

The effect of hydrostatic vs. shock pressure treatment of plant seeds

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Abstract. The hydrostatic pressure and shock response of plant seeds has been investigated antecedently, primarily driven by interest in reducing bacterial contamination of crops and the theory of panspermia, respectively. However, comparisons have not previously been made between these two methods of applying pressure to plant seeds. Here such a comparison has been undertaken based on the premise that any correlations in collected data may provide a route to inform understanding of damage mechanisms in the seeds under test. In this work two varieties of plant seeds were subjected to hydrostatic pressure via a non-end-loaded piston cylinder setup and shock compression via employment of a 50 mm bore, single stage gas gun using the flyer plate technique. Results from germination tests of recovered seed samples have been compared and contrasted, and initial conclusions made regarding causes of trends in the resultant data-set. Data collected has shown that cress seeds are extremely resilient to static loading, whereas the difference in the two forms of loading is negligible for lettuce seeds. Germination time has been seen to extend dramatically following static loading of cress seeds to greater than 0.4 GPa. In addition, the cut-off pressure previously seen to cause 0% germination in dynamic experiments performed on cress seeds has now also been seen in lettuce seeds.

1. Introduction

A growing interest is developing in the area of high-pressure biology [1, 2]. However, there is a relatively small body of work in the literature concerning the effects of high pressure on plant seeds. The motivation for this work with regards to high hydrostatic pressure has mainly come from the desire to remove unwanted food spoilage bacteria from seed crops [3, 4]. The study by Mori *et al.* in particular, saw *Brassica oleracea - italica* (broccoli), *Brassica rapa - perviridis* (turnip leaf) and *Brassica rapa - nipposinica* (potherb mustard) treated to high static pressures of 5.5 GPa for 15 minutes, using a cubic anvil press. A 20 - 30% drop in germination was seen when compared to control seeds, however no drop in the rate of change of height was observed in those that did successfully germinate [3].

Some of the research studying the effects of pressure on plant seeds has been motivated by the idea of life being seeded on our planet by biological material being deposited through asteroid impact [5]. As a result, there is some published work investigating the ability of plant seeds to survive high dynamic pressure treatment, although the variety of samples tested is very limited. The present authors have previously published data regarding the ability of *Lepidium sativum* (cress) to survive dynamic



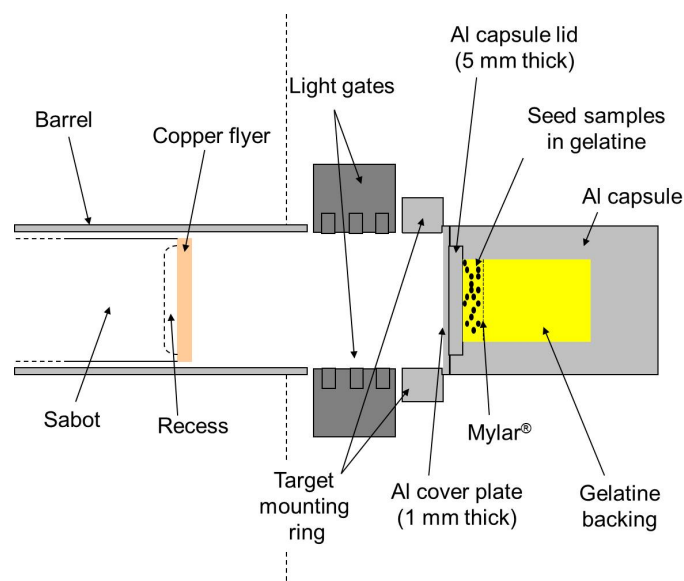


Figure 1. A basic diagram showing the target setup for shockwave experiments.

pressures [6]. This study concluded that cress seeds do not have the ability to survive shockwave treatment with peak shock pressures (PSP) over ~ 0.8 GPa. Another study looked at the ability of plant seeds to withstand high dynamic pressures caused by high velocity impacts [7]. This investigation employed a two stage gas gun in order to accelerate cress, alfalfa and tobacco seeds at water-filled targets. Projectile velocities ranged from $1.0 - 2.9 \text{ km s}^{-1}$, with 2 - 3 seeds being launched in each case. Peak pressures corresponding to these impact velocities ranged from $0.24 - 2.42$ GPa, although as these were calculated numerically, there were large error factors involved. In this investigation, all tests resulted in 0% germination as only fragments of seeds were recovered.

Finally, although there has been previous interest in both the static and dynamic response of various plant seeds to high pressure, the two different regimes have not been compared or contrasted. This study set out to subject the same species of seed to both shock and hydrostatic loading, in order to compare the two datasets and interrogate the damage mechanisms causing any reduction in germination.

2. Experimental Design and Method

Seed samples tested in this study were from *Lepidium sativum* (cress) and *Latuca sativum* (lettuce). These were off-the-shelf plant seeds sourced from Sutton Seeds, UK. Cress and lettuce seed sizes were typically 2 - 3 mm and 3 - 4 mm along the length, respectively. For dynamic experiments, plant seeds were shock loaded using the plate impact technique and a 50 mm bore, 5 m barrel single stage gas gun. Samples were held in a gelatine support medium (Cooking gelatine, Dr Oetker UK Ltd.), set inside an Al shock-recovery capsule (see figure 1), which has been employed previously to study the shock response of porcine muscle tissue [8] and to test the survivability of plant seeds to shock loading [6]. Dynamic experiments saw projectiles (5 mm thick Al flyer plates) accelerated to velocities of $200 - 400 \text{ m s}^{-1}$, corresponding to peak sample pressures of $0.3 - 0.9$ GPa. The sample size for dynamic experiments was ~ 400 seeds per shot. Peak shock pressures were calculated using 2D, axially symmetric ANSYS Autodyn[®] hydrocode models, with a cell size of $0.2 \text{ mm} \times 0.2 \text{ mm}$.

For hydrostatic experiments, seeds were set in a gelatine support medium within a teflon capsule. Samples were loaded using a 100 tonne, non-end-loaded piston cylinder, capable of reaching sample pressures of up to 4.0 GPa. This setup has been employed previously to study the effects of high hydrostatic pressure on bacterial samples [9]. Each hydrostatic test used 20 - 40 seeds, which were pressurised to $0.2 - 4.0$ GPa for 15 minutes. The capsule was pressurised by forcing a tungsten carbide

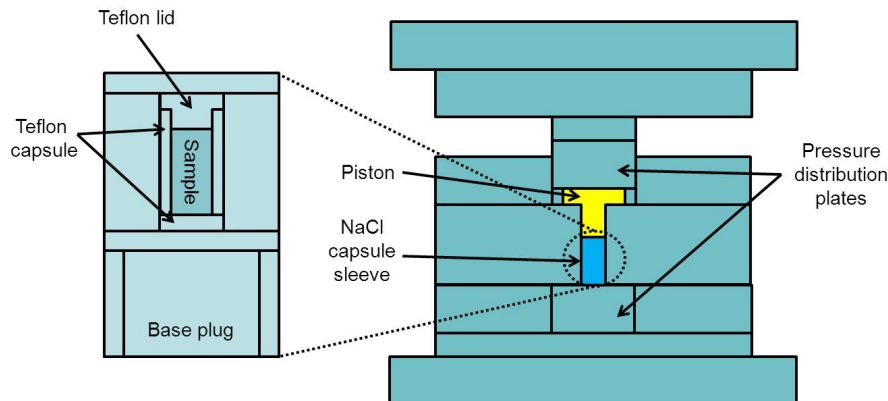


Figure 2. A schematic diagram showing the key parts of the piston cylinder experimental setup.

piston inside the cylinder containing the sample capsule, using a hydraulic ram. Figure 2 shows a schematic illustration of the piston cylinder experimental setup. In both static and dynamic experiments, seeds were recovered from the gelatine support medium and spread on water-saturated paper towels within a petri dish to test for germination. Each petri dish was stored in a laboratory environment and watered once-daily with 30 ml tap water. In this investigation, germination was said to be present when there were visible signs of penetration of the seed coat by either the root or shoot [10]. Each experiment was also paired with a control set, in order to account for off-the-shelf seed packets not producing 100% germination under standard conditions. Shocked germination values were then normalised against their respective control result to remove any effects in variation of growing conditions (i.e. light-levels etc.), using equation 1 (applied previously by Leighs *et al.* [6]).

$$G_a = \frac{G_b}{G_c} \quad (1)$$

where G_a = the adjusted germination value for the shocked sample, G_b = the observed value for shocked germination and G_c = the observed germination value for the control sample. However, this adjustment technique was not performed on results gathered from the hydrostatic experiments to avoid skewing the data as the sample numbers were considerably lower.

3. Results

The complex nature of the target arrangement meant pressure-time histories could not be directly recorded during dynamic experiments. Consequently, peak shock pressures were calculated using ANSYS Autodyn[®]; typical resultant traces are shown in figure 3. The pressures were recorded at a nodal monitoring point placed near the front of the sample region (indicated in the inset diagram in figure 3). Percentage germination results from both hydrostatic and dynamic pressure treatment of plant seeds are shown in figure 4, highlighting the trend of decreasing survival with increasing pressure in all cases. The time required for germination of cress seeds in the static experiments was tracked in order to see if there was an increase or decrease in germination time as a result of such loading. The data collected is shown in figure 5, for a variety of pressures.

4. Discussion

Both graphs in figure 4 show that germination was not seen in any case above peak shock pressures of 0.8 GPa, a pressure at which it is thought the seeds are being damaged beyond repair. This was seen in previous experiments studying the effects of shockwaves on cress seeds [6], but this work also extends the 0.8 GPa cut-off pressure to lettuce seeds (as shown in figure 4). It is postulated that this could be due

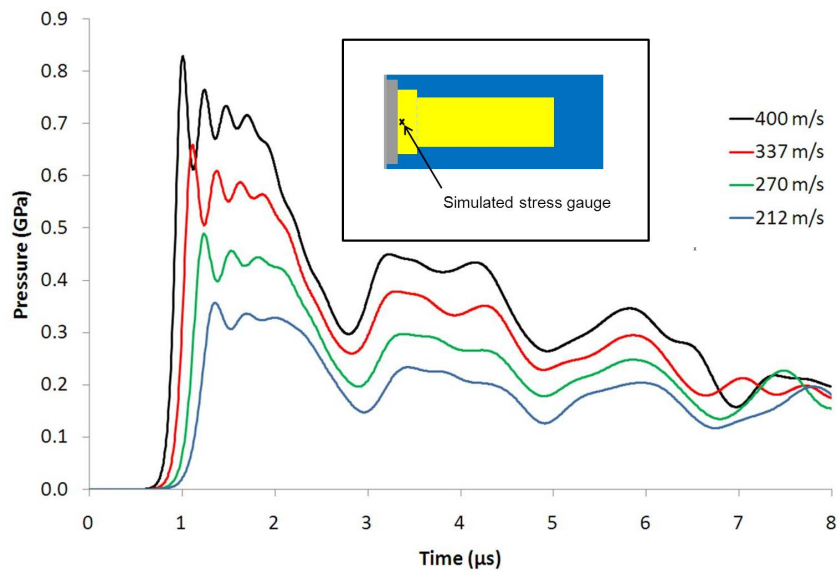


Figure 3. Numerically calculated (ANSYS Autodyn[®]) loading histories for dynamic experiments performed on cress seeds at a range of projectile velocities. Inset: a schematic target diagram highlighting the position of the nodal monitoring point in the hydrocode model.

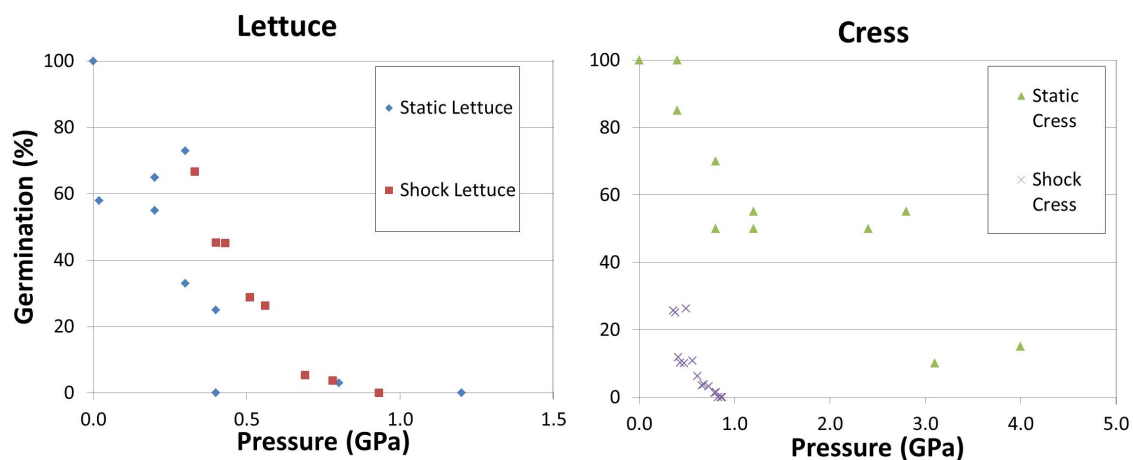


Figure 4. Percentage germination of lettuce (left) and cress (right) seed samples as a function of pressure in the hydrostatic and dynamic regimes.

to denaturing of enzymes within the seed sample, as many of the seed coats were still intact. Figure 4 (left) shows that although lettuce seeds appear to be more affected by static loading than shockwave, the difference is relatively small and negligible. However, for cress seeds (figure 4 - right) this difference is much more pronounced. Despite cress being completely inviable once shock loaded to 0.8 GPa or above, > 10% survival was still seen following hydrostatic treatment of 4.0 GPa.

A series of the experiments were performed on cress seeds to study the effects of high pressure static loading on the length of time required for visible signs of germination to appear. As shown in figure 5, static loading has a considerable effect on germination time. The control set reached 100% germination relatively quickly, whereas seeds pressurised to 0.4 GPa still reached 100% germination, but this took around three times as long. Experiments performed with pressures of 0.8 GPa and higher

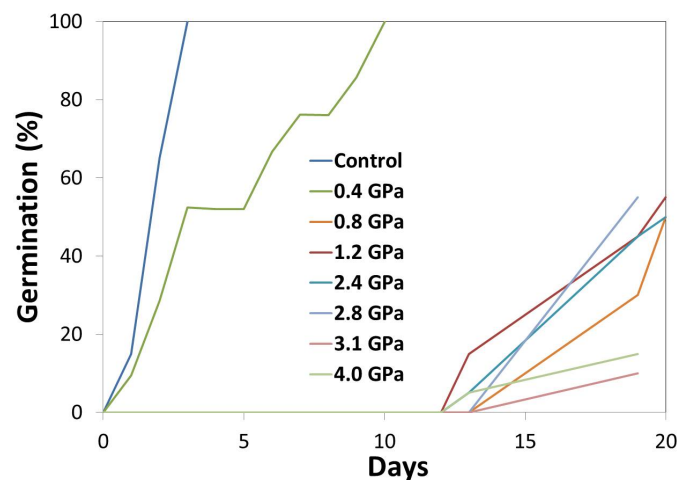


Figure 5. Germination time of cress seed samples following hydrostatic pressure treatment, highlighting the prolongation of germination time above 0.4 GPa.

take considerably longer, causing no signs of germination before 12 - 13 days. This is of particular interest as this is the cut-off pressure seen to cause inviability in the dynamic experiments. Beyond this, increasing the pressure does not appear to have any further effect on germination time until a pressure of greater than 3.0 GPa is reached. Initial results suggest that greater pressures extend germination time considerably more, although there are relatively few observations at this point. It should be noted that Mori *et al.* [3], did not see any noticeable increase in average germination time in their experiments, when hydrostatically loading plant seeds. It is thought this discrepancy is likely due to difference in experimental technique and varieties of seeds studied. Mori *et al.* employed a cubic anvil press to hydrostatically pressure-load broccoli, turnip leaf and potherb mustard seeds in three dimensions; whereas in this investigation, a non-end-loaded piston cylinder was used to hydrostatically pressure-load cress and lettuce seeds in one dimension. Finally, in this work rate of growth was studied in terms of the number of germinated seeds with respect to time, whereas Mori *et al.*, observed changes in height of germinated plants, thus a direct comparison of the two data-sets is inherently complex.

5. Conclusion

Cress and lettuce seeds have been subjected to hydrostatic and dynamic pressures of up to 4.0 GPa. Results from this study follow the hypothesis that the 0.8 GPa cut-off pressure in dynamic experiments extends across many, if not all seeds. It has been postulated that at this pressure the enzymes within the seed are being severely denatured and the seed is being damaged beyond repair, however there is currently no firm evidence to back this idea. The main aim of this paper was to compare the differences between effects of dynamic and static pressure loading on seeds. Although lettuce seeds appear to be slightly more susceptible to damage caused by dynamic loading, the difference looks to be negligible. Conversely, the difference between static and dynamic pressure loading on cress seeds appears to be much greater. As stated antecedently, cress seeds are unviable after dynamic loading with PSPs of ≥ 0.8 GPa, however after static treatment of 4.0 GPa for 15 minutes, there was still $> 15\%$ germination.

Although this investigation has not provided additional insight into the exact damage mechanisms, it has confirmed the 0.8 GPa cut-off pressure for germination extends to 2 seed types (lettuce and cress), with the possibility of more. In addition, it has shown that different seeds are affected differently by static and dynamic pressure loading. It would appear that on the whole, damage seen is not complete destruction of the seed testa. As a result, further work is underway to assess the possibility of damage

mechanisms causing inviability from within the seed.

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