in darkness (Sasaki *et al.* 1999a). Thus one possibility was that the change in light sensitivity behaviour could be caused by the high moisture content of the seeds.

According to Milberg *et al.* (1996) when the aim of the experiment is to explain a physiological process or response, the inclusion of heavy and light seeds contributes to increase the variation of the data, thereby confounding interpretation of the results. To test the possible relationship between light sensitivity and high moisture content of the achenes, only seeds of median size, 9 and 10 mm, were used. Sasaki *et al.* (1999a) have shown that the achenes were fully imbibed after five hours from the beginning of the imbibition tests. Achenes of 1997 harvest were imbibed at 25 °C for 1 h (partially imbibed) and 24 h (totally imbibed) and stored in closed bottles at 4 °C in darkness for 10 and 35 days; dry achenes were stored at the same conditions. The moisture content was determined immediately after imbibition and after 10 and 35 days storage: dry achenes presented a moisture content of 10% independent of period of storage. The moisture content of achenes imbibed for 1 h was 25% immediately after imbibition and when stored for either 10 or 35 days; however, when imbibition lasted for 24 h, storage during either 10 or 35 days led to 47% moisture content. Thus achenes imbibed for 24 h and kept at 4 °C in darkness for 10 and 35 days presented a moisture content very similar to that of achenes stored in soil for more than one month (Sasaki *et al.* 1999a). The results of the germination tests of these treatments are shown in figure 1. The achenes not previously imbibed (dry) showed sensitivity to light after 10 and 35 days storage at 4 °C (figure 1A,B). The light sensitivity was not altered by previously imbibing the achenes for 1 h or 24 h and storing them for 10 or 35 days (figure 1C-F). Thus the high moisture content of the achenes buried in the soil for one month should not be responsible for changing the seed requirement to light reported by Sasaki *et al.* (1999a).

A second possibility to explain why the achenes lose their light sensitivity when stored in soil is soil temperature. Ortolani & Pinto (1972) reported alternating temperatures in a cerrado soil in the State of São Paulo at 5 or 10 cm deep (*ca.* 22 °C at 8.00 h and 35 °C at 16.00 h) but the temperatures were nearly

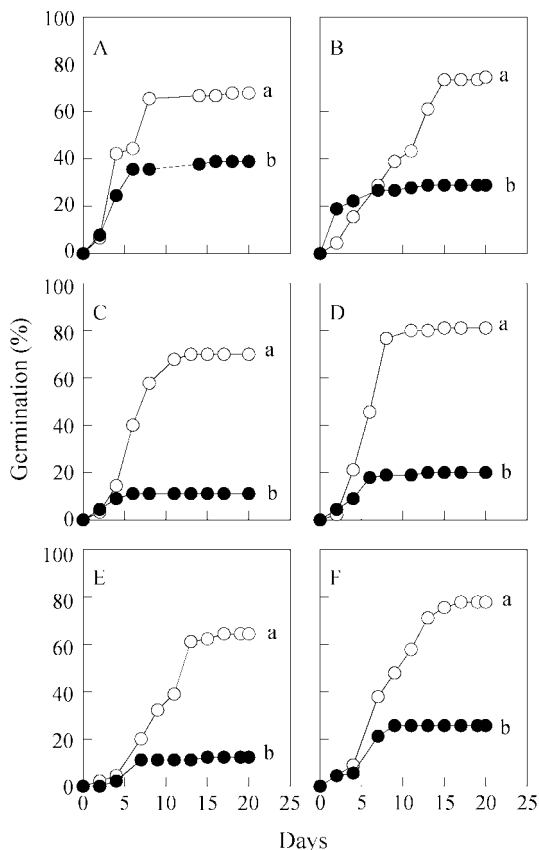


Figure 1. Germination at 25 °C in light (open symbols) and in darkness (closed symbols) of newly collected (harvest 1997) achenes of *B. gardneri* stored (dry) at 4 °C in darkness for 10 (A) and 35 days (B), control treatment; artificially imbibed for 1 h and stored at 4 °C in darkness for 10 (C) and 35 days (D); artificially imbibed for 24 h and stored at 4 °C in darkness for 10 (E) and 35 days (F). Small letters compare germination in light and darkness in each treatment.

constant at 20 cm deep (*ca.* 25 °C). In the experiments conducted by Sasaki *et al.* (1999a) the achenes were stored at 10 cm deep in the cerrado or in the forest. The achenes of *B. gardneri* are usually stored dry in closed bottles, in dark, at 4 °C (Felippe 1990). Taking all this information into account, in this work dry and imbibed achenes were stored at the temperatures of 4 °C, 25 °C and 20/30 °C.

In one experiment achenes (1998A) were stored dry or imbibed at 4 °C, 25 °C and 20/30 °C and stored for 1, 10 and 30 days and the germination was carried out in light and in darkness at 25 °C and 20/30 °C.

The germination results are presented in table 1. Achenes imbibed at 25 °C and 20/30 °C and stored for 30 days deteriorated during storage and could not be used for germination (dead achenes). The majority of the achenes imbibed at 25 °C and 20/30 °C and stored for 10 days deteriorated during storage; the number of viable achenes was not enough to prepare three replicates, so just one replicate was used for germination and these treatments could not be submitted to statistical analysis (in table 1 they are marked with *). Temperature and period of storage had no effect upon the light sensitivity of the dry achenes when the germination was tested at 25 °C and 20/30 °C: germination was significantly higher in light than in darkness, with one exception in which germination in the dark at 20/30 °C was significantly

Table 1. Germination (on day 30) at 25 °C and 20/30 °C in light and darkness of newly collected achenes of *B. gardneri* (1998A) and stored dry (10% moisture content) and imbibed (see table 3 for moisture content) at 4 °C, 25 °C and 20/30 °C in darkness for 1, 10 and 30 days.

Storage condition	Storage period (days)	Germination (%)			
		25 °C		20/30 °C	
		light	darkness	light	darkness
4 °C dry	1	96.6a	13.3bD	90.0a	14.4bD
	10	62.2a	5.5bE	68.8a	12.2bD
	30	80.0a	8.8bE	97.7a	16.6bD
4 °C imbibed	1	84.4a	16.6bD	94.4a	26.6bC
	10	77.7a	16.6bD	96.6a	16.6bD
	30	78.8a	16.6bD	92.2a	33.3bB
25 °C dry	1	75.5a	20.0bD	90.0a	14.4bD
	10	91.1a	22.2bD	98.8a	21.1bD
	30	85.5a	20.0aD	90.0a	22.2bD
25 °C imbibed	1	87.7a	28.8bC	97.7a	27.7bC
	10	100.0*	20.0*	100.0*	27.0*
20/30 °C dry	1	83.3a	10.0bD	100.0a	13.3bD
	10	74.4a	20.0bD	98.8a	11.1bD
	30	78.8a	15.5bD	87.7a	32.2bB
20/30 °C imbibed	1	92.2a	25.5bD	100.0a	68.8bA
	10	100.0*	100.0*	100.0*	92.0*

*: one replicate only; no statistical analysis in these cases

Small letters: Student's test between light and darkness in each treatment. Capital letters: values of germination in darkness analysed by ANOVA, different letters mean significant differences. F_{5%} not significant for all values of germination in light.

higher than at 25 °C, this including achenes stored dry at 20/30 °C for 30 days. Thus the use of alternating temperature during the germination test does not affect the light sensitivity of newly collected achenes with low moisture content. In relation to the achenes stored imbibed (around 50% moisture content) the light sensitivity was lost only when the imbibed achenes were stored at 20/30 °C for 10 days and the germination tests were carried out both at 25 °C and at 20/30 °C (table 1). But as mentioned above no statistical analysis could be applied in these cases. When the achenes were stored imbibed at 20/30 °C for one day and the germination test was carried out also at 20/30 °C there was a promotion of germination in darkness (a value of 68.8%), a value statistically different from all other values obtained during germination in darkness (see last column in table 1). However, achenes of *B. gardneri* collected in Moji Guaçu (1998B) showed a different behaviour: the dry and the imbibed achenes stored at 4 °C, 25 °C and 20/30 °C lost their light sensitivity when germination was carried out at 20/30 °C (table 2): thus for the 1998B achenes the important was the temperature during germination instead of the storage temperature. Pandley & Dubey (1988) have shown differences in the sensitivity to light and alternate temperatures in achenes collected from different populations in India. These authors concluded that

Table 2. Germination (on day 30) at 25 °C and 20/30 °C in light and darkness of achenes of *B. gardneri* (1998B) collected in Moji Guaçu, stored in darkness dry and imbibed for one day and kept for one day at 4 °C, 25 °C and 20/30 °C.

	Storage temperature	Germination (%)			
		25 °C		20/30 °C	
		light	darkness	light	darkness
dry	4 °C	55.5a	23.3bC	65.5a	43.3aA
	25 °C	64.4a	18.8bC	81.1a	32.2bAB
	20/30 °C	77.7a	23.3bC	67.7a	51.1aA
imbibed	4 °C	72.2a	14.4bC	75.5a	46.6aA
	25 °C	75.5a	12.2bC	61.1a	51.1aA
	20/30 °C	73.3a	21.1bC	61.1a	58.9aA

Small letters: Student's test between light and darkness in each treatment. Capital letters: values of germination in darkness analysed by ANOVA, different letters mean significant differences. F_{5%} not significant for all values of germination in light.

this behaviour was due to the effects of different disturbance levels and environmental conditions in the distinct areas. However these conclusions can not be used for the present study as only parts of one population in Itirapina and one in Moji Guaçu were tested. To make the same assumptions a more detailed research studying several populations from these two areas is necessary.

An imbibition experiment was carried out with newly collected (1998A) achenes in the three temperature regimes used here: 4 °C, 25 °C and 20/30 °C in constant white light. It was important to find out if these three temperatures could affect the imbibition of the achenes. The imbibition proceeded for five hours when 60% imbibition was reached by the achenes in all the temperature treatments. No statistical difference (ANOVA) between imbibition at these three temperature regimes was detected (data not shown). Thus if these temperatures had an effect upon response to light during germination this was not because of differences in imbibition of the achenes. Storage temperature had a very strong effect on the germination in light of achenes stored in darkness with high (45%) moisture content as can be seen in table 3. Storage temperature in darkness had no effect upon germination in light in low moisture (10%) content achenes stored for 1, 10 and 30 days.

Table 3. Germination (on day 30) at 25 °C in light of achenes of *B. gardneri* collected in 1997 stored dry (10% mc) or imbibed for 24 h (45% mc) at 4 °C, 25 °C and 20/30 °C in darkness for 1, 10, 30 and 40 days. Small letters compare germination between control and imbibed achenes in each storage condition.

Moisture content (mc) (stored achenes)	Germination (%)			
	storage period (days)			
	1	10	30	40
storage temperature 4 °C				
dry (10% mc)	93.2a	88.8a	93.3a	92.2a
imbibed (45% mc)	87.7a	91.1a	85.5a	38.8b
storage temperature 25 °C				
dry (10% mc)	93.3a	83.3a	88.8a	91.1a
imbibed (45% mc)	86.6a	47.7b	11.1b	1.1b
storage temperature 20/30 °C				
dry (10% mc)	100.0a	98.8a	87.7a	-
imbibed (45% mc)	98.8a	41.0b	0.0b	-

-.: not tested.

No effect was observed even up to day 40 of storage at 4 °C and 25 °C (table 3). With the imbibed achenes, however, germination was strongly reduced when the achenes were stored at 4 °C for at least 40 days (but not before). The reduction in germination could be seen already in imbibed achenes stored for 10 days when the temperature during storage was 25 °C or 20/30 °C (table 3). Also a large number of the imbibed achenes died during storage at 25 °C and 20/30 °C for periods longer than 10 days.

To find out if the achenes that did not germinate shown in table 3 were dead, the tetrazolium test was applied in the achenes stored dry and imbibed at 4 °C, 25 °C and 20/30 °C for 10 and 30 days. The results can be seen in table 4: there was a strong reduction in the reaction to the tetrazolium in imbibed achenes stored for 10 and 30 days at 25 °C and 20/30 °C; after 30 days storage the majority of the achenes must have been dead. This effect was not observed in imbibed achenes stored at 4 °C and in all dry achenes stored at 4 °C, 25 °C and 20/30 °C for 10 and 30 days. The moisture content of newly collected achenes is presented in table 5. The data show small differences in the moisture content of achenes imbibed for 1, 10 or 30 days in the three temperature regimes tested. Taking into account tables 3 and 5, high moisture content of the achenes and higher temperatures during storage (25 °C or 20/30 °C) seem to be responsible for the death of the achenes of *B. gardneri*. Low temperature (4 °C) during storage of achenes with high moisture content delays the death of the achenes when compared to higher temperatures during storage (table 3).

It seems that light is also important for seed viability during storage of imbibed achenes at controlled temperatures, as can be seen in figure 2. The presence of light during storage did not change viability when the imbibed achenes were stored at 4 °C

Table 4. Results of the tetrazolium test of dry and imbibed achenes of *B. gardneri* (1998A) stored at 4 °C, 25 °C and 20/30 °C in darkness for 10 and 30 days.

Storage (days)	red-stained embryos(%)					
	4 °C		25 °C		20/30 °C	
	dry	imbibed	dry	imbibed	dry	imbibed
10	81.0	85.5	92.2	39.9	93.3	49.9
30	79.9	87.7	78.8	6.6	82.2	2.2

Table 5. Moisture content (%) of dry and imbibed achenes of *B. gardneri* (1998A) stored at 4 °C, 25 °C and 20/30 °C for 1, 10 and 30 days.

Storage (days)	moisture content (%)					
	4 °C		25 °C		20/30 °C	
	dry	imbibed	dry	imbibed	dry	imbibed
1	10.6	46.1	10.6	49.0	10.9	48.6
10	10.7	49.6	10.7	50.6	10.7	52.0
30	10.9	49.0	10.7	56.8	10.6	58.4

(figures 2A, B). But at 25 °C storage of imbibed achenes has a inhibitory effect in germination: achenes imbibed and stored for 10 days in light at 25 °C showed the same final germination as the dry achenes (figure 2C) and a strong reduction of germination occurs when achenes are stored for 20 days (figure 2D); in imbibed achenes stored in darkness for 10 days germination was very low (figure 2C) and when stored for 20 days germination was nihil (figure 2D).

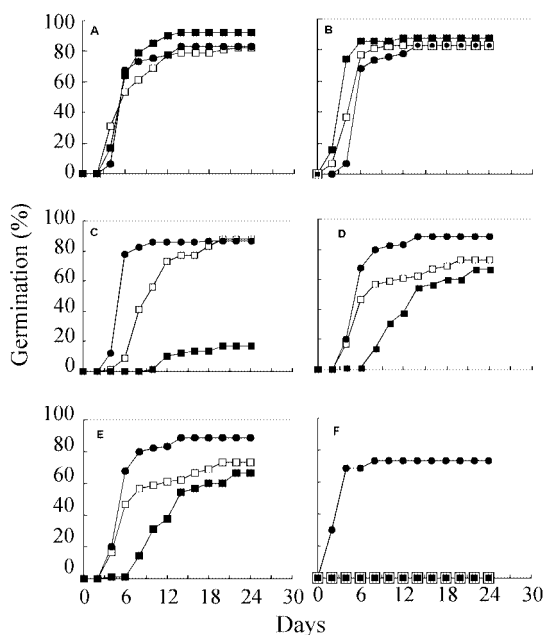


Figure 2. Germination in light at 25 °C of achenes of *B. gardneri* (harvest 1998A) stored dry (10% moisture content) in darkness and stored imbibed (50% moisture content) in darkness and in white light for 10 and 20 days at 4 °C (A, B), at 25 °C (C, D) and at 20/30 °C (E, F). Closed circles: dry achenes; open squares: imbibed achenes stored in light; closed squares: imbibed achenes stored in darkness.

During storage in light at 25 °C some imbibed achenes germinated: 45% of the achenes germinated during the 10 days storage period and 46% during the 20 days storage period (although germination only occurred during the first 10 days). When the imbibed achenes were stored at 20/30 °C (figures 2E, F) germination was inhibited with storage for 20 days. Figure 2E shows a non-significant reduction in germination for imbibed achenes stored at 20/30 °C (in relation do dry achenes) in light and in darkness for 10 days; no germination occurred in achenes stored for 20 days, in light and darkness (figure 2F).

The viability of median sized achenes of *B. gardneri* is reduced when they are stored at 20/30 °C in darkness with high moisture content for 10 days and longer (see table 3). When very short and very long achenes (1998A) were stored imbibed at 20/30 °C for 10, 20 and 30 days dead achenes were only found among the short achenes stored for 30 days (41.2% were alive); in relation to long achenes 25% of them were alive after storage for 20 days against only 1.2% when storage lasted for 30 days. Compared to the dry achenes germination (carried out at 25 °C) was reduced in all the imbibed achenes stored for 10 days (table 6); both long and short achenes were sensitive to light (see data for the achenes stored for 10 days). Germination was further reduced when the short achenes were stored for 20 and 30 days and no germination occurred in imbibed long achenes stored for 20 days (table 6). Felipe (1990) has shown that the newly collected short achenes of *B. gardneri* were dormant when compared to the long ones and that with the removal of the seed coat the short achenes did germinate. In the present work it has been shown that the short achenes

Table 6. Germination (on day 30) at 25 °C in light and darkness of short (5-6 mm long) and long (12-13 mm long) achenes of *B. gardneri* (1998A) stored dry and imbibed, at 20/30 °C in darkness for 10, 20 and 30 days.

Storage conditions		Germination (%)				
		short achenes		long achenes		
		light	dark	light	dark	
dry	10 days	80	30	70	30	
	imbibed	10 days	28	13	20	7
		20 days	5	5	0	0
	30 days	3	0	-	-	

survive for a longer period of time (41.2% of short achenes were alive after storage for 30 days while only 1.2% of long achenes were alive with the same storage period) than the long ones when artificially imbibed, but no differences could be seen in their germination. No differences in germination between short and long achenes which were not imbibed and stored at 4 °C were also reported previously (Sasaki *et al.* 1999a). Differences in germination of short and long achenes have been also shown for *Bidens pilosa* (Forsyth & Brown 1982). In *Bidens odorata* long achenes germinate better and lose their light sensitivity during storage faster than the short ones (Corkidi *et al.* 1991).

The present results could be resumed as follows:

1. high moisture content does not affect the light sensitivity of the achenes of *B. gardneri* during germination;
2. alternate temperatures during storage of achenes with low moisture content do not change their photoblastim when the achenes were germinated at 25 °C;
3. alternate temperatures during storage of achenes collected in Itirapina with high moisture content followed by alternate temperatures during germination change the light sensitivity of the achenes (this can explain the germination of *B. gardneri* achenes during storage in the soil, as reported by Sasaki *et al.* 1999a). Also alternate temperatures during storage of achenes, change the light sensitivity of the achenes when germination is carried out at 25 °C. This explains why the intact achenes removed from the soil lose their photoblastism after 1 and 3 months storage as related by Sasaki *et al.* (1999a);
4. alternate temperatures of 20/30 °C during germination do not change the light sensitivity of achenes collected in Itirapina and stored for short periods of time (up to 30 days) at 4 °C;
5. high moisture content together with storage temperatures of 25 °C and 20/30 °C have a deleterious effect upon the longevity of the achenes (life span strongly reduced).

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