

# Salicylhydroxamic Acid Potentiates Germination of 'Waldmann's Green' Lettuce Seed<sup>1</sup>

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## ABSTRACT

A combination of salicylhydroxamic acid (SHAM) + cyanide (CN) is known to stimulate dark germination of *Lactuca sativa* L. seeds. Further studies were done to characterize SHAM and CN action in stimulating dark germination of lettuce seed. Germination was stimulated slightly by either SHAM or CN, whereas when SHAM and CN were combined germination was greatly enhanced. Treatment of seeds with SHAM + CN only during the first 8 hours of hydration stimulated germination as much as did treatment for 72 hours. During the first 8 hours of incubation in SHAM + CN, potentiation (*i.e.* dormancy-breaking) of germination occurs. SHAM alone stimulated potentiation nearly to the level of SHAM + CN but inhibited subsequent radicle elongation, thereby decreasing germination when present for 72 hours. Oxygen must be present for SHAM or SHAM + CN to potentiate dark germination. The ability of SHAM and SHAM + CN to potentiate germination is influenced by O<sub>2</sub> concentration and the timing of chemical treatment.

Cyanide has been reported to mildly stimulate germination of lettuce seeds (3, 8, 12). The mechanism is unknown but researchers have hypothesized that CN<sup>3</sup> may cause a shift in intermediary metabolism from glycolysis to the pentose phosphate pathway (5). We previously reported that SHAM + CN stimulates lettuce seed germination (1, 10, 11). Since SHAM is an inhibitor of the CN-insensitive respiratory path and CN is an inhibitor of the CN-sensitive respiratory path, the focus of attention has been on effects of SHAM and CN on oxidative metabolism in breaking dormancy. As a first step in determining if SHAM + CN break dormancy by means of effects on oxidative metabolism, we have addressed the following questions in this investigation: What are the effects of SHAM and CN during the potentiation *versus* the germination phase? Potentiation is the phase during which dormancy is broken, thereby altering the physiological state of seeds to that which allows germination to occur. Is O<sub>2</sub> necessary for SHAM and CN to potentiate germination? What is the effect of SHAM and CN on O<sub>2</sub> consumption? Can a respiratory inhibitor with a different site of action also stimulate germination in combination with SHAM and/or CN?

## MATERIALS AND METHODS

To study the effect of respiratory inhibitors on germination of light-sensitive *Lactuca sativa* L., seeds from two similar lots of

'Waldmann's Green' were used. Batches of 50 seeds were placed in 6-cm-diameter plastic disposable Petri dishes containing two sheets of Whatman No. 1 filter paper moistened with 1.8 ml of either deionized-distilled H<sub>2</sub>O, 4 mM SHAM (Sigma Chemical Co.), or 10 mM malonate (Aldrich Chemical Co.). For CN treatments, we pipetted 0.1 ml of 1 mM KCN into a centerwell in each Petri dish and then added 0.1 ml of 1 N H<sub>2</sub>SO<sub>4</sub> to the KCN to generate HCN. Details of this method for generating CN have been described previously (9). All Petri dishes were sealed with thermoplastic film, which had no effect on germination under the conditions of the experiment.

Incubation conditions were as follows: Seeds were imbibed in darkness for 1 h, and then, to ensure dormancy, were given FR for either 4 or 10 min depending on seed lot. To ascertain seed viability, we gave 2 min of R to three dishes in each experiment. The R and FR sources have been described previously (10). Seeds were then incubated in darkness at 22°C for a total of 72 h unless specified otherwise. All operations for the experiments described were conducted in a darkroom illuminated with green safe lights. Germination was scored as any visible protrusion of the radicle from the seed coat and data were expressed as per cent germination.

Since, in the experimental procedure described above, SHAM and CN were present for the entire 72-h incubation period, it was not clear whether these chemicals need be present both during and after potentiation to stimulate germination. To determine if SHAM and CN, alone or in combination, could potentiate germination, treated seeds were rinsed at 4, 6, 8, or 10 h after the start of imbibition. To remove SHAM and CN, seeds were washed out of the Petri dish with deionized-distilled H<sub>2</sub>O onto a disc of filter paper within a Büchner funnel. The seeds were rinsed 3 times and then gently transferred to fresh filter paper and rinsed 3 more times. The seeds were then transferred to Petri dishes containing filter paper moistened with water. The dishes were sealed with thermoplastic film for the remainder of the 72-h incubation period.

To determine the effect of SHAM, CN, or SHAM + CN on potentiation at low O<sub>2</sub> tension, seeds were imbibed in test solutions for 8 h in a glove bag which was continuously purged with flowing N<sub>2</sub>. Before imbibition, the glove bag was flushed several times with humidified N<sub>2</sub> until the O<sub>2</sub> concentration was <0.5% (v/v) (Carle Respiratory Gas Chromatograph GC 8700). Dry seeds were then sprinkled onto premoistened filter paper in Petri dishes in the glove bag, the dishes sealed with thermoplastic film, and CN treatment initiated in three dishes. After 1 h, the sealed dishes were removed from the glove bag for brief FR or R treatments. The dishes were then returned to the glove bag and the bag again flushed with N<sub>2</sub>. After 7 h of additional incubation, the dishes were removed from the glove bag, the seeds were rinsed, and transferred to fresh Petri dishes as described above.

For O<sub>2</sub> consumption studies, six sheets of 4-cm-diameter Whatman No. 1 filter paper were fitted in the bottom of each

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<sup>3</sup> Abbreviations: CN, cyanide; SHAM, salicylhydroxamic acid; R, red light; FR, far-red light.

respirometer flask and moistened with 2.0 ml of test solution. SHAM concentration was 4 mM, and HCN was generated according to the method of Robbie (4), which maintains an equilibrium concentration of HCN in the headspace of flasks during measurement of O<sub>2</sub> consumption. Seeds imbibed for 1 h before receiving FR and were incubated in darkness for another 7 h prior to O<sub>2</sub> consumption measurement. O<sub>2</sub> consumption was measured for 3 h at 22°C with a 12-port respirometer (Gilson Medical Electronics, Middleton, WI). To express O<sub>2</sub> consumption on a fresh weight basis, seeds were weighed immediately after completion of O<sub>2</sub> consumption measurements. Seeds were washed from the respirometer flasks onto a disc of filter paper within a Büchner funnel. The filter disc was placed on paper toweling to blot excess moisture and the seeds were weighed with an analytical balance.

## RESULTS

Germination was stimulated slightly by either SHAM alone or CN alone (Table I). Malonate by itself, however, did not stimulate germination. A 2 × 2 factorial analysis of variance revealed significant interactions between SHAM and CN, SHAM and malonate, and CN and malonate. In all cases examined, combination of any one respiratory inhibitor with a second synergistically stimulated germination. Combination of malonate with either SHAM or CN stimulated germination to the same extent, whereas the combination of SHAM with CN stimulated germination even more. A 2<sup>3</sup> factorial analysis of variance shows a significant three-way interaction between SHAM, CN, and malonate. SHAM + CN + malonate stimulated germination more than any two-inhibitor combination.

Stimulatory effects of SHAM + CN were already evident after 4-h incubation (Fig. 1). As the incubation period in SHAM + CN was lengthened from 4 to 10 h, per cent germination increased, peaking at 8-h incubation. Germination of seeds incubated in SHAM alone also increased with increasing incubation time, although germination was less than that of seeds incubated in SHAM + CN. Cyanide did not stimulate germination until seeds had been incubated for at least 8 h, and then germination was stimulated only slightly. Incubation for 10 h in SHAM, CN, or SHAM + CN did not enhance germination more than did incubation for 8 h.

Incubation of seeds in SHAM + CN for either 8 or 72 h resulted in 50% germination, whereas incubation in SHAM alone for either 8 or 72 h resulted in 33 and 9% germination, respec-

Table I. Effect of Respiratory Inhibitors on Dark Germination of 'Waldmann's Green' Lettuce Seeds

Seeds were incubated in test solutions for 72 h in darkness at 22°C. Means represent data from four separate experiments with three replicates per treatment per experiment. An arcsin transformation of percentage data was conducted prior to a 2<sup>3</sup> factorial analysis of variance (6). Data were reconverted to per cent germination for presentation.

Treatment	Germination
	%
H <sub>2</sub> O	4 <sup>a</sup>
Malonate <sup>b</sup>	1
SHAM <sup>c</sup>	14
CN <sup>d</sup>	15
SHAM + malonate	44
CN + malonate	45
SHAM + CN	64
SHAM + CN + malonate	85

<sup>a</sup> LSD at the 5% level = 2%. <sup>b</sup> 10 mM malonate. <sup>c</sup> 4 mM SHAM. <sup>d</sup> CN provided by placing 0.1 ml of 1 mM KCN + 0.1 ml of 1 N H<sub>2</sub>SO<sub>4</sub> in the center well of a Petri dish.

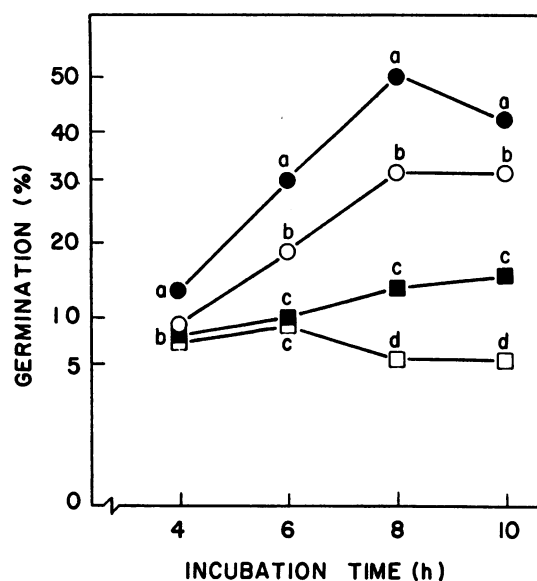


FIG. 1. Effect of incubation time in test solutions of SHAM and CN on dark germination of 'Waldmann's Green' lettuce seeds. Seeds were incubated in test solutions for either 4, 6, 8, or 10 h, and germination was scored after 72-h total incubation. Means represent data from three separate experiments with three replicates per treatment per experiment. An arcsin transformation of percentage data was conducted prior to analysis of variance. Data were converted to percentage germination for presentation. Different letters within incubation times indicate significant differences at the 5% level as separated by protected LSD. (□), H<sub>2</sub>O; (■), CN; (○), SHAM; (●), SHAM + CN.

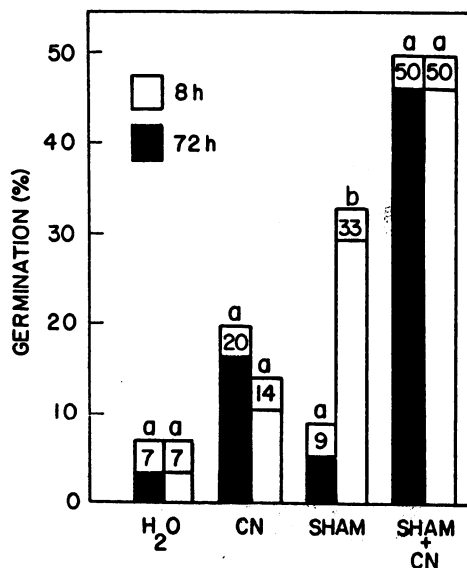


FIG. 2. Effect of SHAM and CN on potentiation and germination of 'Waldmann's Green' lettuce seeds. Seeds were imbibed in test solutions for either the first 8 h or the entire 72 h of incubation. Germination was scored 72 h after the start of imbibition. Means represent data from three separate experiments with three replicates per treatment per experiment. Means within bar pairs having different letters are significantly different at the 5% level according to *F* test.

tively (Fig. 2). There was no significant difference in germination between seeds incubated in CN for 8 or 72 h. The rinsing procedure had no effect on germination of water controls. Controls germinated 7% regardless of whether the seeds were rinsed after the first 8-h imbibition or remained undisturbed for the entire 72-h incubation period.

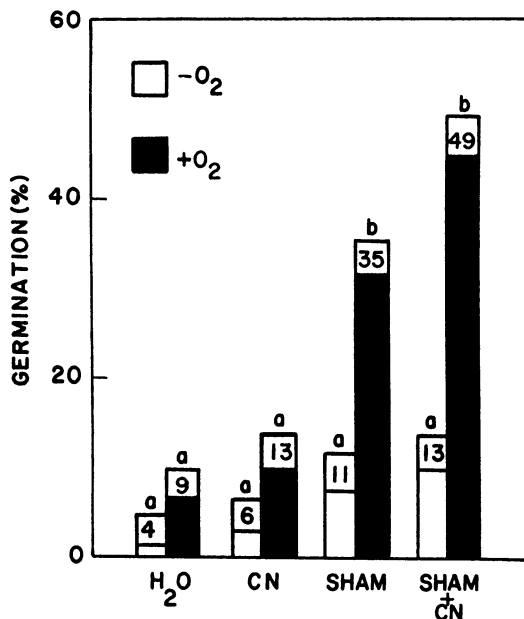


FIG. 3. Effect of O<sub>2</sub> with SHAM and CN on germination of 'Waldmann's Green' lettuce seeds. Seeds were imbibed in test solutions for 8 h while under either ambient or hypoxic O<sub>2</sub> levels (<0.5% O<sub>2</sub>). After 8 h, seeds were rinsed with water and incubated under ambient O<sub>2</sub> for the remainder of the 72-h period. Means represent data from three separate experiments with three replicates per treatment per experiment. Different letters within bar pairs represent significant differences at the 5% level according to *F* test.

Table II. Effect of SHAM and CN on O<sub>2</sub> Consumption by 'Waldmann's Green' Lettuce Seeds

O<sub>2</sub> consumption was measured after 8-h imbibition in test solutions. Each treatment was replicated 3 times. Data represent means pooled from seven separate experiments. Means followed by different letters are significantly different at the 5% level as separated by protected LSD.

Treatment	O <sub>2</sub> Consumption Rate $\mu\text{mol g}^{-1} \text{h}^{-1}$
H <sub>2</sub> O	7.8 b
SHAM <sup>a</sup>	8.8 a
CN <sup>b</sup>	7.7 b
SHAM + CN	6.0 c

<sup>a</sup> 4 mM SHAM. <sup>b</sup> CN provided by placing 0.4 ml of 0.715 M KCN made up in 0.5 M KOH in the center well of a respirometer flask.

Germination of seeds exposed to hypoxic conditions during 8 h of incubation in SHAM or SHAM + CN was greatly decreased compared to that of seeds under ambient O<sub>2</sub> level (Fig. 3). The presence or absence of O<sub>2</sub> during this period had no significant effect on germination of seeds treated with CN or H<sub>2</sub>O. Water controls which received R were not affected by hypoxia during the 8-h potentiation period when germination was scored at 72 h (data not shown).

Treatment of lettuce seeds with SHAM significantly increased their O<sub>2</sub> consumption rate compared to that of H<sub>2</sub>O controls, but treatment with SHAM + CN consistently reduced O<sub>2</sub> consumption in each of seven experiments conducted (Table II).

## DISCUSSION

We previously reported that SHAM + CN stimulates lettuce seed germination (1, 10, 11), and this finding has been confirmed

by Tanno (7). However, the difference in germination response of seeds imbibed in SHAM alone for 8 or 72 h suggests that SHAM without CN potentiates germination during the first 8 h, but retards subsequent radicle emergence (Fig. 3). These results might be explained by the observation that SHAM inhibits radicle elongation. SHAM may potentiate germination during the first 8 h, but with continued exposure to SHAM subsequent radicle elongation is inhibited and the radicle does not penetrate the seed coat. However, the presence of CN with SHAM during the 72-h incubation period negates the otherwise inhibitory effect of SHAM alone.

The action of SHAM or SHAM + CN in breaking lettuce seed dormancy requires O<sub>2</sub> (Fig. 3). One interpretation is that SHAM or SHAM + CN inhibits an O<sub>2</sub>-consuming process which competes for O<sub>2</sub> with other processes that are not inhibited by SHAM or CN but are necessary to break dormancy. When SHAM or SHAM + CN are present, thereby diminishing competition for O<sub>2</sub>, germination is permitted. However, when SHAM or SHAM + CN are present in an hypoxic environment, germination does not occur.

Esashi (2) proposed that the coat-imposed dormancy of *Xanthium* seed is broken by a certain ratio of respiration occurring through the CN-insensitive and CN-sensitive electron transport paths, with a high flux of electrons through the alternative path favoring germination. Our finding that SHAM alone can potentiate germination suggests that increased flow of electrons through the alternative path is not necessary to break dormancy of lettuce seed. In fact, SHAM stimulated O<sub>2</sub> consumption rate of seeds compared to that of H<sub>2</sub>O controls. In addition, the observation that malonate, an inhibitor of succinate dehydrogenase, stimulated germination equally whether combined with SHAM or CN indicates that a certain ratio of electron flux through the CN-sensitive and CN-insensitive paths is not necessary to break dormancy in lettuce. Whether SHAM and CN act via electron transport to break dormancy is further clouded by the observation that seeds treated with SHAM + CN have the least O<sub>2</sub> consumption but germinate the most. Since our experiments, as well as those of Esashi, have been conducted with whole seeds, results are difficult to interpret at a biochemical level. Future studies need to determine the site of action of SHAM and SHAM + CN to accurately determine how these agents break dormancy.

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