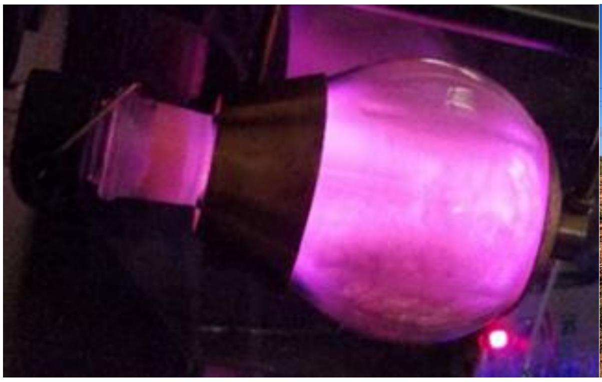
Short summary of final report on the Australian Flora Foundation funded project:

Improving germination success for Australian native plant seeds using plasma treatments



Dr Bryan Coad[1] & Dr Jenny Guerin[2]

1. School of Agriculture Food & Wine, University of Adelaide, Adelaide 5000 SA

2. Seed Research Officer, South Australian Seed Conservation Centre, Botanic Gardens and State Herbarium, Adelaide 5000 SA

August 2023

Complex seed dormancy is common in Australian native plants and can result in low rates of seed germination, limiting the number of plants that can be produced for restoration programs. Seed dormancy can be broken by several human interventions such scarification, exposing to cold, heat including fire, and treatments such as soaking, boiling, or applying smoke compounds.

In this project a form of matter known as cold plasma was investigated as a treatment to break seed dormancy.

Plasma is a state of matter. While it may not as familiar to us as solids, liquids and gasses, we can gain a basic understanding of what plasma is by thinking about what happens when liquid water is transformed into gaseous steam with energy input. Analogously, a gas can be brought into the plasma phase with energy input. This gives us the impression that all plasmas require high energy input and are capable of ignition or burning. However, it is possible to sustain a plasma discharge at low energy if the plasma is generated in a vessel under partial vacuum. Indeed, the plasma glow in fluorescent light tubes is generated using this principle. These are cold plasmas and they can be used as a treatment. In contrast to thermal (hot) plasmas, cold plasma process can be run just slightly warmer than room temperature, using only tens of watts of power. In the figure above left, a 500 mL glass vessel contains a glowing pink air plasma discharge and some seeds. A unique feature that ensures the seeds are evenly treated in the plasma discharge is a motor that ensures the seeds tumble in the plasma like clothes in a tumble dryer.

Why would one want to use plasma as a seed treatment? Plasmas generated from air contain energetic oxygen species that can oxidise surfaces that they contact. Like familiar oxidising substances such as bleach and hydrogen peroxide, air plasmas can destroy microbes, superficially etch and scarify surfaces on a molecular scale, and break down biological substances. In contrast to those wet chemical treatments, plasma treatments can change the properties of seed coats through etching without ever getting seeds wet.

In this project we studied plasma's effect on the seed of 13 native plant species' seed. We observed the physical changes to the seed coat with microscopy. To understand if plasma had a benefit to breaking dormancy for hard-to-germinate seeds, rates of germination were compared to control seeds that had "best practice" germination treatments applied.

For the 13 different plant seed species treated, results from 9 showed no increase in germination percent. Two of the species indicated an adverse effect of plasma treatment likely caused by physical damage to delicate seed tissues. However, 2 seed species showed a large increase in germination after plasma treatments.

For *Acrotriche halmaturina* (Kangaroo Island groundberry) the best-practice treatment for improving germination is to use gibberellic acid soaking. We observed 15% germination for this treatment. After plasma treatment, and application of the same gibberellic acid soaking treatment, the resulting germination was 77% – a greater than 5-fold increase.

The second notable seed species was *Commersonia craurophylla* (Brittle-Leaf Rulingia). *C. craurophylla* is a vulnerable plant with only one extant native plant known in natural habitat in SA. The germination for untreated seeds was 0%; however, after applying plasma, germination success could be increased to approximately 80%. We conducted a detailed analysis of water imbibition for these seeds. We found that the plasma treated seeds could imbibe more than 2 times as much water as untreated seeds. We also measured changes to how water enters the seed and found that the plasma treatment reversed the mode of water entry from capillary-driven to diffusion-driven.

The successes and failures of plasma to increase germination success in all tested seeds provided many first-of-its kind observations helpful for gauging the future prospect of the technology as a treatment for Australian native plant seeds. The scientific knowledge gained was particularly valuable since learnings can be applied in future studies to understand better the relationship between increased water uptake in plasma treated seeds and germination success.

Finally, the benefit of plasma treatments for conservation efforts was demonstrated as an outcome from this research. Thousands of plasma-treated *C. craurophylla* were directly seeded in a revegetation project on a heritage property on Eyre Peninsula (photo above right). An exciting legacy of this project will be to monitor seedling emergence in the near future.

Image legend:

Left. Seeds tumbling in and through the glowing pink air plasma discharge. Image courtesy of Dr Melanie Ford.

Right. Direct seeding of plasma-treated *Commersonia craurophylla* seeds in a revegetation project on Eyre peninsula by SA Seed Conservation Centre workers in June 2023.