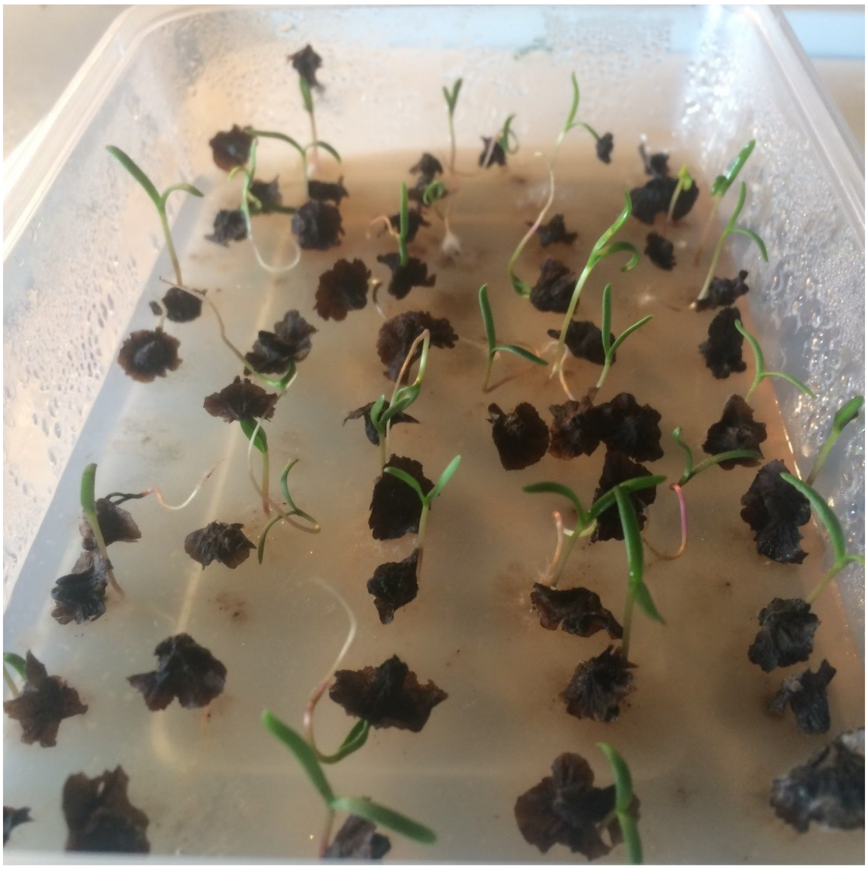
Seed Storage Longevity of Black Bluebush (*Maireana pyramidata*) collections: 2019 Progress Report B



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Report Title: Seed Storage Longevity of Black Bluebush (*Maireana pyramidata*) collections: 2019 Progress Report B

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Executive Summary

Tronox Mining Australia Ltd. (Tronox, previously Cristal Mining Australia Ltd.) currently conducts broad-scale rehabilitation at Ginkgo and Snapper mines, located in western New South Wales. To date rehabilitation has been achieved by (a) storing and returning stockpiled subsoil and topsoil, (b) mechanically broadcasting seed and (c) planting tubestock for selected species.

Prior to broadcasting, all seeds are stored within controlled conditions (two air-conditioned shipping containers held at ~20°C and ambient humidity). However, it has been reported that the seeds of chenopod species (e.g. Bluebush) have a short longevity (only remain viable for 1-2 years), even when stored in controlled conditions. As such, a trial began in 2015 to determine how long the seed of important chenopod species could be stored within the Ginkgo seed stores before significant loss of viability.

This initially included single collections of *Maireana pyramidata* (Black Bluebush) and *M. sedifolia* (Pearl Bluebush). After just one year in storage both Bluebush species showed significant losses in viability, with minimal seed remaining viable after 2 years in storage. It was also shown that both collections used in the trial had high proportions of immature seeds, which may have contributed to the rapid decline in viability.

As a result of the above findings it was initially recommended that *M. pyramidata* and *M. sedifolia* should not be stored beyond 1 year, each year collecting only the required quantities for that rehabilitation season. While *M. pyramidata* is available for collection most years, *M. sedifolia* seeds infrequently and the lack of storage capability may create a 'bottleneck' in being able to return this species to the rehabilitated landscape.

In 2016 four additional Bluebush species (*M. georgei, M. pentatropis, M. trichoptera* and *M. turbinata)* were added to the trial. While all species had small declines in viability during storage, each of these species have been deemed capable of storage for at least 3 years (Quarmby, 2019).

Since 2017 6 additional collections of *M. pyramidata* have been added to the trial (when seed has been available) in order to clarify if the trends seen in the initial collections hold true with varying seasons and/or populations. Note additional tests have not yet been conducted on *M. sedifolia* as seed has not been available to do so.

This report details the effect of storage on viability and germination of all *M. pyramidata* collections tested to date.

A summary of the current findings and current recommendations are provided below:

- ONS 279 was the only *M. pyramidata* collection to show a rapid decline in viability during storage, with all other collections tested capable of storage in the Ginkgo Seed Store for at least 1-2 years. It is noted, however, that Duncan *et al.* (2019a, 2019b), also reported a decline of greater than 50% within 12 months of storage at 20^{o}C for another collection of this species.

- Single collections of four other Bluebush species have also been shown to be capable of storage in the Ginkgo Seed Store for at least 3 years. However, as this study has shown variation between individual collections, it is recommended further collections of these species are added to the trial as seed becomes available.

- The ONS 279 results may have been affected by increased seed age, with seeds potentially 3 months older (or more) than other collections at each test interval. The results for this collection are also less reliable than all other collections due to the low number of replicates that were tested. These factors may have also attributed to the results by Duncan *et al.* (2019a, 2019b), with tests conducted on seeds with a similar age to ONS 279 (8 months old), and with low numbers of replicates and seed tested (4 replicates of 25 seeds).

- Variation in the seed longevity may also be attributed to varying seed maturity as well as environmental factors experienced by the parent plants during seed development. Both of these factors are difficult to control and will continue to vary with individual collections.

- The current Tronox seed store conditions may have reduced the potential shelf-life of individual batches or species. It is expected that improvements to the temperature and humidity would increase the shelf life of all species held within the seeds stores. As such it is recommended that at a minimum, the current air-conditioners should be upgraded so that a constant temperature is maintained year round. If possible, the RH should also be controlled and maintained at less than 15%. When further facilities are commissioned (due to increased seed volume requirements), it is recommended that they should be capable of a temperature of approximately 5°C and less than 15% RH. Improved conditions will be of particular benefit to species with short longevities under the current conditions (such as *M. sedifolia*), and allow storage of other species on a medium term basis (5-10 years), especially for species that seed infrequently.

- Current *M. pyramidata* collections should be continued to be monitored for at least 1-2 years under the current storage conditions in order to gain a better understanding of the average expected shelf-life for these species. Given the variation in results for *M. pyramidata*, additional collections of *M. sedifolia* should be added to the study (when seed becomes available) to verify existing results, as the potential lack of storage capability under the current conditions may create a 'bottleneck' in being able to return this species to the rehabilitated landscape.

- It is also recommended that, following any upgrades to the Tronox seed storage conditions, the storage performance of the above species (plus others) are monitored to ascertain changes in shelf-life. As storage space within each seed store is limited, investigations will also determine priority species that benefit the most from storage under the premium conditions (e.g. species currently with the shortest seed longevities).

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1. Background

Tronox Mining Australia Ltd. (Tronox, previously Cristal Mining Australia Ltd.) own and operate two mineral sand mines the Murray Darling basin (southwestern NSW). Ginkgo Mine was established in 2006 and broad-scale rehabilitation began in 2009. Snapper mine was established in 2010 and rehabilitation began in 2012. To date rehabilitation has been achieved by (a) storing and returning stockpiled subsoil and topsoil, (b) mechanically broadcasting seed and (c) planting tubestock for selected species.

Seeds that are used in mechanical broadcasting are currently stored within controlled conditions (air-conditioned shipping containers held at ~20°C and ambient humidity) at the Ginkgo Mine prior to use. For many species, the seed longevity (viability) remains stable for several years (up to 10 years) when stored in such conditions (Gunn, 2001, Crawford, 2007). As such, seeds are often stored for 1-3 years in the Ginkgo Seed store prior to broadcasting (Zwiersen, per.comm.).

However, it has been reported that the seeds of chenopod species (e.g. Bluebush and Saltbush) have a short longevity (only remain viable for a few years), even when stored in controlled conditions (Barrett-Lennard, 2003, Cockerton, 2014). As such, a trial began in 2015 to determine how long the seed of important chenopod species could be stored within the Ginkgo seed store before significant loss of viability.

This initially included single collections of *Maireana pyramidata* (Black Bluebush), *M. sedifolia* (Pearl Bluebush)*, Atriplex rhagodioides* Silver Saltbush) and *A. vesicaria* (Bladder Saltbush). After just one year in storage both Bluebush species showed significant losses in viability, with minimal seed remaining viable after 2 years in storage. It was also shown that both collections used in the trial had high proportions of immature seeds, which may have contributed to the rapid decline in viability (Quarmby 2017).

As a result of the above findings it was recommended that *M. pyramidata*, *M. sedifolia* should not be stored beyond 1 year, each year collecting only the required quantities for that rehabilitation season. While *M. pyramidata* is available for collection most years, *M. sedifolia* seeds infrequently and the lack of storage capability may create a ‘bottleneck’ in being able to return this species to the rehabilitated landscape.

In 2016 four additional Bluebush species were added, including *M. turbinata* (Slit-wing Bluebush), *M. pentatropis* (Erect Mallee Bluebush), *M. trichoptera* (Downy Bluebush) and *M. georgei* (Satiny

Bluebush). While all species had small declines in viability during storage, each of these species have been deemed capable of storage for at least 3 years (Quarmby, 2019).

Since 2017 6 additional collections of *M. pyramidata* have been added to the trial (when seed has been available) in order to clarify if the trends seen in the initial collections hold true with varying seasons and/or populations. Note additional tests have not yet been conducted on *M. sedifolia* as seed has not been available to do so.

Seven *M. pyramidata* collections have now been included in the study, with the collections shown in Table 1 included in the current report. Note that both ONS 434 and ONS 435 were collected in 2019, therefore only their initial viability and germination results are included within this report.

Table 1.   Black Bluebush collections included in current report.

Collection Number	Year Collected	Storage Lengths Tested
ONS 279	2014	0 (fresh), 1yr, 2 yrs
ONS 386	2017	0 (fresh), 1yr, 2 yrs
ONS 387	2017	0 (fresh), 1yr, 2 yrs
ONS 413	2018	0 (fresh), 1yr
ONS 414	2018	0 (fresh), 1yr
ONS 434	2019	0 (fresh)
ONS 435	2019	0 (fresh)

2. Methods

On receiving the initial samples (fresh seeds) of each collection bracts (containing a single seed) were dissected under a stereo microscope to determine the initial seed fill (presence of an embryo) and seed heath (e.g. healthy & mature (creamy/yellow embryo), immature (green embryo), dead (brown embryo), or undeveloped (shrivelled seed or empty fruit). For ONS 279 there was 1 replicate of 150 fruit, however, for all other collections there were 8 replicates of 50 fruit.

A germination test was then conducted by plating bracts on a medium of 1% water agar and placing them in a germination cabinet at 17°C maximum/ 6°C minimum and 14 hours of light (simulating average Winter diurnal temperature and light settings at Pooncarie (from BOM, 2017)) for 4 weeks.

At the end of the germination test any non-germinated fruit were dissected to determine their current viability (referred herein as post-germination viability test). For ONS 279 there were 3 replicates of 50 fruit per test, however, for all other collections there were 8 replicates of 50 fruit per test.

After each storage duration samples were re-tested using a germination test was repeated to assess changes in viability and germination

2.1 Analysis

Differences in the seed fill, viability (post-germination) and viability adjusted germination (germination of only viable seeds only) of fresh seed between individual collections were analysed using a generalized linear model (glm). The effect of storage on viability (post-germination) and viability adjusted germination (germination of only viable seeds only) within collections and between collections was analysed using a generalized linear model with mixed effects (glmer).

Comparisons between storage lengths and/or collections were analysed using a Tukey's HSD post-hoc test using the "glht" function in the "mult-comp" package of R software. (R. 2019, version: 3.4.30) software.

3. Results

3.1 Initial seed fill, viability and germination results

The initial seed fill, viability and germination (viability adjusted) of individual collections is presented in Figure 1 and Table 2. Seed fill and viability was found to vary significantly (P>0.05) between collections as indicated by the dis-similar letters in Figure 1. Seed fill was highest in ONS 279, ONS 386 and ONS 387, however, the slight differences between these collections and ONS 416 and ONS 434 were not considered significant. Differences in the seed fill of ONS 416, ONS 434, ONS 435 and ONS 279 were also considered to be non-significant, however, the supposed similarity for ONS 279 was likely caused by the low number of replicates used weakening the strength of the data. ONS 413 was considered to have a significantly lower seed fill (72.50%) than all other collections.

Viability was highest in ONS 279, ONS 386, ONS 387 and ONS 416, with differences between these collections not considered significant (Figure 1 and Table 2). ONS 413, ONS 434 and ONS 435 were found to have significantly lower viabilities than all other collections (47.25%, 78.75% and 64.50% respectively). This was mainly attributed to a higher proportion of fresh seed that were considered dead or empty (un-developed) as shown in the initial viability cut tests (pre-germination) (Figure 1 (Appendix 1)) and the post- germination viability cut tests (Figure 2 (Appendix 1)). All collections were also found to vary in the proportion of immature seeds, with nearly half of all healthy seeds for ONS 279 and ONS 435 considered immature.

Germination (viability adjusted) of fresh seed was also found to vary significantly between collections. ONS 435 had the lowest germination (85.49%), with greater than 90% of viable seed germinating in all other collections. Note that the apparent significant differences or similarities between certain collections have been exacerbated by the varying levels of viability between collections (as described above).

3.2 Effect of Storage on viability and germination

The effect of storage on viability for each of the *M. pyramidata* collections is shown in Figure 2 and Table 3, with varying responses between individual collections. ONS 279 incurred a rapid decline in viability during storage, with a loss in viability of 40% after 1 year in storage and further dramatic loss after 2 years with only 7.33% considered viable. In contrast, the viability of ONS 386 remained stable after 1 and 2 years in storage, with changes in viability not considered significant. After 1 year of storage ONS 387 was found to have declined by 5% (considered significant), however, the viability after 2 years was considered similar to that of fresh seed. Therefore both 2017 collections were considered to have remained stable after 2 years of storage. After 1 year of storage both of the 2018 collections (ONS 413 and ONS 416) also remained stable (no decline in viability). Note that ONS 413 was considered significantly different to all other collections due to the difference in initial viability, compared to all other collections (Figure 2, Table 3).

Storage was not found have any effect on the germination of viable seeds for any of the collections (Figure 2, Table 3). Viable seeds germinated readily, regardless of storage length or collection, with germination mainly occurring within 14 days of plating (data not shown).

Table 2. Initial seed fill, viability (post germination) and germination (viability adjusted) test results ± St. Error. Dissimilar letters indicate significant differences (P< 0.05) between collections.

Collection Number	Germination Test Treatment	Seed Fill % (± St. Error)	Viability % (± St. Error)	Germination % (± St. Error)
ONS 279	Fresh Seed	94.67 (±2.40) bc	91.33 (±1.76) cd	100.00 (±0.00) bd
ONS 386	Fresh Seed	93.50 (±1.72) c	88.75 (±1.92) d	98.62 (±0.58) cd
ONS 387	Fresh Seed	93.25 (±1.56) c	89.25 (±1.81) d	98.29 (±0.56) acd
ONS 413	Fresh Seed	72.50 (±2.26) a	47.25 (±1.77) a	93.29 (±2.44) ab
ONS 416	Fresh Seed	88.50 (±1.35) bc	84.50 (±1.05) d	99.43 (±0.38) d
ONS 434	Fresh Seed	89.50 (±1.84) bc	78.75 (±2.45) c	91.69 (±1.01) b
ONS 435	Fresh Seed	83.00 (±1.85) b	64.50 (±1.50) b	85.49 (±2.27) bc

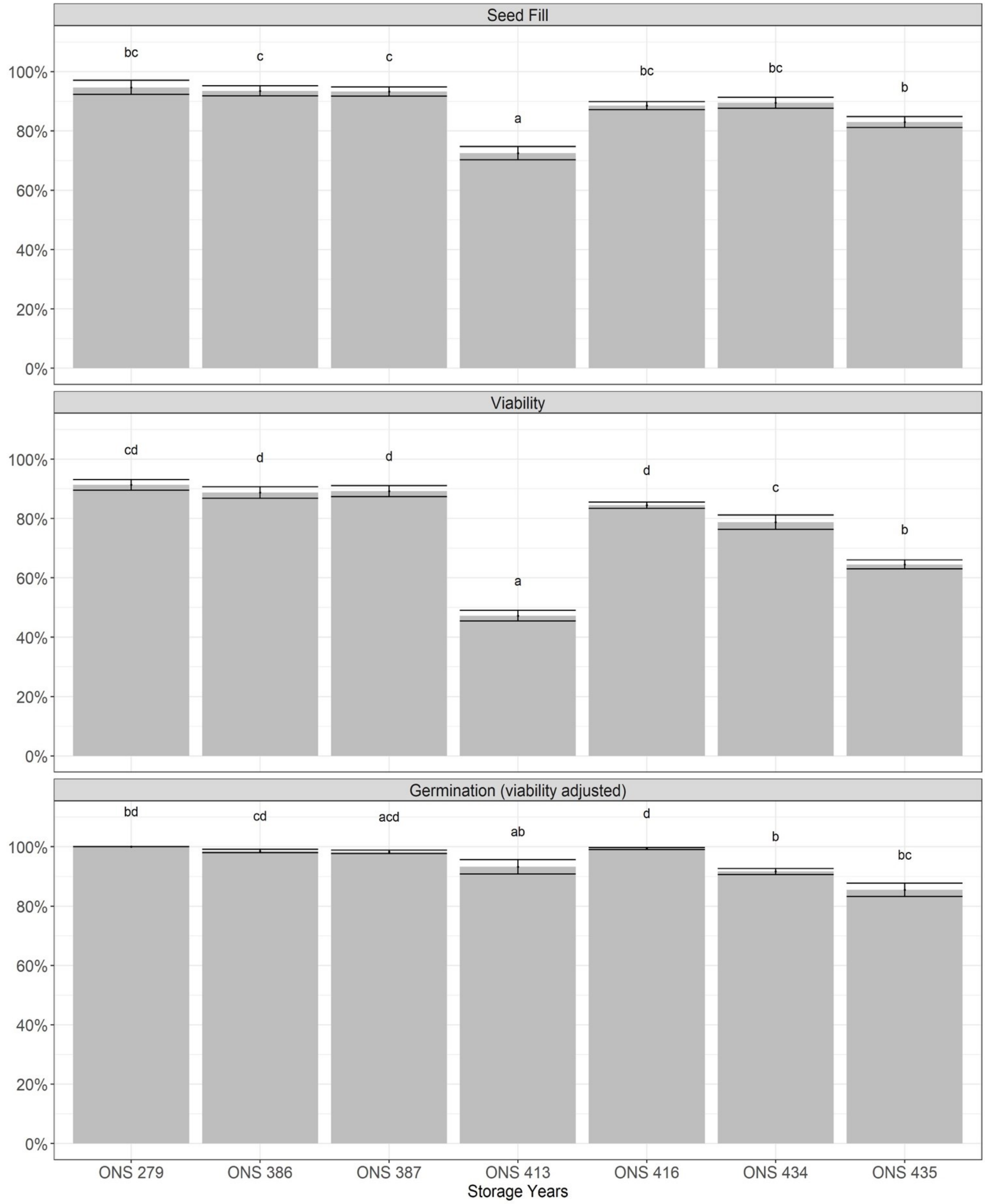

Figure 1. Initial Seed Fill, Viability (post germination) and Germination (viability adjusted) test results ± St. Error. Dissimilar letters indicate significant differences (P< 0.05) between collections.

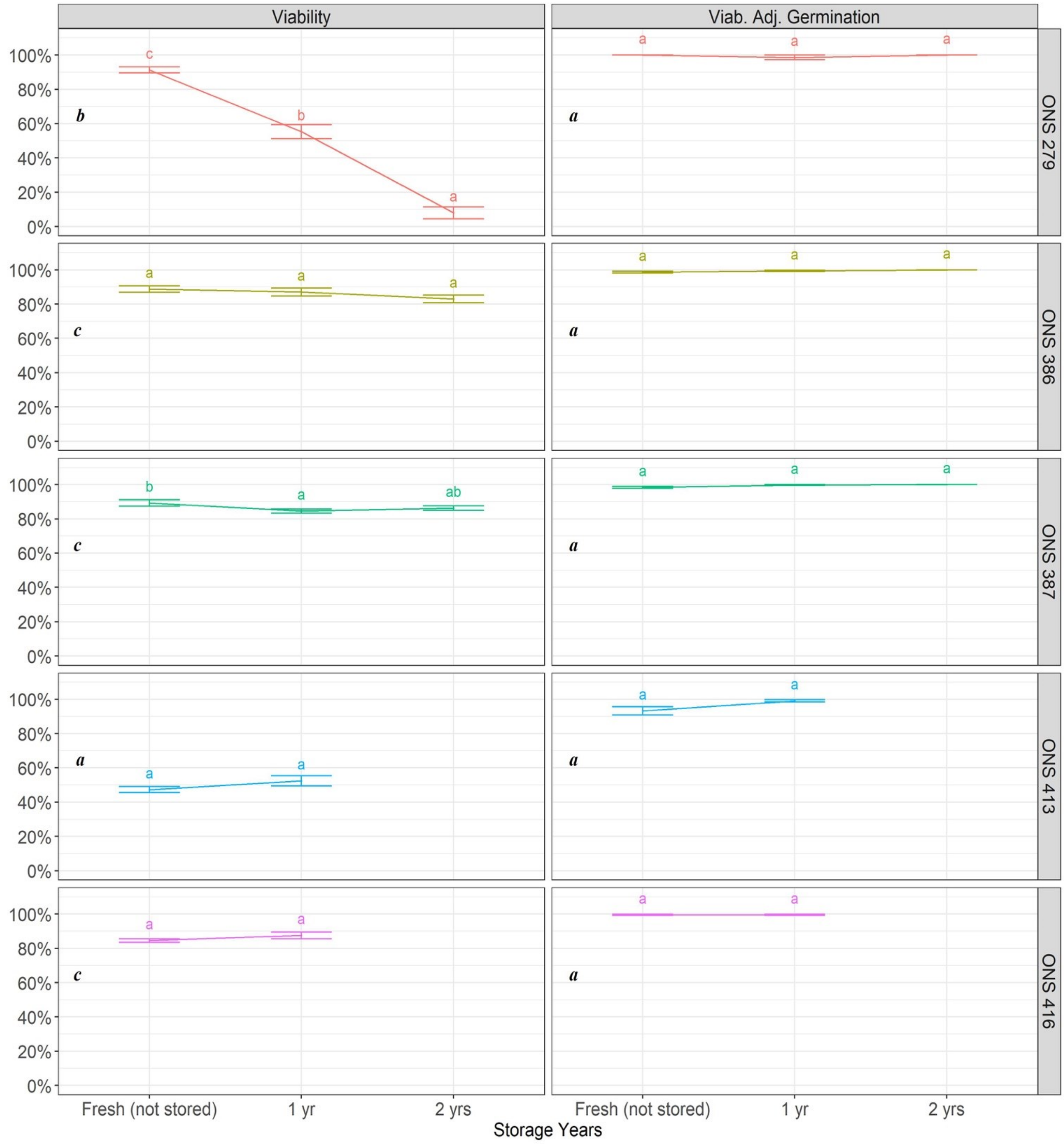

Figure 2. Effect of Storage on Viability (post germination) and Germination (viability adjusted). ± St. Error. Dissimilar letters indicate significant differences (P< 0.05) between collections and storage length.

Table 3. Effect of storage on viability (post germination) and germination (viability adjusted) for individual collections ± St. Error. Dissimilar letters indicate significant differences (P< 0.05) between storage lengths.

Collection Number	Germination Test Treatment	Viability % (± St. Error)	Viab. Adj. Germination % (± St. Error)
ONS 279	Fresh Seed	91.33 (±1.76) c	100.00 (±0.00) a
	Stored 1 yr	55.33 (±4.06) b	98.61 (±1.39) a
	Stored 2 yrs	8.00 (±3.46) a	100.00 (±0.00) a
ONS 386	Fresh Seed	88.75 (±1.92) a	98.62 (±0.58) a
	Stored 1 yr	87.00 (±2.30) a	99.39 (±0.40) a
	Stored 2 yrs	83.00 (±2.30) a	100.00 (±0.00) a
ONS 387	Fresh Seed	89.25 (±1.81) b	98.29 (±0.56) a
	Stored 1 yr	84.50 (±1.18) a	99.71 (±0.29) a
	Stored 2 yrs	86.25 (±1.28) ab	100.00 (±0.00) a
ONS 413	Fresh Seed	47.25 (±1.77) a	93.29 (±2.44) a
	Stored 1 yr	52.50 (±2.97) a	99.09 (±0.60) a
ONS 416	Fresh Seed	84.50 (±1.05) a	99.44 (±0.37) a
	Stored 1 yr	87.50 (±1.95) a	99.46 (±0.36) a

4. Discussion

The initial seed fill, viability and germination was found to vary significantly between individual collections. This is likely due to environmental conditions experienced by the parent plants (e.g. water supply, temperature, mineral nutrition and light) during seed development (Waller *et al.,* 1983; Mayer and Poljakoff-Mayber 1989; Wotton, 1993). The collections with greatest seed fill and viability (ONS 279, ONS 386 and ONS 387) were collected in the years with greatest rainfall. The latter collections were collected during drought years (2018-2019).

Regardless of the initial viability, four of the five *M. pyramidata* collections were found capable of storage in the current Ginkgo Seed Store for at least 1-2 years. This is contrary to the initial findings for this species, with the 2014 collection (ONS 279) aging rapidly, with few seed remaining viable after 2 years in storage. This also contradicts the findings of a study by Duncan *et al.* (2019a, 2019b), where *M. pyramidata* seeds were found to decline by >50% within 12 months of storage at 20^{o}C. The current results are, however, in line with other Bluebush species being currently studied, with small declines in viability during storage but overall capable of storage for at least 3 years (Quarmby, 2019).

A possible cause for the difference in results between the ONS 279, Duncan *et al.* (2019a, 2019b) results, and all other collections is the different age of the seeds tested. ONS 279 was collected in Spring 2014 (August-September) and were 7-8 months old when initially tested in April 2015. Subsequent re-tests were every 12 months. The seeds used in the study by Duncan *et al.* (2019a, 2019b), collected in September 2015, and were also 8 months old when tested. All other collections tested were initially tested within 3 months of collecting, with tests conducted in October or November of each year. If the age difference alone was the main cause for differing results, it would have been expected that ONS 386 and ONS 387 should have shown significant declines after 2 years of storage.

Another potential cause for the differing results is seed maturity. Seed maturity at the time of collection has been known to affect storage longevity of seeds (Merritt, 2006). The high proportion of immature seeds present in ONS 279 (47% of all healthy seeds) may have therefore attributed to the rapid decline for this collection, with most other collections having approximately a third of healthy seed considered immature. However, ONS 387 showed no decline in viability after 2 years of storage even though 43% of the seeds were considered immature. As 45% of the healthy ONS 435 seeds were also considered immature (collected 2019), the future results for this collection may assist in determining if seed maturity has attributed to the rapid decline for ONS 279. It should be noted that immature seeds are difficult to avoid during collection of this species due to progressive ripening on the plant, however, all of these collections were harvested on the point of dispersal (fruits falling from the plants), indicating that they were ready to collect (Zwiersen, pers. comm., Quarmby, pers. obs.). In some instances it can be necessary to collect seeds prior to peak maturity. This can occur when adverse weather conditions (winds and/or rain) have been forecast, with a risk of maturing seed being knocked off the plants.

The environmental conditions experienced by the parent plant (e.g. water supply, temperature, mineral nutrition and light) can also affect seed longevity, with varying fruiting events resulting in varying seed longevity (Waller *et al.,* 1983, Mayer and Poljakoff-Mayber 1989, Woller, 1993). Collections tested were from disjunct locations and harvested across varying years, with differing rainfall and temperatures during development. This highlights the need to replicate tests using collections from varying collection years as well as locations.

It is also possible that the current storage conditions may have impacted the shelf-life of individual batches and species. Temperature and relative humidity (RH) are considered as two of the most important factors in maintaining seed quality during storage, with cool, dry storage essential for maximising seed longevity (Delouche *et al*, 1973; Martyn *et al.,* 2009). This is emphasised by Harrington's "Rule of Thumb" where seed longevity is approximately doubled for every reduction in temperature of 10°C and seed moisture content of 1% (Martyn *et al.,* 2009). Wide fluctuations in temperature during storage can therefore significantly reduce seed longevity (Ralph, 2006). Ambient conditions are considered dry when RH is <50%, with seed aging occurring rapidly with higher humidity (Martyn *et al.,* 2009). Unfortunately, up until June 2019 the actual temperature and RH within the two seed stores had not been monitored. Data to date (Appendix 1, Figures 3 and 4) shows that temperatures and humidity were found to vary between the two seed stores. This may be due to the northern seed store having greater heat exposure and having to work harder to maintain temperatures, with the southern seed store partially shielded by the northern one and therefore working more efficiently. Regardless, during warmer months both seed stores have maintained a temperature of 18°C or 20°C (depending on the store), however, during cooler months there have been daily fluctuations of approximately 4°C, with an overall range of approximately 12°C (20°C and 8°C), depending on external conditions. This is due to the fact that the air conditioner units are set to cool, therefore, if the thermostat has been sensing that the temperature is above 20°C it will cool the seed store to 20°C. Due to the cool setting, heating has not occurred when temperatures have been below 20°C. During heat waves the air-conditioners have also struggled to maintain a maximum of 20°C, with temperatures reaching in excess of 30°C in some instances. The humidity has ranged mostly between 50-60% RH during cooler months, with some spikes to near 70% RH. During warmer months humidity has ranged mostly between 30-45%.

It is therefore possible that the current Tronox seed store conditions may have reduced the potential shelf-life of individual batches or species. As such it is recommended that at a minimum, air-conditioners should be upgraded so that a constant temperature is maintained year round. If possible, the RH should also be controlled and maintained at less than 15%. When further facilities are commissioned (due to increased seed volume requirements), it is recommended that they should be capable of a temperature of approximately 5°C and less than 15% RH. This should increase the shelf-life of species with short longevities (such as *M. sedifolia*), and allow storage of other species on a medium term basis (5-10 years), especially targeting species that seed infrequently.

Finally it should be mentioned that the data for the ONS 279 collection is also considered less reliable than the data presented for all other collections. This is due to the difference in number of replicates tested, with three replicates of 50 seed for ONS 279 and 8 replicates of 50 seed for all other collections. It should also be noted that the study by Duncan *et al.* (2019a, 2019b) used 4 replicates of 25 seed. A lack in replicates may not have enabled a true representation of overall seed traits, evident in ONS 279, where there was the supposed changes in the proportion of undeveloped seed (shrivelled seed or empty fruit) after storage (compared to initial (pre-germination) viability results (Figure 3 and 4)). While seed ageing can occur with time, seed development (seed fill) is at its maximum potential prior to dispersal (harvesting). It should be acknowledged, however, that differences in the proportion of seed in the categories between Figures 3 and 4 (across all collections) were also due in part to difficulty in differentiating between dead, shrivelled and empty seeds, especially when seeds had been dead for an increased length of time. Although this has occurred as seeds in all three categories are ultimately considered non-viable the accuracy of differentiation between these categories is not deemed critical.

Despite the above it appears that the optimal storage length for *M. pyramidata* can be increased to at least 1-2 years, rather than the previous recommendation of storage of less than 12 months. This study should be continued in order to further clarify seed storage longevity and corresponding collection regimes for this species. Given the variation in results for *M. pyramidata* it is imperative that additional collections of *M. sedifolia* (Pearl Bluebush) are added to the study as seed becomes available. As *M. sedifolia* seeds infrequently the potential lack of storage capability may create a ‘bottleneck’ in being able to return this species to the rehabilitated landscape. It is also recommended that further collections are added to the current investigation on other Bluebush species, given the importance of testing multiple collections before ascertaining the overall storage behaviour of individual species. It is also recommended that monitoring occurs to ascertain changes in shelf-life, following any upgrades to the Tronox seed storage conditions.

5. Summary findings and recommendations

A summary of the current findings and current recommendations are provided below:

- ONS 279 was the only *M. pyramidata* collection to show a rapid decline in viability during storage, with all other collections tested capable of storage in the Ginkgo Seed Store for at least 1-2 years. It is noted, however, that Duncan *et al.* (2019a, 2019b), also reported a decline of greater than 50% within 12 months of storage at 20^{o}C for another collection of this species.

- Single collections of four other Bluebush species have also been shown to be capable of storage in the Ginkgo Seed Store for at least 3 years. However, as this study has shown variation between individual collections, it is recommended further collections of these species are added to the trial as seed becomes available.

- The ONS 279 results may have been affected by increased seed age, with seeds potentially 3 months older (or more) than other collections at each test interval. The results for this collection are also less reliable than all other collections due to the low number of replicates that were tested. These factors may have also attributed to the results by Duncan *et al.* (2019a, 2019b), with tests conducted on seeds with a similar age to ONS 279 (8 months old), and with low numbers of replicates and seed tested (4 replicates of 25 seeds).

- Variation in the seed longevity may also be attributed to varying seed maturity as well as environmental factors experienced by the parent plants during seed development. Both of these factors are difficult to control and will continue to vary with individual collections.

- The current Tronox seed store conditions may have reduced the potential shelf-life of individual batches or species. It is expected that improvements to the temperature and humidity would increase the shelf life of all species held within the seeds stores. As such it is recommended that at a minimum, the current air-conditioners should be upgraded so that a constant temperature is maintained year round. If possible, the RH should also be controlled and maintained at less than 15%. When further facilities are commissioned (due to increased seed volume requirements), it is recommended that they should be capable of a temperature of approximately 5°C and less than 15% RH. Improved conditions will be of particular benefit to species with short longevities under the current conditions (such as *M. sedifolia*), and allow storage of other species on a medium term basis (5-10 years), especially for species that seed infrequently.

- Current *M. pyramidata* collections should be continued to be monitored for at least 1-2 years under the current storage conditions in order to gain a better understanding of the average expected shelf-life for these species. Given the variation in results for *M. pyramidata*, additional collections of *M. sedifolia* should be added to the study (when seed becomes available) to verify existing results, as the potential lack of storage capability under the current conditions may create a 'bottleneck' in being able to return this species to the rehabilitated landscape.

- It is also recommended that, following any upgrades to the Tronox seed storage conditions, the storage performance of the above species (plus others) are monitored to ascertain changes in shelf-life. As storage space within each seed store is limited, investigations will also determine priority species that benefit the most from storage under the premium conditions (e.g. species currently with the shortest seed longevities).

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Appendix 1

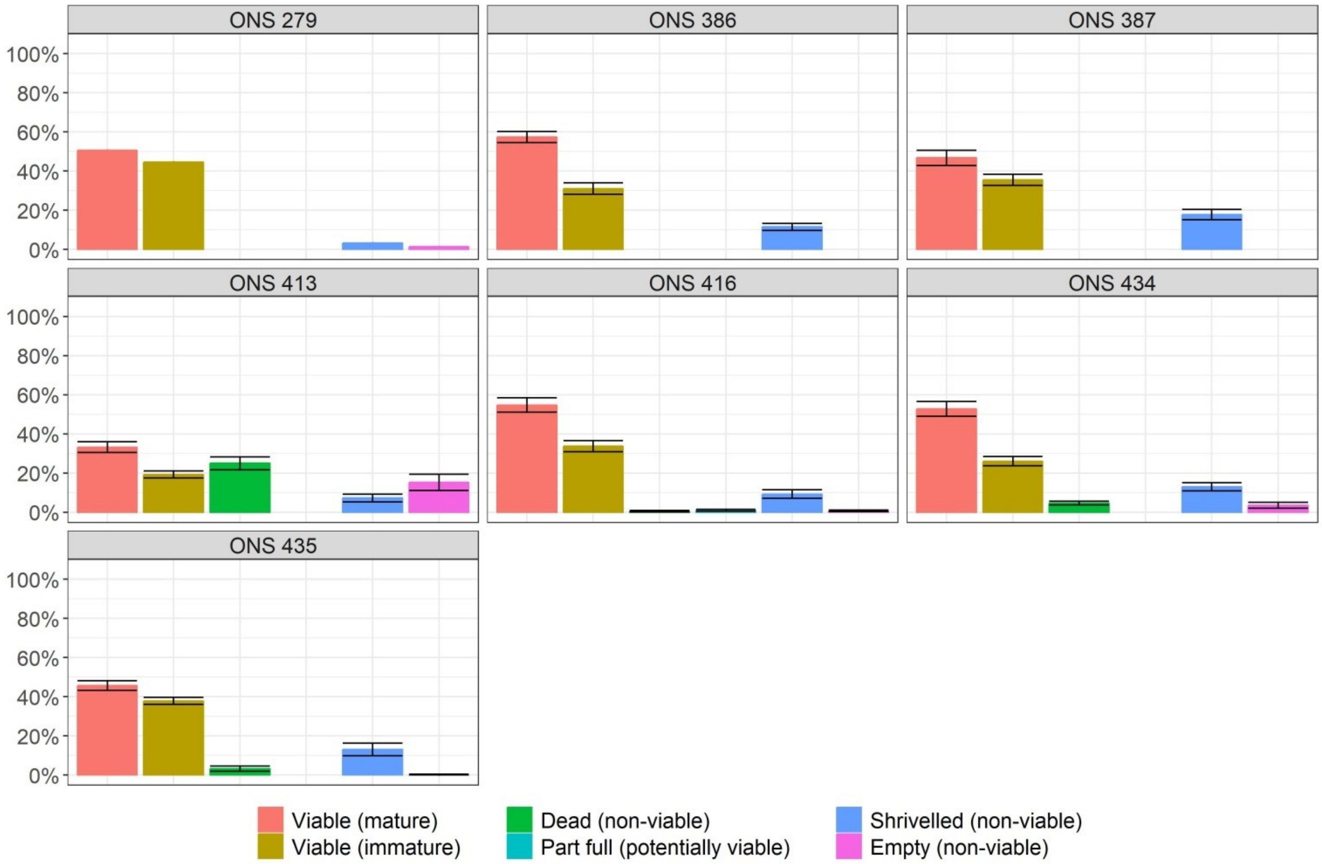

Figure 1. Pre-Germination Viability Test Categories ± St. Error

Figure 2. Post Germination Viability Test Categories

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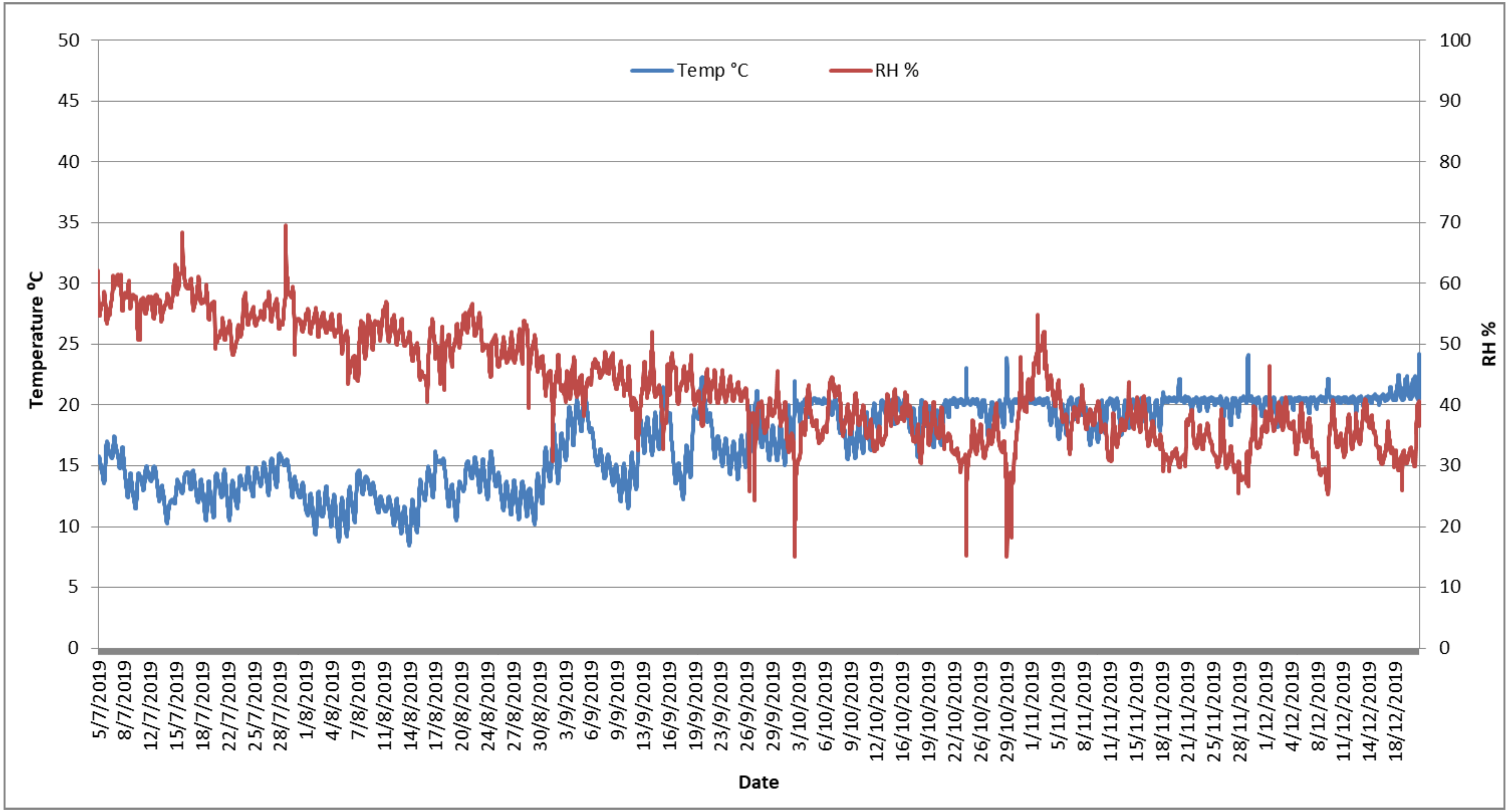

Figure 3. Temperature and Relative Humidity of Tronox Seed Store 1 July –Dec 2019 (southern seed store)

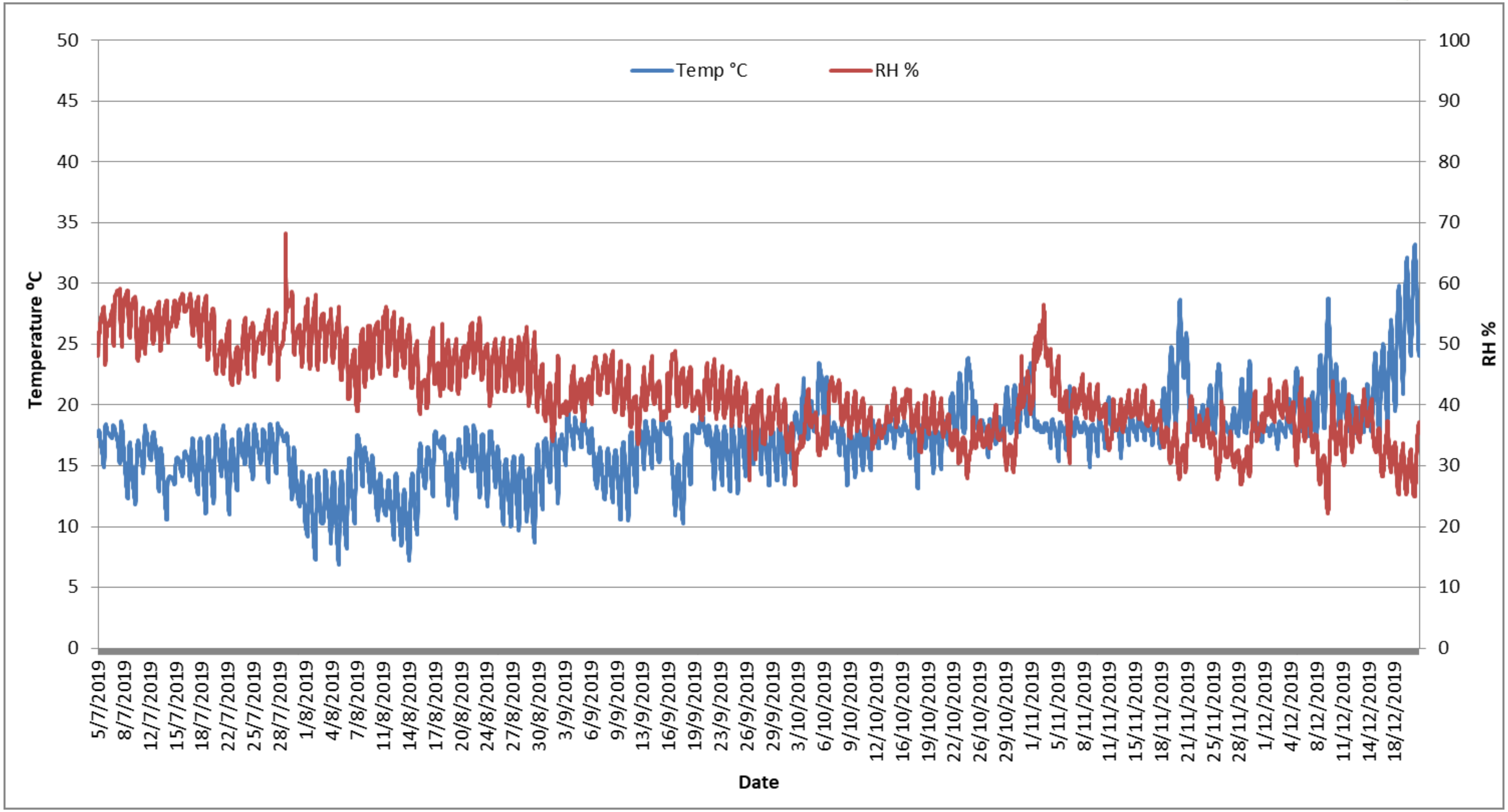

Figure 4. Temperature and Relative Humidity of Tronox Seed Store 2 July –Dec 2019 2019 (northern seed store)