



Role of seed environment and covering structures on large crabgrass germination

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ABSTRACT

The success of large crabgrass (*Digitaria sanguinalis*) growing among summer crops in Argentina, may be partly explained by its escape from weed controls related to the emergence of different seedlings cohorts determined by seed dormancy and germination requirements. The objectives of this work were to evaluate the effect of temperature, red (R):far-red (FR) ratio and the possible role of the caryopses covering structures on the release of seed dormancy in *D. sanguinalis*. Therefore, the effects of moist pre-treatment duration, light and temperature, as well as the caryopsis covering structures, and imbibition with H₂O₂ and the extract of caryopses covers on seed germination, were investigated. Moist pre-treatment at 5 and 20 °C promoted dormancy release and fluctuating temperatures between 20/30 °C and light promoted germination. However, exposure to 30 min of light with a high R:FR ratio reduced germination. Removing or puncturing some of the caryopsis covering structures, as well as imbibition with 2.6 M H₂O₂ enhanced seed germination. Results suggest that the extended seedling emergence throughout the season could be due to the influence of the environmental factors studied here on dormancy release and germination, and that seed covering structures have an important role in seed dormancy imposition for this species.

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1. Introduction

Weed population shifts were observed during the last twenty years when conventional tillage systems were changed to permanent no-tillage in the main agricultural region of Argentina. In the absence of tillage, grassy annual populations increased and broad-leaved populations decreased (de la Fuente et al., 2006; Scursoni and Satorre, 2010). However, *Digitaria sanguinalis* (L.) Scop. has maintained or even increased its constancy, becoming an important problematic weed species among local soybean and maize crops, probably because of its extended period of emergence in the field, which allows some seedling cohorts to escape from weed controls (Oreja and de la Fuente, 2005). This summer annual weed grows in both temperate and tropical regions of the world, between 50°N and 40°S (Holm et al., 1977). Its presence produces significant yield losses in different crops (Monks and Schultheis, 1998; Fu and Ashley, 2006; Oreja and González-Andújar, 2007).

Seedling emergence is one of the most critical stages in a weed life cycle, because it determines successful competition and reproduction by the end of the growing season (Forcella et al., 2000). On the other hand, the seedling stage is also the most vulnerable and, consequently, the stage where control measures are most effective. The timing of

seedling emergence in the field is determined by previous processes which include seed dormancy, germination and pre-emergence seedling growth (Benech-Arnold et al., 2000; Forcella et al., 2000). Knowledge about how environmental conditions affect seed dormancy and germination would be useful to predict *D. sanguinalis* emergence and, thus, to plan successful weed management strategies.

Temperature is an important factor regulating seed dormancy under field conditions (Batlla and Benech-Arnold, 2010). For example, the seeds of summer annual species generally require exposure to low temperatures during autumn and winter to diminish their dormancy level. This mechanism allows seed germination from spring to early summer and, as a consequence, plant growth occurs during summer and seed dispersion in autumn. This timing of stages ensures the reproductive success of these species in temperate climates (Benech-Arnold et al., 2000; Bewley et al., 2013). Once seeds reach a low dormancy level, many weed seeds require additional factors to terminate dormancy and allow germination, such as light and alternating temperatures (Benech-Arnold et al., 2000). There is evidence indicating that the perception of these signals allows seeds to detect an overlying canopy or excessive burial depth, thus avoiding futile germination (Fenner, 1980; Thompson and Grime, 1983; Batlla et al., 2000). For example, fluctuating temperatures of the upper layers of the soil are reduced by the presence of a crop canopy, thus inhibiting germination of species that require fluctuating temperatures to terminate dormancy (Benech-Arnold et al., 1988; Huarte and Benech-Arnold, 2003). On the

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other hand, the presence of a crop canopy filters sun light reducing the red (R):far-red (FR) ratio of the light reaching the soil surface. This FR enriched canopy-filtered light would reduce the germination of weed seeds located at the surface of species sensitive to environments with low R:FR ratios through the action of phytochrome (Bewley et al., 2013).

Environmental conditions promoting seed dormancy release and germination of *D. sanguinalis* are quite different among published studies (Turner et al., 2012). Successful treatments include exposure to low temperatures, such as 3 °C for 28 days (Toole and Toole, 1941), 4 °C for 2 weeks (Hsu et al., 1985) or 2–4 °C for two months (Delouche, 1956) and moist conditions, and hot temperatures of 50–60 °C and dry conditions (Taylorson and Brown, 1977). According to Toole and Toole (1941), Hsu et al. (1985) and King and Oliver (1994), fluctuating temperatures of 20°/35 °C and 20°/30 °C (18 h/6 h) with light (18 h/6 h) are the best conditions for *D. sanguinalis* seed germination. Zhang et al. (2012) reported that, in addition to fluctuating temperatures of 20°/30 °C (12 h/12 h), germination can also be maximal at constant temperatures of 25 °C and 30 °C (12 h light/12 h darkness). Although most evidence indicates that light promotes seed germination of *D. sanguinalis* seeds, the role of light quality in seed dormancy has not yet been explored for this species.

In most grasses, primary dormancy (innate dormancy of seeds recently dispersed, Benech-Arnold et al., 2000) imposition is related to seed coats. The surrounding tissues can impose dormancy i) preventing water uptake (common in dicotyledonous), gaseous exchange (through seed coats and pericarp tissues surrounding the caryopsis) or embryo expansion (hard tissues of the pericarp and seed coats) or ii) as a source of germination inhibitors (present in different seed outer tissues but mainly in glumes) (Adkins et al., 2002). In *D. sanguinalis*, evidence shows that primary dormancy imposed by the seed coat is not due to permeability barriers to water uptake (Gianfagna and Pridham, 1951; Delouche, 1956; Biswas et al., 1978). Primary dormancy could be due to germination inhibitors in the surrounding structures but the mechanism is still unclear (Gianfagna and Pridham, 1951; Delouche, 1956; Gallart et al., 2008). Among the surrounding structures, the lemma seems to be the most important structure responsible for dormancy imposition (Gallart et al., 2008).

In many grasses, such as barley (Lenoir et al., 1986) and oats (Corbineau et al., 1986), dormancy has been explained by oxygen trapping by phenolic compounds present in the surrounding structures. When in contact with oxygen, these compounds are oxidised, reducing the diffusion of oxygen towards the embryo and thus imposing seed dormancy. The exogenous application of hydrogen peroxide (H₂O₂), a highly oxidative compound, has resulted in a successful technique to promote germination in species showing phenolic compounds, such as sorghum (Benech-Arnold et al., 1992), barley, wheat, rice (Naredo et al., 1998) and *Zinia elegans* (Ogawa and Iwabuchi, 2001). Dormancy imposition in *D. sanguinalis* could possibly be related to the presence of oxidizable compounds in fruit structures, as shown in many other grasses, although this possibility has not been tested yet.

The objectives of this work were to evaluate i) the effect of temperature on primary seed dormancy release, ii) the effect of R:FR ratio on seed germination and iii) the possible role of the surrounding caryopsis structures on seed dormancy of local biotypes of *D. sanguinalis*. To achieve these objectives, experiments in germination chambers under different environmental conditions were carried out.

2. Materials and methods

2.1. Seed material

D. sanguinalis dispersal unit is usually called “seed” but it is actually the spikelet composed of the caryopsis enclosed within the lemma and palea, and all these structures are covered by the glumes. Hereafter, spikelets will be referred to as seeds unless otherwise specified. Seeds were collected during the season of natural dispersal: March 2008 (for

experiment 1) and March 2009 (for experiments 2, 3, 4, and 5), from 10 plants randomly selected in a field located at Roque Pérez (35°20'S lat, 59°23'W long), Province of Buenos Aires, Argentina. Mature seeds were collected by shaking the panicles into a paper bag. After collection, seed samples were winnowed with a seed blower to eliminate very small seeds and plant residues. Seeds were later stored in paper bags at room temperature (20–25 °C) and relative humidity (<20%) for 40 days approximately, until each experiment was performed, considering that seeds stored under dry conditions for at least 2 months do not have increased germination capacity (Toole and Toole, 1941; Gallart et al., 2009).

2.2. General experimental procedures

Several experiments were carried out in germination chambers at the Faculty of Agronomy of Buenos Aires University, Argentina. Experimental units consisted of 50 seeds per replicate, placed in 9 cm diameter Petri dishes with two paper filters (Double Rings, Argentina). Distilled water (4 mL) was added into each dish at the beginning of the tests, and afterwards dishes were sealed with Parafilm to avoid evaporation. For experiments where seeds underwent moist pre-treatment, seeds were kept in darkness by wrapping the dishes into aluminium foil. Germination (radicle emergence) was recorded at regular intervals in experiments with light until no further seeds germinated, and water was added as required, when germination was checked. For treatments in darkness, germination was recorded 20 days after experiment initiation. At the end of each experiment incubation period, the viability of non-germinated seeds was tested with a 1% tetrazolium (2,3,5-triphenyl-2H-tetrazolium chloride) solution (International Seed Testing Association, ISTA, 1999). Germination percentage of each treatment was calculated based on viable seeds.

2.2.1. Experiment 1: effect of pre-treatment conditions on seed dormancy release and germination

A completely randomised factorial experiment (Exp. 1), with five replicates, was performed. Seeds were pre-treated under moist conditions at three temperatures (5°, 20° and 30 °C) for different periods of time (14, 21 and 28 days). Following pre-treatment, dishes were incubated under three fluctuating temperature regimes (8/16 h: 10°/20 °C, 15°/25 °C and 20°/30 °C) and two light conditions (8 h of darkness and 16 h of light and continuous darkness). For treatments under light, dishes were placed into a germination chamber with six fluorescent tubes providing 40 μmol m⁻² s⁻¹.

2.2.2. Experiment 2: effect of light quality on germination

A completely randomised factorial experiment, with five replicates was performed. Seeds were pre-treated in darkness at 5 °C under moist conditions for different periods of time (0, 15 and 30 days) and exposed to different light treatments: i) red light for 60 min, ii) far-red light for 30 min, iii) a cycle of red light for 60 min/darkness for 30 min/far-red light for 30 min and iv) always in darkness. After light treatments, seeds were kept in darkness and incubated at 20/30 °C (8/16 h) for 20 days.

Red light (calculated proportion of the phytochrome FR-absorbing form (Pfr) and the phytochrome R-absorbing form (Pr) as [Pfr:Pr]: 87%, 35 μmol m⁻² s⁻¹) was provided by red fluorescent tubes (Phillips 40/15, Germany). Far-red light (calculated proportion of phytochrome as Pfr [Pfr:Pr]: 2.7%, 42 μmol m⁻² s⁻¹) was provided by a 150 W incandescent reflector lamp (Phillips R95, Buenos Aires, Argentina) in combination with an 8-cm water filter and an RG9 filter (Schott, Mainz, Germany).

2.2.3. Experiment 3: the role of the caryopsis covers on seed dormancy

An experiment in a completely randomised design with three replicates was performed. The treatments were i) whole seeds, ii) naked caryopses and iii) whole seeds after moist pre-treatment in darkness

at 5 °C for 30 days. These latter conditions are considered to be the best for a pre-chilled treatment, based on the results from Experiment 1. Naked caryopses are the seeds without glumes, lemma and palea, which were removed using a scalpel and fine tweezers. The dishes were placed in a chamber at 20 °C/30 °C (8/16 h) with light (8 h of darkness and 16 h of light) to evaluate seed germination, conditions chosen as the best for germination according to results from Experiment 1.

2.2.4. Experiment 4: the role of the different caryopsis covering structures on seed dormancy

In order to evaluate the role of the different caryopsis covering structures on seed dormancy, a completely randomised experiment with 5 replicates was performed. Treatments were i) whole seeds, ii) naked caryopses, iii) caryopses without the glumes, iv) caryopses without the glumes and the lemma, v) seeds with a puncture in the endosperm, and vi) naked caryopses imbibed with extract from caryopsis covering structures. The extract was prepared by soaking the covering structures of 250 naked seeds in 15 mL of distilled water at 30 °C for 24 h in darkness. Dishes were placed in a chamber at 20 °C/30 °C (8/16 h) with light (8 h of darkness and 16 h of light), conditions chosen as the best for germination according to results from Experiment 1.

2.2.5. Experiment 5: role of germination inhibitors in the caryopsis covering structures

A completely randomised factorial experiment with 3 replicates was performed. Factors were i) H₂O₂ concentrations with 5 levels (0 M (distilled water), 0.5 M, 1 M, 1.5 M and 2.6 M) and imbibition times on H₂O₂ with 3 levels (2, 4 and 6 h). After imbibition treatments, seeds were transferred to a germination chamber at 20/30 °C (8/16 h) with light (8 h of darkness and 16 h of light and continuous darkness), conditions chosen as the best for germination according to results from Experiment 1.

2.3. Data analysis

As germination data follows a binomial distribution (probability ranging from 0 to 1), the best way to achieve linearity is the use of a generalised linear model with logit link function and binomial error distribution, setting the variance to “mean (1-mean)” (Venables and Ripley, 1994; Schütz and Rave, 1999). This was followed by a DGC's post-test (Di Rienzo et al., 2002) for mean separation with a significance level of 5%. Statistical analysis was performed using InfoStat software (InfoStat 2010 version. InfoStat Group, FCA, National University of Córdoba, Argentina) assisted by R (R version 2.11.1 Copyright 2010; The R Foundation for Statistical Computing).

3. Results

3.1. Effect of pre-treatment conditions on seed dormancy release and germination

Results of Exp. 1 showed significant ($p < 0.001$) interactions between moist pre-treatment temperatures, days of treatment, fluctuating temperatures and light (Table 1). Seeds pre-treated at 5 or 20 °C for 28 days, followed by incubation with light at 20/30 °C, showed the highest germination percentage. Seeds germinated in darkness, regardless of pre-treatment temperatures and fluctuating temperatures, showed lower germination values. However, maximal germination was achieved under similar conditions as those observed for seeds germinated in light (5 °C pre-treatment and 20/30 °C germination) (Fig. 1).

3.2. Effect of light quality on germination

Significant ($p < 0.001$) interactions between factors were observed in Exp. 2 (Table 2). Seeds pre-treated for 30 days under moist conditions at 5 °C and with different light treatments showed high germination

Table 1

Generalised linear models test summary of a) germination percentage of large crabgrass seeds from Exp. 1 at different pre-treatment temperatures (PtT), pre-treatment duration (PtD), fluctuating temperature (FT) and with or without light (L).

Factor or interaction	Degrees of freedom	Deviance	p
a)			
Pre-treatment temperature (PtT)	2	347.13	<0.0001
Pre-treatment days (PtD)	2	4.16	0.1248
Fluctuating temperature (FT)	2	3208.33	<0.0001
Light (L)	1	857.67	<0.0001
PtT × SD	4	82.82	<0.0001
PtT × FT	4	319.24	<0.0001
PtD × FT	4	14.04	0.0072
PtT × L	2	20.87	<0.0001
PtD × L	2	30.18	<0.0001
FT × L	2	62.84	<0.0001
PtT × PtD × FT	8	34.41	<0.0001
PtT × PtD × L	4	9.00	0.0611
PtT × FT × L	4	29.96	<0.0001
PtD × FT × L	4	9.35	0.0530
PtT × PtD × FT × L	8	28.43	0.0004

(above 90%), except for those exposed to 30 min of far red light, which showed a lower germination value (72%) ($p < 0.05$). The inhibitory effect of far red light was not observed in seeds pre-treated for 15 days. Seeds without pre-treatment showed almost no germination (Fig. 2).

3.3. The role of the caryopsis covering structures on seed dormancy

In Exp. 3 naked caryopses, and whole seeds pre-treated at 5 °C under moist conditions, showed higher ($p < 0.05$) germination percentages than whole seeds without pre-treatment (GLM degrees of freedom: 6, deviance: 184.03 and p value: <0.05, Fig. 3). On the other hand, in Exp. 4 seed germination was increased by removing or puncturing some of the caryopsis covering structures. Seeds punctured by the endosperm reached the highest germination value (36%) and showed significant differences ($p < 0.05$) with the seeds and the naked caryopses (GLM degrees of freedom: 5, deviance: 221.57 and p value: <0.05, Fig. 4). When only the glumes were removed, germination was low and not significantly different ($p < 0.05$) from the germination of the whole seeds, which was zero. Germination percentage of seeds without the glumes and the lemma was higher than the germination of whole seeds and similar to naked caryopses (Fig. 4). Naked caryopsis with the extract of the covering structures showed no differences ($p < 0.05$) compared with the naked caryopses alone (Fig. 4).

3.4. Role of germination inhibitors in the caryopsis covering structures

In Exp. 5, significant ($p < 0.05$) interactions between treatment factors were observed (Table 3). Imbibition of seeds on 2.6 M H₂O₂ increased the germination percentage, regardless of imbibition time, and imbibition on 1.5 M H₂O₂ for 6 h increased seed germination when compared to 2 h of imbibition (Fig. 5).

4. Discussion

Our results showed that the best conditions for primary dormancy release were seed pre-treatment at 5 °C under moist conditions and incubation at fluctuating temperatures of 20/30 °C (8/16 h) with light (Fig. 1). Similar conditions promoting dormancy release in *D. sanguinalis* seeds were previously reported by Toole and Toole (1941) and Delouche (1956).

Interactions among the duration of pre-treatment, fluctuating temperatures and light were observed (Table 1). Results reported in Fig. 2 show that as duration increased (30 days), germination tended to be higher in comparison to shorter periods of pre-treatment (15 days). An increase in seed germination with extended exposure to low

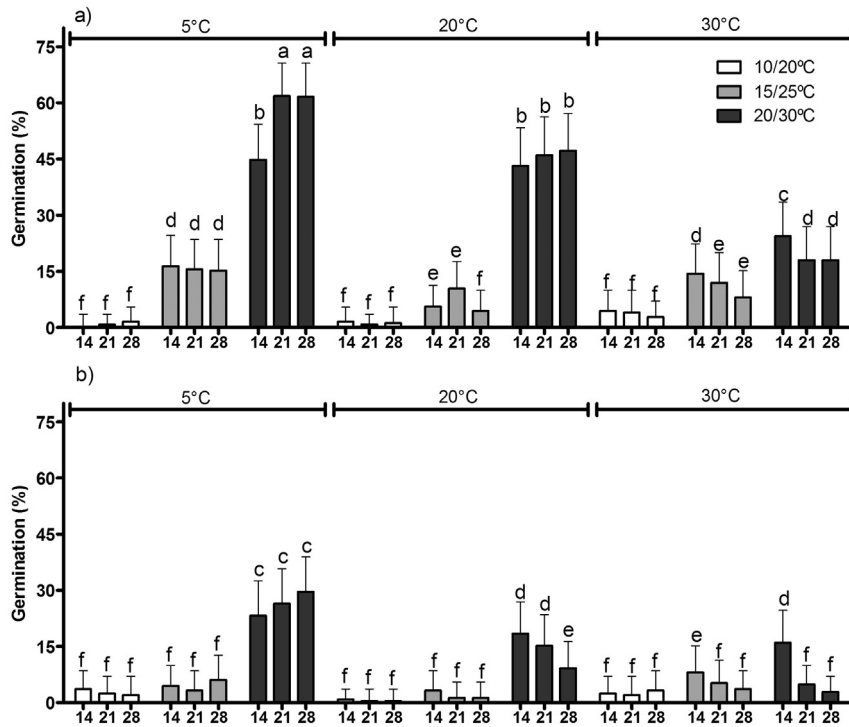


Fig. 1. Germination of *D. sanguinalis* at fluctuating temperatures (FT) of 10/20 °C, 15/25 °C and 20/30 °C (8/16 h) after different moist pre-treatment durations (SD) of 14, 21 and 28 days (14, 21 and 28 respectively), at temperatures (ST) of 5 °C, 20 °C and 30 °C, with 8 h of light (a) and in darkness (b). Values are the means and vertical bars are binomial confidence intervals. Columns with the same letters are not significantly different according to DGC's test ($p < 0.05$).

temperatures has been reported in many spring emerging weed species (Baskin and Baskin, 1998), such as *Polygonum aviculare* (Courtney, 1968; Batlla et al., 2007). In summary, *D. sanguinalis* seeds showed the characteristic response observed in summer annual weeds from temperate climates, in which dormancy release is promoted by exposure to low temperatures (Baskin and Baskin, 1998; Bencch-Arnold et al., 2000).

Although the seeds lost dormancy at low temperatures, results showed that dormancy release can also occur after pre-treatment at relatively “high temperatures” of 20 °C (Fig. 1), a temperature that was not previously reported as favourable to release dormancy in *D. sanguinalis* seeds (Turner et al., 2012). This wide thermal range (5–20 °C) that enables dormancy release may probably allow this species to extend its germination up to late spring in the field. Therefore, under cropping conditions, in which the first cohorts are usually controlled by pre sowing or sowing applications of herbicides, late-emerging cohorts may avoid such control measures.

The highest germination observed when seeds were pre-treated at cold (5 °C) and warm (20 °C) temperatures and then incubated at fluctuating temperatures of 20/30 °C and light (Fig. 1) could explain the peak of emergence usually observed in the field during mid and late spring (Oreja and de la Fuente, 2005; Gallart et al., 2010). These results partially agree with Toole and Toole (1941), King and Oliver (1994) and Zhang et al. (2012), who also reported fluctuating temperatures of 20/35 °C and 30/40 °C as successful thermal regimes for *D. sanguinalis*

germination. However, present results showed that a small proportion of seeds are also capable of germinating at lower temperatures (15/25 °C) (Fig. 1). This could probably determine the first cohorts of seedlings emerging in the field during early spring. On the other hand changes in dormancy level in seeds are likely to comprise changes in sensitivity to fluctuating temperatures. For example, seeds after-ripened in the soil during winter require lower amplitudes and smaller number of cycles of fluctuating temperatures to release dormancy than recently shed seeds (Bencch-Arnold et al., 2000). This increase in seed response to fluctuating temperatures as a consequence of after-ripening could be also explaining, at least in part, the extended seedling emergence period (different cohorts over the season) reported under field conditions for this species.

Table 2

Generalised linear models test summary of germination percentage of large crabgrass seeds from Exp. 2 at different pre-treatment duration (SD), and with different light treatments (L).

Factor or interaction	Degrees of freedom	Deviance	p
Pre-treatment duration (PtD)	2	3322.64	<0.0001
Light (L)	3	50.22	<0.0001
SD × L	6	82.11	<0.0001

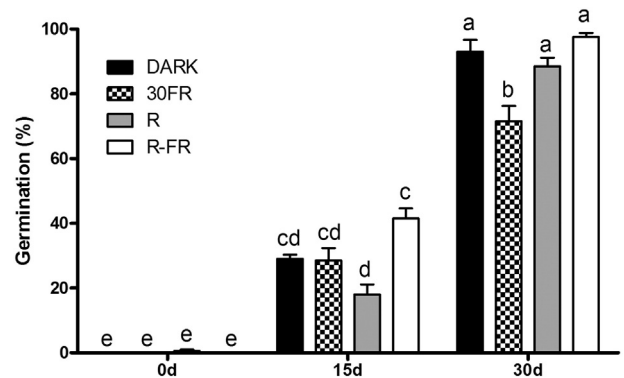


Fig. 2. Germination of *D. sanguinalis* at 20/30 °C (8/16 h), moist pre-treatment duration (SD) of 0 days (0 d), 15 days (15 d) and 30 days (30 d), and exposed to darkness (DARK), 30 min far red light (30FR), 60 min red light (60R) and a cycle of 60 min red light/30 min darkness/30 min far red light (R:FR). Values are the means and vertical bars are binomial confidence intervals. Columns with the same letters are not significantly different according to DGC's test ($p < 0.05$).

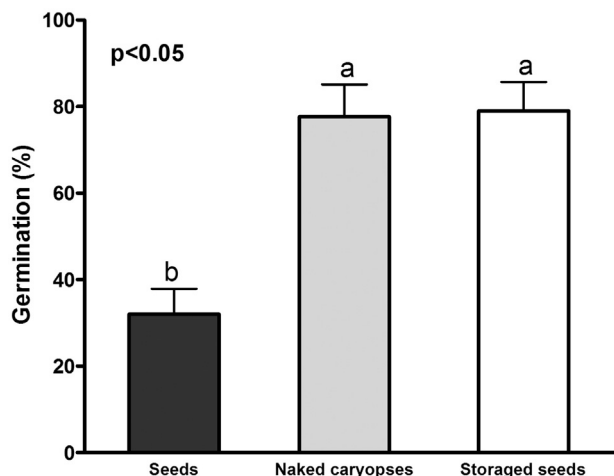


Fig. 3. Germination percentage of *D. sanguinalis* seeds at 20/30 °C (8/16 h) with light (8 h of darkness and 16 h of light), for whole seeds (no pre-treatment), naked caryopses and whole seeds after moist pre-treatment for 30 days at 5 °C. Values are the means and vertical bars are binomial confidence intervals. Columns with the same letters are not significantly different according to DGC's test ($p < 0.05$).

Cyclic light treatment (a cycle of red light for 60 min/darkness for 30 min/far-red light for 30 min) was included to verify if the short exposure to the red light, previous to canopy closure could be enough to allow germination (Benech-Arnold et al., 2000). An inhibitory effect of FR light was observed in seeds pre-treated for 30 days at 5 °C but not for 15 days, probably due to the higher dormancy level of these seeds (Fig. 2). Although the inhibitory effect of FR light on seed germination has been reported for many weed species, such as *Lolium multiflorum* (Deregibus et al., 1994), *Silene gallica* and *Brassica campestris* (Batlla et al., 2000), and *Amaranthus palmeri* (Jha and Norsworthy, 2009), this is the first body of evidence of the germination inhibitory effect of low R:FR ratio on *D. sanguinalis* seeds. The ecological role of the fluctuating temperatures plus the effect of R:FR ratio could be related to the detection of canopy gaps (Fenner, 1980) as well as burial depth (Benech-Arnold et al., 1988).

Caryopsis covering structures had an important role in *D. sanguinalis* seed dormancy (Fig. 3). Within the caryopsis covering structures, the lemma seems to be the most important structure imposing dormancy (Fig. 4), in agreement with Gallart et al. (2008). The lack of differences in germination percentage of naked caryopses alone and naked caryopsis with the extract of the covering structures suggest no presence of soluble inhibitors in the covering structures. These results contradicts those presented by Gianfagna and Pridham (1951), who found that seed extracts inhibited *D. sanguinalis* seeds germination. These

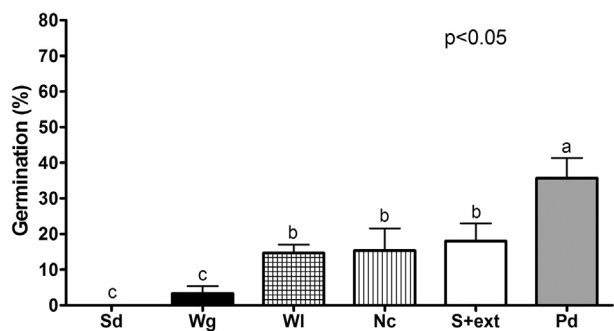


Fig. 4. Germination percentage of *D. sanguinalis* whole seeds (Sd), caryopses without the glumes (Wg), caryopses without the lemma and the glumes (WI), naked caryopses (Nc), seeds imbibed with extract from caryopses covering structures (S + ext) and seeds punctured (Pd) at 20/30 °C (8/16 h) with light (8 h of darkness and 16 h of light). Values are the means and vertical bars are binomial confidence intervals. Columns with the same letters are not significantly different according to DGC's test ($p < 0.05$).

Table 3

Generalised linear models test summary of germination percentage of large crabgrass seeds from Exp. 5 at different H_2O_2 concentrations ($[H_2O_2]$) and imbibition time (IT).

Factor or interaction	Degrees of freedom	Deviance	P
$[H_2O_2]$	4	886.13	<0.0001
Imbibition time (IT)	2	53.45	<0.0001
$[H_2O_2] \times IT$	8	64.52	<0.0001

differences between results could be related to differences in the solutions used in each treatment (extracts of the covering structures in our work and whole seeds in cited work).

Moreover, results obtained in Exp. 5, in which imbibition of seeds on 2.6 M of H_2O_2 increased the germination percentage (Fig. 5), also support the presence of inhibitors in the covering structures of *D. sanguinalis*. According to Ogawa and Iwabuchi (2001) the positive effect of H_2O_2 on seed germination is associated with oxidation of germination inhibitors (e.g. phenolic compounds) present in the caryopses covering structures which reduces the oxygen diffusion towards embryo. The germination percentage observed in punctured seeds (which probably increases oxygen diffusion towards embryo) similar to that of seeds without lemma and naked seeds, supports this latter assumption. Results suggest that a similar mechanism as that observed for barley (Lenoir et al., 1986) and oats (Corbineau et al., 1986) could be partly responsible for dormancy in *D. sanguinalis*, but further experimentation will be necessary to test this hypothesis.

In summary, results indicate that i) the extended seedling emergence pattern of *D. sanguinalis* along the season could be due to the influence of wide thermal range of temperatures (5–20 °C) and temperature fluctuations (15/25 °C and 20/30 °C) that enable seed dormancy release and germination, ii) the environment enriched with far-red light would reduce the germination of seeds with low dormancy level and iii) the seed covering structures, mainly the lemma, and inhibitors may have an important role on seed dormancy imposition in this species.

In terms of implications for management, the presence of stubble in no-till systems reduces the amplitude of fluctuating temperatures on soil surface compared with bare soil (Faccini and Vitta, 2007). Furthermore, a crop canopy not only reduces fluctuating temperatures, but also the R:FR ratio (Batlla et al., 2000; Huarte and Benech-Arnold, 2003; Norsworthy, 2004), which could reduce seedling emergence in the field (Batlla and Benech-Arnold, 2014). Based on responses of *D. sanguinalis* seeds to fluctuating temperatures and light observed in the present work, stubble and crop canopy can be managed in order to reduce seedling emergence in the field. For example, the adoption of no-tillage system with stubble on the surface would reduce the soil fluctuating temperatures and delay *D. sanguinalis* seedling emergence

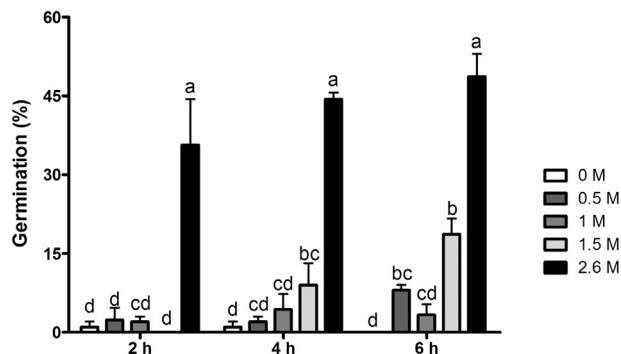


Fig. 5. *D. sanguinalis* seeds germination percentage at 20/30 °C (8/16 h) with light (8 h of darkness and 16 h of light) imbibed with H_2O_2 concentrations of 0, 0.5, 1, 1.5, and 2.6 M; for 2, 4 and 6 h imbibition time. Values are the means and vertical bars are binomial confidence intervals. Columns with the same letters are not significantly different according to DGC's test ($p < 0.05$).

as reported by Norsworthy and Oliveira (2007) for *Xanthium strumarium*. Using spring cover crops, early sowing, increasing crop density (Huarte and Benech-Arnold, 2003) and reducing inter-row distance (Norsworthy, 2004) of summer crops are management decisions that would reduce fluctuating temperatures and R:FR ratio on the seed micro-environment (Huarte and Benech-Arnold, 2003; Batlla and Benech-Arnold, 2014). These decisions could in turn reduce and delay *D. sanguinalis* emergence in the field.

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