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Respiration in dormant and non-dormant bitterbrush seeds

D. T. Booth* & S. Sowa†‡

**United States Department of Agriculture, Agriculture Research Service, High Plains Grasslands Research Station, 8408 Hildreth Road, Cheyenne, WY, 82009, U.S.A.*
†National Seed Storage Laboratory, Fort Collins, CO, U.S.A.

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Bitterbrush (*Purshia tridentata* dc.) seed dormancy is not understood but may result from a metabolic block by a chemical inhibitor. To determine whether dormancy affects seed respiration, we compared CO_2 evolution from individual imbibed dormant and non-dormant seeds and from germinating seeds, using Fourier transform infrared photoacoustic spectroscopy. We found CO_2 evolution did not differ between dormant and non-dormant seeds, and that it accelerated with germination and growth. Our results are in agreement with other studies indicating that dormant seeds respire. We conclude that high respiration rates in dormant bitterbrush seeds can decrease seedling vigor, and we recommend bitterbrush be sown when soil temperatures are cool enough to prevent significant losses of respiratory substrate from imbibed-dormant seeds.

Keywords: dormancy; Fourier Transform Infrared Spectroscopy; photoacoustic spectroscopy

Introduction

Bitterbrush (*Purshia tridentata* dc.) is a deciduous or evergreen North American member of the rose family. It has intricately branched stems and leaves that are alternate, simple, apically three-toothed, and glandular. Bitterbrush usually exists on well-drained soils in areas receiving 30–64 cm annual precipitation from California eastward through the Rocky Mountains, and from British Columbia south to Baja California. At lower elevations it is found with sagebrush (*Artemisia*) and pinyon-juniper (*Pinus cembroides* Zucc., *P. edulis* Engelm., or *P. monophylla* T. & F., with *Juniper* sp. L.) and is particularly valued on big-game winter ranges as cover and high-protein browse.

Seed dormancy is influenced by the seed environment and by select chemical treatments (Pearson, 1957; Young & Evans, 1976; Meyer, 1989). The mechanism(s) for dormancy are not fully known (Booth, 1992). Nord (1965) postulated a water-soluble

‡Present address: Indiana University of Pennsylvania, Indiana, PA, 15705-1076, U.S.A.

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inhibitor in the seed coat and Dreyer and Trousdale (1978) identified the triterpenes, cucurbitacins D(1) and I(2), as water-soluble seed-coat constituents. They were unable to show that either cucurbitacin inhibited germination; however, the aqueous extract containing the two cucurbitacins inhibited lettuce germination. Booth (1999) reported imbibition temperature affected post-imbibition seed weight of dormant and non-dormant seeds. He suggested the effect was the result of seed respiration.

Among the tools available for studying seed respiration are instruments using infrared spectroscopy, e.g. infrared gas analyser (IRGA) technology that is used extensively to measure CO_2 concentrations. Infrared spectroscopy measures the vibrational transitions of molecular dipoles; the energy of the transitions is determined by the strength of the bond holding the atoms together and their reduced mass (Griffiths & de Haseth, 1986). In other words, molecules gain energy from infrared light at a discrete frequency unique to the absorbing substance. Thus, when CO_2 absorbs infrared energy it produces a signature that allows quantification of the amount of CO_2 present. Fourier transform infrared (FTIR) instrumentation uses this principle, but expands the technology to allow simultaneous collection of spectral information from all frequencies so that many accurate scans can be averaged in a short time (Griffiths & de Haseth, 1986). Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS) is designed specifically for solid samples. The sample is irradiated by a modulated infrared beam. Absorbed energy is transferred to the atmosphere above the solid sample as heat, causing pressure changes that are detected by a sensitive microphone. The resulting acoustical signal is converted into a spectrum.

Sowa & Roos (1991, 1992) concluded that FTIR-PAS accurately and non-invasively measured the onset of CO_2 evolution within 30 min of imbibition in viable, intact seeds of *Phaseolus* and found the signal increased significantly with root emergence. Griffiths & de Haseth (1986. 314–315) address the utility of FTIR-PAS for monitoring small amounts of gas, especially CO_2. Since FTIR-PAS measures CO_2 evolution from single seeds as it occurs, it can be used as a direct measure of seed metabolism. We used FTIR-PAS to measure CO_2 evolution from imbibed bitterbrush seeds which were dormant, non-dormant, and germinated, to test the null hypothesis of no differences in CO_2 evolution among those seed conditions.

Materials and methods

Plant material

We used bitterbrush seeds collected from Utah and Oregon (Booth, 1999). Twenty un-dried seeds from the Utah collection had a weight of 436 $\pm$ 22 mg (mean $\pm$ S.D.) and a tetrazolium chloride test (TZ) of 98%. Twenty un-dried seeds from the Oregon collection weighed 492 $\pm$ 44 mg, and a TZ = 93%. Non-dormant, germinating seeds were produced by holding the seeds at 2°C under cool moist conditions for 28 days (Young & Evans, 1976). This treatment resulted in 82 $\pm$ 8 and 73 $\pm$ 10% (mean $\pm$ S.D.) germination for the Utah and Oregon collections in a related study (Booth, 1999). Imbibed-dormant seeds were obtained by imbibing seeds for 48 h at 15°C. Ten dormant and 10 non-dormant seeds of each accession were tested and compared with germinated seeds (germinants) having root lengths between 1 and 26 mm.

Instrumentation and procedure

We used a Nicolet 740 FTIR with a MTEC 200 photoacoustic detector. Single seeds were used, and 32 scans at 4 cm^{-1} resolution were averaged for each seed. Carbon black

was used for the background, mirror velocity was 10, gain 64 for the MTEC 200 and 32 for the Nicolet 740. A 5-min nitrogen purge of the instrument chamber was used before a scan. We measured gaseous CO_2 which absorbs as a double peak with a wavenumber centered near 2350 cm^{-1} (CO_2 dissolved in water absorbs as a single peak at 2343 cm^{-1}) (Griffiths & de Haseth, 1986. 314; Sowa & Towill, 1991). Seed temperature during the scans was about 25°C. Data are unitless PAS signals indicating the relative CO_2 production among seeds in this test. (Our measurements are like those from a precise clock not set to a standard time zone. The clock can be used to precisely measure the passage of time but cannot be used to accurately tell the time of day. Likewise, our instrument was not set to a standard but it very precisely measured CO_2 production.) We assumed equal variance among test populations and used two-tailed *t*-tests to compare CO_2 evolution between imbibed dormant and non-dormant seeds of each collection. We used simple linear regression to test for a significant increase in respiration with increasing germinant root length.

Results and discussion

We found no difference in CO_2 production between imbibed dormant and non-dormant seeds of either seed collection (Table 1). Therefore we conclude that seed dormancy does not prevent active respiration. We also found that germinants (root > 2 mm) produced more CO_2 than either dormant or non-dormant seeds (Fig. 1), and as expected (Sowa & Roos, 1991, 1992), germinants with longer roots produced greater amounts of CO_2. The latter findings confirm our ability to detected differences in CO_2 evolution.

Comparable rates of gas exchange between dormant and non-dormant seeds have been reported from other studies using different techniques. Bewley & Black (1982. 200–204) reviewed the evidence that oxygen uptake is similar among germinable and dormant seeds of wild oat (*Avena fatua* L.) and lettuce (*Lactuca sativa* L.).

Table 1. *Relative CO_2 production by imbibed dormant and non-dormant bitterbrush seeds as measured by FTIR-PAS*

	Oregon		Utah	
Seed	Dormant	Non-dormant	Dormant	Non-dormant
		PAS signal		
1	7	11	14	10·5
2	15	9	13	16
3	14	11	10	10
4	6	15	24	11
5	8·5	14	17	26
6	14	5·5	28	10
7	12	9	7·5	16
8	10	9	11	9·5
9	9	9	8·5	10
10	7·5	8	8	7
Mean ± S.D.	10 ± 3	10 ± 3	14 ± 7	13 ± 5
Observed significance level*		$p = 0{\cdot}86$		$p = 0{\cdot}60$

*Two tailed *t*-test for difference between dormant and non-dormant seed.

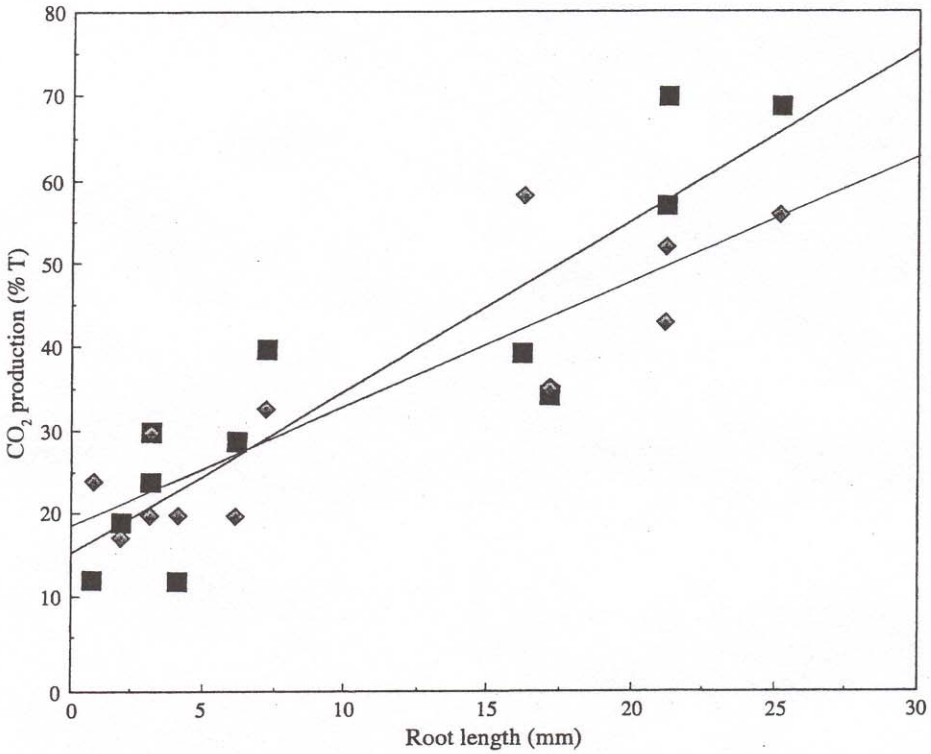

Figure 1. CO_2 evolution from bitterbrush germinants with various root lengths as measured by FTIR-PAS. Increasing CO_2 is indicated by increasing strength of the PAS signal. Oregon (■), $y = 14{\cdot}66 + 2{\cdot}10x$, $r^2 = 0{\cdot}82$, $p = < 0{\cdot}001$; Utah (◆), $y = 18{\cdot}76 + 1{\cdot}46x$, $r^2 = 0{\cdot}85$, $p = < 0{\cdot}001$.

In a separate study that included the same seed lots as tested here, Booth (1999) found significant dry-weight losses from dormant bitterbrush seeds imbibed at 20°C and significant decreases in post-germination vigor. The weight and vigor-loss data measured by Booth, and the evolution of CO_2 from imbibed-dormant seeds measured in this study are evidence that dormant-seed respiration has the potential to reduce seedling growth by consuming stored food.

Bitterbrush seeds are dispersed in July and August which allows for uptake of warm-season precipitation. The reduction in growth that accompanies warm imbibition seems contrary to the general trend for plants to evolve mechanisms that protect reproductive potential. However, there may be factors in the bitterbrush seedbed ecology that mitigate the apparent negative effects to seedling vigor of a summer or early fall seed-imbibition event. If so, these factors need to be understood and used to further our ability to replenish lost or depleted bitterbrush stands.

Conclusions and recommendations

Respiration rates of imbibed dormant and germinable bitterbrush seeds are comparable and it is likely that similar amounts of stored food are used during respiration by the respective seeds. Therefore we recommend bitterbrush be seeded when low soil temperatures will reduce respiration by imbibed-dormant seeds.

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