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The Wounding Response of Dormant Bamyardgrass (*Echinochloa crus-galli*) Seeds[1]

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Abstract. Induction of germination in dormant barnyardgrass seeds by wounding was investigated. Previous research indicated that a volatile compound was emitted during imbibition of wounded caryopses. When wounded caryopses were submerged in agar, total germination and speed of germination were stimulated, and the stimulation was dependent upon the concentration of agar. A twofold increase in germination occurred in 1% agar versus water, and a fivefold increase with caryopses placed in 5% agar. When wounded caryopses were imbibed, there was a fourfold increase in respiration over that of intact caryopses after 1 h. This increased rate of respiration of wounded caryopses continued for 7 h, while there was a gradual increase in respiration of intact caryopses. Carbon dioxide induced intact dormant caryopses to germinate but was not effective in stimulating germination of the seed (spikelet). High levels of abscisic acid found in the hulls of dormant seeds may have prevented the action of carbon dioxide. These results suggest that increased respiration resulting from wounding provides elevated levels of carbon dioxide in the microenvironment of the seed, thus stimulating germination. Removal of the hulls is necessary for germination even in high levels of carbon dioxide. Nomenclature: Barnyardgrass, *Echinochloa crus-galli* (L.) Beauv. #[3] ECHCG.

Additional index words. Carbon dioxide, germination, abscisic acid, respiration, ECHCG.

INTRODUCTION

Dormant barnyardgrass seeds (spikelets) can be induced to germinate by puncturing through the caryopsis coat into the scutellum (13). Surgical cuts, including removal of a portion of the endosperm, stimulated germination of barnyardgrass caryopses; cuts made closer to the embryo were more effective (14). Similar results have been obtained with other weed species, including wild oats (*Avena fatua* L. # AVEFA) (3). These results (13, 14) and those of others (3, 9) suggested that the limiting factor for germination was water uptake by the embryo which was altered by puncturing the seed (caryopsis) coat. However, we determined that this factor was not a barrier such as an impermeable caryopses coat. We proposed that the stimulatory effect of cutting the caryopses of bamyardgrass was a physiological response to wounding and possibly involved a volatile compound (14).

During the course of our investigations with barnyardgrass seeds, we were searching for a germination medium to provide anaerobic conditions and at the same time be able to limit the available water for germination. We found that high concentrations (3 to 5%) of agar in water was the desired medium. However, when wounded barnyardgrass caryopses were submerged in the agar, germination was increased (12).

The objective of these experiments was to determine the effect(s) of agar on germination and to further elucidate the mechanism of the wounding response in bamyardgrass seeds.

MATERIALS AND METHODS

Bamyardgrass seeds used in this research were obtained from a single mother plant as described previously (13, 14). Unless specified, all experiments were conducted using dormant dehulled seeds (caryopses).

Germination in agar. Bacto-agar[4] was prepared in water at concentrations of 1 to 5%, heated in a water bath, poured into 100- by 15-mm plastic petri dishes, and allowed to cool. Bamyardgrass caryopses that had been wounded by cutting away the apex portion of the endosperm with a razor blade at half the distance to the scutellum (1/2 cut), or by cutting adjacent to the scutelhun parallel to the embryo (cut scutellum) (14), were immersed in the cooled agar. The plates with lids were maintained in a germinator at alternating day/night temperatures of 30/20 C, 86% RH, with 8-h photoperiods (18.8 $\mu E\ m^{-2}\ s^{-1}$). Germination was scored each day at the same hour for 7 d. Each treatment contained 25 caryopses and was replicated four times. The germination index (GI)[5] was calculated according to the method of Timson (16), whereby germination percentage was summed (Σ) daily. A higher index value indicate8 greater speed of germination.

Respiration. Respiration was determined using a differential respirometer. Twenty-five intact or 25 wounded (cut scutellum) ciuyopses were placed in 15-ml respirometer flasks with 1 ml of water as the incubation medium. Six flasks, three with intact caryopses and three with wounded caryopses contained 0.2 ml of a 10% solution of KOH in the center well. To facilitate CO_2 absorption, a fluted piece Of filter paper was immersed into the KOH well. Six additional flasks were established in like manner but without KOH. Two flasks

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[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

[4]Difco Laboratories, Detroit, ML Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of tbe product by the U.S. Dep. Agric. and does not imply its approval to the exclusion of other products of vendors that may also be Suitable.

[5]Abbreviations: GI, germination index; ABA, abscisic acid; HPLC, high-performance liquid chromatography.

containing water and with KOH in wells served as blanks. The water bath was maintained at 28 C, and the flasks were allowed to equilibrate for 0.5 h before the first manometer reading. Manometers were read at 0.5-h intervals for 7 h (the first visible indication of germination occurs at 8 h after the beginning of imbibition), and respiration quantified as µl O_2 h^{-1} (25 caryopses)$^{-1}$.

Germination in gaseous atmosphere. Barnyardgrass germinates well under both anaerobic and aerobic atmospheres (6). To have constant atmospheres, these studies were performed with the caryopses under anaerobic conditions. Twenty-five dormant caryopses were placed in 40-ml gas collection vials with 2 ml distilled water. The vials were sealed with serum caps and flushed with N_2 (99.9% purity) for 5 min via a 23-gauge needle; a second needle served as the exit port. Different percentages of CO_2 were obtained by first removing the required amount of gas from the vial with a needle and syringe and then replacing with the same amount of CO_2. For 100% CO_2, the flasks were flushed for 5 min as previously described. Flasks containing the caryopses were incubated in a germinator as described above. Getmination was scored each day at the same hour for 14 d. Emergence of the radicle was the criterion for germination. Each treatment was replicated three times.

Abscisic acid analysis. Abscisic acid (ABA)5 was extracted and analyzed qualitatively by high-performance liquid chromatography (HPLC)[5] as described by Thompson et al. (15). ABA was quantified using a Phytodetek™-ABA enzyme immunoassay[6]. All extraction and assay procedures were performed in a Venetian blind-darkened laboratory to prevent isomerization of the ABA.

Germination with ABA and CO_2. Twenty-five dormant caryopses were placed in 40-ml glass collection vials with 2 ml ABA solution at concentrations of 0, 1, 10, or 100 µM, sealed, and flushed with N_2(–) or CO_2(+). After 48 h, vials containing CO_2 were flushed with N_2. All treatments were replicated three times.

All experiments were repeated at least once, the results combined, and the data analyzed by the Student's t-test or analysis of variance.

RESULTS AND DISCUSSION

Germination in agar. As the concentration of agar was increased, germination (total % and speed) of the wounded dormant caryopses immersed in agar increased (Figures la, lb). Intact caryopses placed in the higher concentrations of agar also germinated (data not shown). Germination experiments with polysaccharides, calcium ions, gibberellic acid (all components of agar), altered pH, or surfactants did not stimulate germination of the dormant caryopses (data not shown). Previous research, including effects of air space volume on germination, suggested that a volatile compound other than ethylene may be involved in the wounding response (14). Because of the physical nature of hydrated agar, we concluded that perhaps a greater quantity of an uncharacterized gaseous compound was being retained in the microenvironment of the caryopsis as the concentration, and thus the cross-linking, of hydrated agar increased.

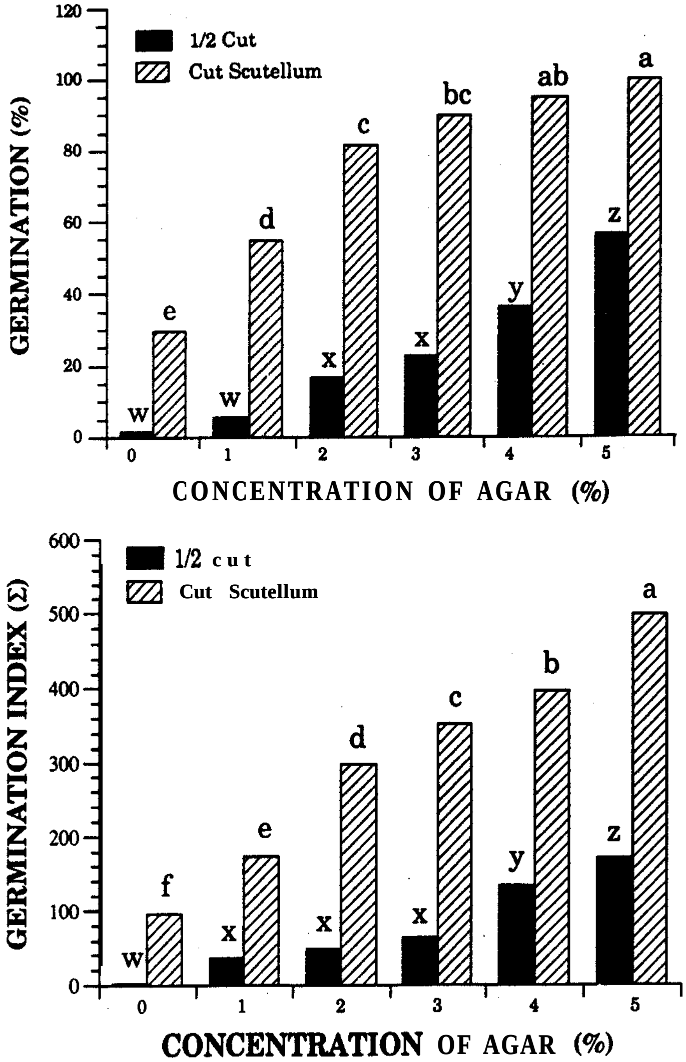
Respiration. Response of plant tissue to wounding is production of ethylene in some and an increase in respiration by most (1, 5, 17). These responses were at first considered unlikely in this case because wounding of the dry caryopsis (< 12% moisture) followed by freezing at -20 C, further drying at 50 C, incubating in a moist 4 C atmosphere for extended periods (6 wk), or other preimbibition manipulations, failed to alter the stimulatory effects of wounding (data not shown). We reasoned that if a volatile compound was emitted, it should dissipate to a level of little consequence by the time of imbibition. It has been reported (5) that the healing processes in wounded plant tissues require the energy produce4.l by increased respiration. since dry seeds respire only at very low levels, we considered that the wounding response occurred only after imbibition of water.

Within the first hour of imbibition, there was a burst of respiration, determined by O_2 uptake, that was 4-fold greater in the wounded bamyardgrass caryopses than in the intact caryopses (Figure 2). Rate of respiration of the wounded caryopses remained higher throughout the imbibition phases, although the intact caryopses showed a steady increase in respiration rate (Figure 2). When the caryopses remained in the respirometer for a total of 24 h: there was an increase in the rate of respiration of the wounded catyopses beginning at 8 h in the vials that did not contain KOH in the center well; respiration of intact caryopses also increased in vials that did not contain KOH; all wounded caryopses in vials without KOH were germinated at 24 h, and the intact caryopses under the same conditions were beginning to germinate; and intact or wounded caryopses in vials with KOH did not germinate (data not shown). The increase in respiration at 8 h would be expected since this has been observed with other seeds during radicle emergence (7, 8). The significant observation was that germination occurred only in the vials without KOH that accumulated respiratory CO_2.

Germination in gaseous atmosphere. Carbon dioxide has been shown to stimulate the germination of a number of seeds (2, 4), and its level in soil is important in the germination of many weed seeds (2). Intact dormant barnyardgrass caryopses were induced to germinate in CO_2 atmospheres (Figure 3). The germination delay observed at higher concentrations may be due to inhibition of radicle growth and not the germination process which we define as occurring prior to radicle protrusion through the seed (in these experiments, the caryopsis) coat (8). We found that the greater germination occurred in vitro if CO_2 was only present for 48 h after the beginning of imbibition (Figure 4).

It appeared that CO, was involved in the wounding response and that it was necessary for breaking dormancy in this species. However, when CO_2 was applied to intact dormant spikelets, no germination occurred even at optimum CO, levels and conditions (100% CO_2 for 48 h) that were required for the germination of caryopses. Dormant spikelets

[6]Idetek, Inc., 1057 Sneath Lane, San Bruno, CA 94066.



1. Germination (a) and **germination index (b) of wounded barnyardgrass caryopses submersed in differing concentrations of agar. Bars for type of wounding having the same letters are no significantly different at P = 0.05 by nonoverlapping confidence limits according to the Student's t-test.**

can be induced to germinate by puncturing through the hulls and caryopsis coat (13), and we conclude from the results reported here that respiratory and applied CO_2 also breaks dormancy and induces germination.

Abscisic acid. In order to determine why CO_2 failed to induce germination in intact spikelets, ABA levels in **glumes, palea,** and lemma, and caryopses of dormant and afterripened spikelets were determined. All of the tissues contained ABA but the greater concentration was found in hulls of dormant spikelets. Extractable **ABA** averaged 0.49 picomoles g^{-1} from the hulls **(glumes, palea,** and lemma) of dormant spikelets and 0.003 picomoles g^{-1} from the hulls of after-ripened spikelets. These data suggest that ABA is also involved in the dormancy of this species and probably inhibits germination until it is lost through afterripening (leaching under field

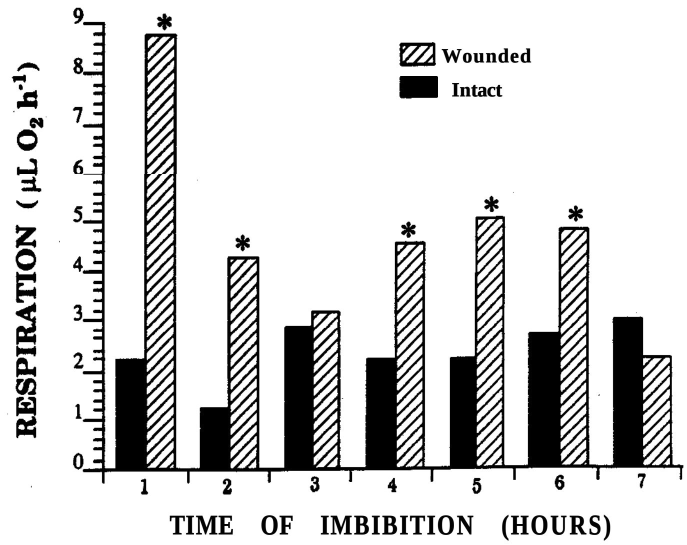


Figure 2. **Respiration of intact and wounded barnyardgrass caryopses during 7 h of imbibition. Bars with a "*" indicate the value for the wounded caryopses are significantly greater than the intact caryopses for the time indicated. Significance determined by ANOVA at P = 0.05.**

conditions) or **removal** of the hulls. When dormant **barnyard**-grass **caryopses were** imbibed with **solutions** of ABA in CO_2 or N_2 atmospheres, CO_2 did not **directly** overcome ABA inhibition of **germination** (Table 1). However, ABA lost its ability to inhibit germination at exogenous concentrations below 10 **μM.**

Germination [defined as the physical and biochemical events which result **in** the final thrust of the radicle through the **testa (8)]** in dormant barnyardgrass seeds can be overcome by puncturing (wounding). Wounding results in greater respiration during imbibition, and the CO_2 produced acts in some way to increase water uptake necessary for radicle elongation **(3, 9).** ABA, which may block the osmotic

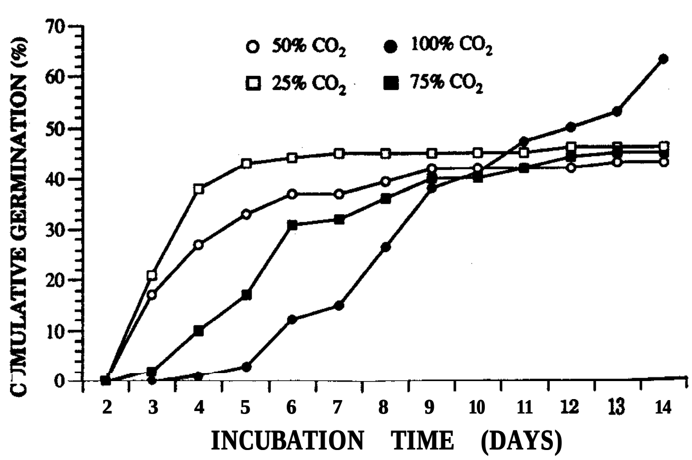


Figure 3. **Germination of barnyardgrass caryopses under 25, SO, 75, or 100% carbon dioxide for 14 d.**

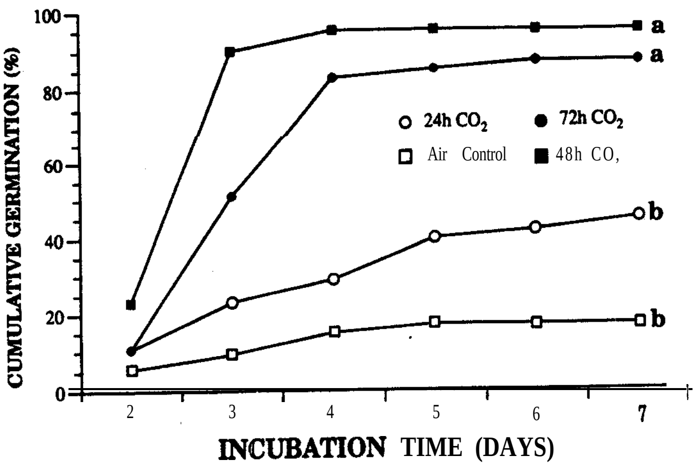


Figure 4. Germination of barnyardgrass caryopscs imbibed for 0, 24, 48, or 72 h under 100% carbon dioxide. Values at day 7 with the same letter are not significantly different at P = 0.05 by nonoverlapping confidence limits according to the Student's t-test.

Table 1. Germination of barnyardgrass caryopses as influenced by abscisic acid and carbon dioxide[a].

ABA	CO_2[b]	Germination 2 day	4 day	7 day
µM		%		
0		0.0 c	6.7 c	10.7 bc
0	+	0.0 c	70.7 b	84.0 a
1	-	4.0 c	10.0 c	15.4 b
1	+	16.6 b	85.3 b	90.6 a
10		1.3 c	13.3 c	13.3 bc
10	+	0.0 c	12.0 c	18.7 b
100		0.0 c	0.0 c	1.3 bc
100	+	0.0 c	0.0 c	0.0 c

[a]Values for each day followed by the same letter are not significantly different at P = .05, as determined by nonoverlapping confidence limits according to the Student's t-test.

[b]Carbon dioxide (100%) was present during the first 48 h of imbibition in treatments indicated with "+".

mobilization of water (10) or the reorientation of the cell wall components of the radicle (11), does not allow germination of barnyardgrass seeds to proceed even with high levels of CO_2. Although we have no direct evidence; we suggest that puncturing the spikelet of bamyardgrass allows free flow of water into the embryo, and that respiratory CO_2 enhances water uptake in the radicle cells through some biochemical pathway that leads to a decrease of water potential in these cells (7, 8). Experiments designed to test this hypothesis are in progress.

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