Influence of selected environmental factors on seed germination of an invasive species, *Opuntia robusta* (wheel cactus).

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Abstract

O*puntia robusta* is an invasive species in Australia as it reduces native species, effects native ecosystems and provides habitat for pest animals and therefore needs to be controlled. Until recently, little was known about the factors affecting germination of this invasive species. Therefore, the objectives of this study were to determine what factors are needed to break the seed dormancy of *O. robusta*, and the effects of a range of environmental factors on germination.

To break the seed dormancy, three different scarification treatments hand scarification, 15 minutes and 30 minutes machine scarification, were applied to *O. robusta* seeds under three different temperatures and two light conditions. Three different factors pH, moisture, and salinity, was studied to determine if they had an effect on *O. robusta* seed germination. A viability test, was conducted to determine what seeds were viable and which were not, and to calculate the viability adjusted germination percentage. Seeds were cut in half and tetrazolium solution was added, if the seeds turned pink, they were classed as viable.

Highest germination was achieved in Hand Scarification, 12 hours light/12 hours dark 30^{o}C-20^{o}C, with 82% germination, while lowest germination was seen in 15 minutes machine scarification, 24 hour dark, 25^{o}C-15^{o}C and 17^{o}C-7^{o}C, and 30 minutes scarification, under both light conditions 17^{o}C-7^{o}C, with 0% germination. These results determined the temperature and light requirements for factors effecting germination study. Moisture seemed to have the most effect on germination with only 7% of seeds germinating in only treatments, followed by salinity, with seed germination decreasing as salinity levels increased, while pH decrease germination by 10 to 26 percent.

This study suggested that *O. robusta* seeds preferred a temperature range between 30^{o}C-20^{o}C, however further studies on higher temperatures need to be conducted to determine if this range is higher. The study also shows that *O. robusta* seeds preferred light to germinate and a form of scarification. Moisture and salinity had a higher effect on the germination of *O. robusta* seeds compared to pH, however, other levels of pH may affect the germination of *O. robusta* seeds, and therefore further studies on other pH levels are needed.

Introduction

Invasive species or weeds are characterised by their ability and rate of spread and the damage they cause to the native environment. In Australia, they cost the economy $4 billion each year in the control and repair of the native environment (Bryson *et al*, 2006). Most invasive species in Australia were deliberately introduced (Campbell *et al*, 2006; Bryson *et al*, 2006) and have the ability to destroy the native environment and the agricultural industry. They impact the habitat and the growth, of native species (Bryson *et al*, 2006), and aid in the survival of invasive animals (Potter, 2011). Invasive species destroy agricultural land, injure stock, and reduce the quality of wool and other animal products (Agnew *et al*, 2010). One such weed in Australia is *Opuntia robusta*, from Mexico, an invasive species of the *Cactaceae* family (Agnew et al, 2010; Bordelon and Mondragon-Jacobo, 1996; Delgado-Sancheiz, 2013).

O. robusta is a perennial (Potter, 2011), succulent shrub (Agriculture Vitoria, 2016) that is long lived (Potter, 2011). They can grow to a size, ranging from 1 metre to 4 metres high (Potter, 2011; Delgado-Sancheiz, 2013; Agriculture Victoria, 2016). *O. robusta* have flowers that are 5 to 8 cm wide,

and are yellow in colour (Potter, 2011; Agriculture Victoria, 2016) with red strips on the underside of each petal. *O. robusta* flowers all year except winter (Potter, 2011). They have a shallow root structure (Chuk, 2010; Agriculture Victoria, 2016), which are fibrous (Agriculture Victoria, 2016) and able to store water (Agnew, 2009). The stems, or pads (Potter, 2011), of *O. robusta* are blue/green in colour, round and flat in shape and grow to 40cm in length (Potter, 2011; Agriculture Victoria, 2016). These stems are covered in areoles that produce spines (Agriculture Victoria, 2016; Nerd and Mizrahi, 1997), which grow to 4cm long (Agriculture Victoria, 2016). These spines provide protection from predators, however, there are very few of those predators in Australia (Chuk, 2010). *O. robusta* plants have many fruit (Agriculture Victoria, 2016) that are seen most of the year (Aguirre *et al*, 2006), are pink to purple in colour, 8cm long (Potter, 2011; Agriculture Victoria, 2016) and 6cm wide (Agriculture Victoria, 2016), with around 518 seeds. They are 4.5mm long, 3.5mm wide (Aguirre *et al*, 2006) and weighs about 0.0178mg (Delgado-Sanchez *et al*, 2013). The seeds have a hard (Potter, 2011), white (Aguirre *et al*, 2006) coat which helps to maintain viability (Potter, 2011) for as long as 15 years (Aguirre *et al*, 2006).

In Australia, *O. robusta* is classified as a weed, because it grows in dense plots resulting in (Potter, 2011; Agriculture Victoria, 2016), the reduction of native species through competition (Agnew *et al,* 2010; Agnew, 2009; Potter, 2011; Agriculture Victoria, 2016), effects native habitat and ecosystems (Agnew, 2009; Campbell *et al*, 2006) especially in arid environments (Edmunds, 2006), blocks waterways, causing erosion (Agnew, 2009) and provides habitat for pest animals (Agriculture Victoria, 2016). *O. robusta* is not classified as a weed of crop or agriculture (Agriculture Vitoria, 2016), however it does cause major problems for farmers (Chuk, 2010). The spines cause damage to stock, which avoid this species and reduces their ability to graze and access water (Potter, 2011; Agriculture Victoria, 2016). It also contaminates animal products, such as wool (Potter, 2011).

O. robusta is a major problem in the Flinders Ranges of South Australia (Agnew, 2009; Potter, 2011; Agnew *et al*, 2010; Edmunds, 2006; Baker *et al*, 2006; Bryson *et al*, 2006). In South Australia, there is over 930,000 ha of land infested by *O. robusta* (Agnew *et al,* 2010), with the worst infestation in the Flinders Ranges (35,000 ha) (Potter, 2011; Agnew *et al*, 2010; Edmunds, 2006; Bryson *et al*, 2006; Agnew, 2009). In the most infested area, there can be up to 200 individual plants per ha (Agnew, 2009).

It is not just the current distribution of *O. robusta* that is a problem, it is the potential distribution and the potential damage that could be caused by *O. robusta* (Agnew *et al*, 2010; Potter, 2011). The distribution in South Australia is only a fifth of its potential distribution (Agnew *et al*, 2010) and could spread and become a problem in Queensland and Western Australia (Potter, 2011). The potential of this species is what makes it such a concerning weed (Agnew *et al*, 2010).

Seed dispersal, in any form is said to be the most prominent form of spread for *O. robusta*, and is the cause of high success rates (Aguirre *et al*, 2006). Seeds are spread through a method call synzoochory, which is seed spread by animals, mainly ants (Rojas-Arechiga and Vazquez-Yanes, 2000), however, many scientists have stated that the main animal vector for seed dispersal in *O. robusta* are birds, with others animals (Agnew, *et al,* 2010; Agriculture Victoria, 2016; Aguirre *et al*, 2006) including foxes, spread seeds through their faeces over large areas (Agriculture Victoria, 2016).

O. robusta seeds have a dormancy period (Aguirre *et al*, 2006; Delgado-Sanchez *et al*, 2013; Alvarez-Fuentes *et al*, 2010; Rojas-Arechiga and Vazquez-Yanes, 2000), which is difficult to break (Delgado-Sanchez el at, 2013). Dormancy for seeds is when all functions terminate, for a period of time, even when there is suitable growing conditions (Rojas-Arechiga and Vazquez-Yanes, 2000). There are different types of dormancies, with *O. robusta* seeds have physical dormancy (Delgado-Sanchez *et al*, 2013; Alvarez-Fuentes *et al*, 2010). For *O. robusta* seeds to germinate, the dormancy of these

seeds need to be broken. In the natural environment, the breaking of *O. robusta* seed dormancy can be caused by chemical reaction (fungi (Delgado-Sanchez *et al*, 2013)), or animal digestion. For seeds to germinate, they also need their preferred weather and temperature (warmer months (Barrads *et al*, 2003; Aguirre *et al*, 2006; Rojas-Arechiga and Vazquez-Yanes, 2000)). Studies conducted by Rojas-Arechiga and Vazquez-Yanes (2000) on the temperature requirements of the species found that there is no germination at or below 10°C and the germination is reduced at, and above 30°C, with an optimal temperature range between 15°C and 25°C. However other studies conducted by Aguirre *et al* (2006) found the optimal germination temperature to be between 24°C and 39°C. Studies conducted on the effects of light requirements showed mixed results with some suggesting the germination rates of the seeds were higher in light than dark, while other found *O. robusta* germinates in the same way with both light and dark and other showed germination rates are higher in dark than light (Delgado-Sanchez *et al*, 2013). Once the dormancy of the seed is broken, *O. robusta* can germinate. Germination occurs during the warmer months, spring and summer (Aguirre *et al*, 2006), but can occur at any time of the year, depending on rain (Potter, 2011).

Until recently, little was known about the factors affecting germination of this invasive species, therefore the aim of this research project was to study the seed ecology of *O. robusta,* to aid in the development of control methods. The objectives of this study was to determine what factors are needed to break the seed dormancy of O. robusta, and the effects of a range of environmental factors on germination.

Methods

Breaking Seed Dormancy

Six different treatments were applied to *O. robusta* seeds, based on a review of previous studies conducted on the germination of *O. robusta* seeds. These treatments were…

- Hand Scarification- 12 hours light/12 hours dark
- Hand Scarification- 24 hours dark
- 15 Minutes Machine Scarification- 12 hours light/12 hours dark
- 15 Minutes Machine Scarification- 24 hours dark
- 30 Minutes Machine Scarification- 12 hours light/12 hours dark
- 30 Minutes Machine Scarification- 24 hours dark

For hand scarification, a nailfile was used to scarify two sides of the *O. robusta* seeds. For machine scarification, the *O. robusta* seeds were placed into a jar containing sharp pieces of marble and were shaken for the relevant time (15 minutes, 30 minutes). No speed rate was indicated on the machine, but for consistency a half turn of the dial was the set speed. Treatments that were 24 hours dark were wrapped in aluminium foil, and for 12 hours light, 12 hours dark treatments the germination cabinets were set on a timer for a day/night replication.

Each of these treatments were conducted in three different germination cabinets, which had a ten degree temperature range. The temperatures were…

- 30°C-20°C
- 25°C-15°C
- 17°C-7°C

In total, 18 treatments (six treatments, in each temperature) were applied to *O. robusta* seeds. For each treatment, there was three replicates, with 20 seeds each, placed in Petri dishes, with sterilised whatman filter paper and 3ml of gibberellic acid (1000ppm) (Gibberellic acid was used in this study as it helps with seed germination) (Chudasama and Thaker, 2007). The *O. robusta* seeds were placed in the germination cabinets for 60 days, and were checked regularly. An additional 1ml of gibberellic acid at a time, was added when checking the seeds, if it was needed to maintain moisture levels.

Environmental Factors Effecting Germination
Three different factors pH, moisture (polyethylene glycol (PEG)) and salinity (sodium chloride (NaCl)), was studied to determine if they had an effect on *O. robusta* seed germination. For each factor, 6 different chemical concentrations were tested. Concentration 4, 5, 6, 8, 9, and 10 of pH, -0.1, -0.2, -0.4, -0.65, -0.85, and -1 of PEG, and 25mM, 50mM, 100mM, 150mM, 200mM, and 250mM of NaCl.

In total, 18 treatments (six per factor) were applied to *O. robusta* seeds. The seeds were soaked for 24 hour in gibberellic acid. For each treatment, there was three replicates, with 20 seeds each, placed in Petri dishes, with sterilised whatman filter paper and 3ml of the chosen chemical. The *O. robusta* seeds were placed in the 30°C-20°C germination cabinet under 12 hours light 12 hours dark conditions, based on the results from the seed germination study results for 57 days, and were checked regularly. An additional 1ml of the chemical at a time, was added when checking the seeds, if it was needed to maintain moisture levels.

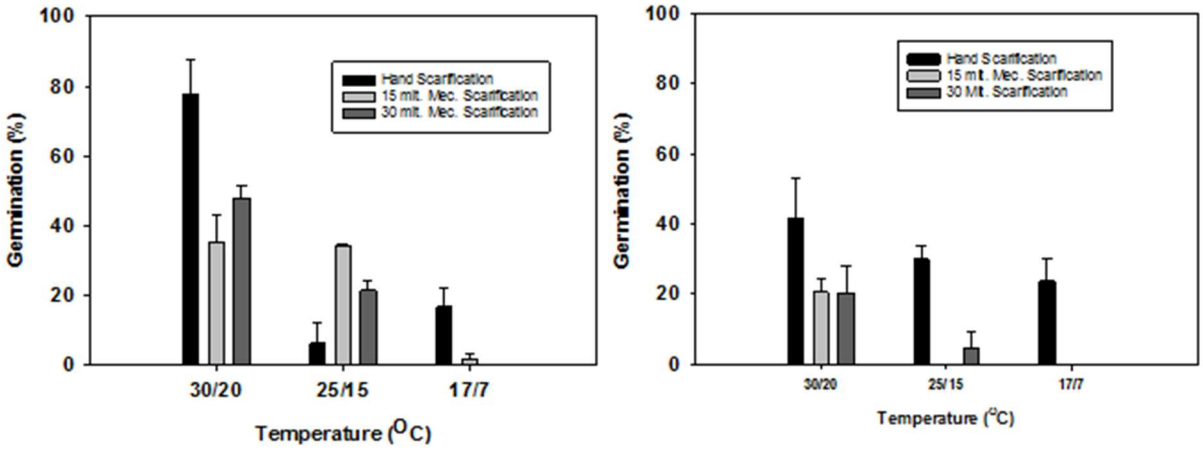
Viability Test
A viability test was conducted to determine what seeds were viable and which were not, and to calculate the Viability Adjusted Germination (VAG) percentage. Non-germinated seeds where cut in-half, placed back in the same Petri dish, with the sterilised whatman filter paper removed and 1ml of tetrazolium added. After 24 hours the seeds were checked to see if the tetrazolium had turned them pink which indicate viability. These seeds were counted and recorded, with the total viable seeds then divided by the total number of germinated seeds and times by 100 to determine the VAG percentage.

Results
Breaking Seed Dormancy
As seen in figure 1 the highest germination for both light conditions was achieved in hand scarification, 30°C-20°C, with 82% seed germination in 12 hours light/12 hours dark and 60% seed germination in 24 hours dark. The lowest germination was seen in 15 minutes machine scarification, 24 hours dark, under 25°C-15°C and 17°C-7°C temperatures, and 30 minutes scarification, under both light conditions, in 17°C-7°C temperatures, with 0% seed germination, as seen in figure 1 and 2.

Temperature 17°C-7°C had the lowest overall germination rate in both 12 hours light/12 hours dark and 24 hours dark, with the highest germination percent of 16 with hand scarification. Four treatments had a germination percent of less than 5%. All treatments with 12 hours light/12 hours dark had a higher germination percent than 24 hours dark, except for 30 minutes machine, 17°C-7°C, with both light conditions having an equal germination percent of 0.

Figure 1. Viability Adjusted Germination of *Opuntia robusta* under three different treatments, three different temperatures and 12 hours light/12 hours dark.

Figure 2. Viability Adjusted Germination of *Opuntia robusta* under three different treatments, three different temperatures and 24 hours dark.



Factors Effecting Germination

pH did not have a large effect on seed germination, however a decrease of 10 to 26 percent was seen. As the pH levels increased to 7, seed germination decreased, then as the pH levels increased past 7, seed germination increased, as seen in figure 3.

Moisture had the biggest effect on *Opuntia robusta* seed germination. The first point on the graph is the control, which was hand scarification under 12 hours light/12 hours dark in 30°C-20°C temperature. The next point is a decrease in the water availability, and each point after that is a greater decrease of water availability. As seen in figure 4, as the water availability decreased, the germination percent decreased, with no seed germination occurring after the fourth point, which is a PEG concentration of 0.4.

Again, the first point on the graph in figure 5 is the control, which was hand scarification under 12 hours light/12 hours dark in 30°C-20°C temperature. The next point is an increase of salinity levels, with each point after that a greater increase of salinity levels. The results of the effect of salinity are, as salinity levels increased, seed germination decreased. At point four, which is a NaCl concentration of 100mM, germination increased compared to the third point, which is a NaCl concentration of 50mM, however, at NaCl concentration of 150mM, which is point five, germination decreased and continued to decrease.

Figure 3. Viability Adjusted Germination of *Opuntia robusta* effected by pH under 30°C-20°C temperature and 12 hours light/ 12 hours dark.

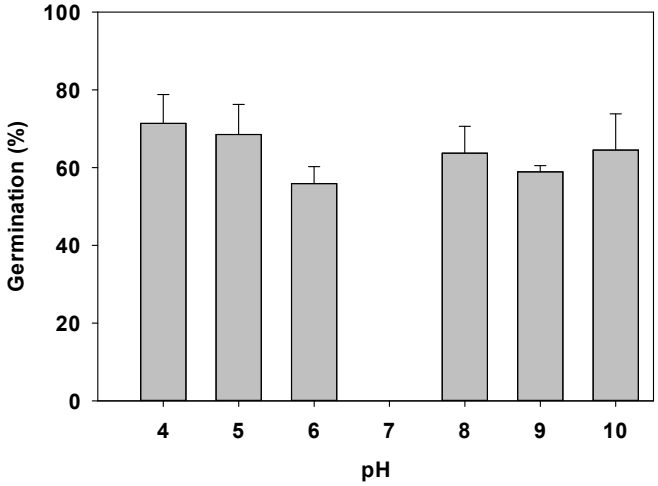


Figure 4. Viability Adjusted Germination of *Opuntia robusta* effected by moisture under 30°C-20°C temperature and 12 hours light/ 12 hours dark.

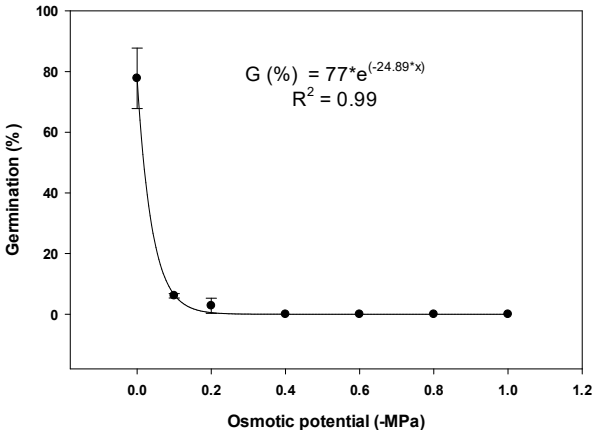
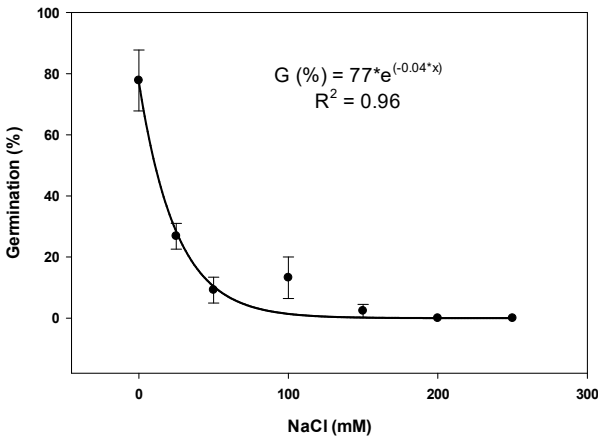


Figure 5. Viability Adjusted Germination of *Opuntia robusta* under effected by salinity 30°C-20°C temperature and 12 hours light/ 12 hours dark.



Discussion

As stated in the introduction, no germination of *O. robusta* seeds occur below 10°C and the optimal temperature of germination is between 15°C and 25°C (Rojas-Arechiga and Vazquez-Yanes, 2000). In this study, germination of *O. robusta* seeds occur between 17°C-7°C, which does not support the previous statement. There was a high seed germination percent in temperature between 25°C-15°C, however there was a higher germination in 30°C-20°C, with Aguirre *et al* (2006) finding similar results (24°C and 39°C). This study found that seeds of *O. robusta* prefer a temperature range between 30°C-20°C, although further studies on higher temperature ranges are needed to conform this.

The light requirements for *O. robusta* seeds, has had mixed results, both in this study and previous studies, however an overall higher germination in 12 hours light/12 hours dark was found in this study and Delgado-Sanchez *et al* (2013). *O. robusta* can germinate in any light conditions, meaning this species has the ability to germinate in the shade, under trees, increasing its ability to effect native tree species, however they prefer some amount of sunlight.

No research has been conducted on the pH requirements of *O. robusta* seeds for germination, although, a study conducted by De Kock (2001) on the genus of *O. robusta* have suggested that the species can tolerate a pH level of 8.5. This study did not support that finding, with *O. robusta* seeds germinating in pH levels above 8.5, at high germination percentage, however, further studies of the effects of pH on seed germination is needed.

Little research has been conducted on the effects of moisture availability on *O. robusta* seed germination. However, one report by Potter (2011) suggested that *O, robusta* seed germination is depended on rain. This study found that *O. robusta* seeds requires a high level of water to germinate, which indicates that the spread of this species through seed is determined somewhat by the water availability. Further studies on the exact amount of water this species requires to germinate is needed to determine how far this species could spread in Australia.

Like all the other factors, there is little research on the effects of salinity on *O. robusta* seeds, however a study on the effects of salinity of a different species within the gene showed that all growth factors of this species decrease when salinity levels were increased (Cortes-avila *et al*, 2001). This could suggest that species within the gene *Opuntia* could be salt sensitive, including *O. robusta*, which was supported by this study.

Conclusion

In conclusion, this study has shown that *O. robusta* has the ability to germinate in a wide variety of temperatures, light and pH levels, suggesting that it has the ability to spread wildly throughout Australia. *O. robusta's* distribution may be affected by the availability of water and the levels of salinity in different ecosystems. This study has also provided a greater understanding of a species that is still unknown.

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