A COMPARISON OF PRE-SOAKING AND PRE-WASHING AS METHODS OF INCREASING BEET (*BETA VULGARIS*) AND NEW ZEALAND SPINACH (*TETRAGONIA TETRAGONIOIDES)* SEED GERMINATION

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ABSTRACT

Water soluble germination inhibitors located in the pericarp of *Beta vulgaris* fruits have historically been reported to be one of the causes of seed dormancy in fruit of this species. Dormancy can lead to low and non-uniform germination, which is a problem in the seed production system. Dormancy-breaking treatments that have been applied to *B. vulgaris* fruit include steeping, priming, rubbing the fruit coat or scarification, and washing and soaking the fruits prior to sowing as a way of leaching out the inhibitors. Anecdotal evidence suggests that soaking the fruits is as effective as the washing dormancy-breaking treatment recommended by the International Seed Testing Association (ISTA).

In this study, the presence or absence of dormancy in 18 *B. vulgaris* (11 vegetable beet, 4 fodder beet, and 3 sugar beet) seed lots was assessed by conducting germination tests on fruits in the absence of dormancy-breaking treatments. There was little or no dormancy in the seed lots tested. Six seed lots had over 90% germination, another six seed lots had more than 80% germination. Four seed lots had germination ranging from 76 to 79% while two seed lots had germination lower than 67%. These low germination percentages were due to empty fruits rather than dormancy. This suggests that dormancy may no longer be a problem in modern *B. vulgaris* cultivars.

Two *B. vulgaris* seed lots were identified as having some residual dormancy as indicated by a high percentage of ungerminated viable fruits. These were Flores sugar beet and an "unnamed" (Kings Seeds) sugar beet cultivar. These had 14% and 16% ungerminated viable fruits respectively. Two other seed lots identified had less than 90% germination, Brigadier and Kyros fodder beets, and had high percentages of ungerminated viable fruits (5%) compared with other seed lots in the same germination range. Dormancy-breaking treatments were conducted on the four seed lots. The dormancy-breaking treatments were i) fruits washed in running water at 25 ˚C for two hours, and ii) fruits soaked in 25 ˚C water for two hours, changing the water every 30 minutes. The aim of this experiment was to compare the effectiveness of the two treatments in removing residual dormancy by leaching inhibitors from the fruit coat. The effectiveness of these dormancy-breaking treatments was determined by measuring the germination percentage, time to 50% germination and the uniformity of germination. No significant differences were found between the germination of soaked or washed fruits compared with the control i.e. 84%, 89%, and 89% for Flores sugar beet, 77%,

80%, and 71% for the Kings Seeds sugar beet, 75%, 80%, and 73% for Brigadier fodder beet, and 93%, 91%, and 91% for Kyros fodder beet for control, soaking and washing treatments respectively. This suggested that water soluble germination inhibitors are absent from the fruit coats as the water treatments did not alleviate the residual dormancy. There were no significant differences in the time to 50% germination (T_{50}) of the seed lots following the treatments, except in Flores sugar beet where the soaking slowed down germination (49.9, 72.26, and 71.17 hours for the control, soaking, and washing treatments respectively).

Tetragonia tetragonioides was the only other species in the ISTA Rules for which the two hour washing treatment was recommended as a dormancy-breaking technique. The same dormancy-breaking treatments were applied to fruits of a *T. tetragonioides* seed lot. There were no significant differences in germination for the control, washing and soaking treatments (72%, 63%, and 70% respectively). The high percentages of ungerminated viable fruits of 26%, 32%, and 28% remaining for untreated, soaked, and washed fruits respectively indicated high levels of dormancy and inability of the dormancy-breaking treatments to alleviate this. A follow up experiment following an ISTA Rules change in 2011 that changed the two hour washing treatment to soaking the fruits for 24 hours was conducted to assess the effectiveness of the new 24 hour soaking recommendation in alleviating dormancy. The 24 hour soaking treatment gave a significantly lower germination (40%) and high percentage of ungerminated fruits (60%) compared with the control, two hour soaking, and two hour washing treatments. Cutting the endocarp of ungerminated fruits at the end of the 35 days germination period alleviated the dormancy in all remaining fresh viable fruits. The study also suggests that *T. tetragonioides* germination is inhibited by the presence of excess water past the early stages of imbibition, but further work is required to confirm this.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. *Beta vulgaris*

Beta vulgaris L. includes economically important cultivated crops like fodder beet (also known as mangel) which is used as a cattle feed, table beets and leaf beets (e.g. Swiss chard) which are used as vegetables, and sugar beet which is used as a source of sucrose (Henry, 2010).

B. vulgaris fruits have however been historically reported as possessing coat imposed dormancy. A number of factors may be involved i.e. physical restriction of radicle protrusion and restriction of gas exchange between the embryo and the environment by the operculum, the presence of a mucilaginous layer that also prevents access of oxygen to the embryo, and the presence of germination inhibitors in the pericarp (Heydecker, Chetram, and Heydecker, 1971; Taylor, *et al.*, 2003). This dormancy has caused germination problems both in the laboratory and in the field (Habib, 2010; Tekrony and Hardin, 1968).

In order to leach out the water soluble inhibitors from the pericarp to obtain full germination potential, the International Seed Testing Association (ISTA) Rules (2010) recommends that *B. vulgaris* fruit should be washed in running water at 25 ˚C for two and four hours for multigerm and monogerm fruits respectively and dried back for two hours prior to sowing. In comparison, New Zealand seed laboratories have reported success with pre-soaking *B. vulgaris* fruit in water at 25 ˚C for two hours, changing the water every half hour then leaving the fruit to dry for two hours before sowing the fruit onto the germination substrate (Diane Bell, 2010, pers. comm.)

However it has been reported that in recent years there has been no dormancy in *B. vulgaris* seed lots (Diane Bell, 2010, pers. comm.) and that rapid and synchronous germination has been selected for in cultivated *B. vulgaris* which has led to a total dormancy elimination (Wagmann, Hautekeete, Piquot, and Van Dijk, 2010).

The objectives of this research were therefore to:

1) assess the presence of dormancy in *B. vulgaris* seed lots and if present, to assess the level of the dormancy.

2) compare the two dormancy-breaking methods (pre-soaking and pre-washing) to determine whether pre-soaking is as effective as pre-washing.

1.2. *Tetragonia tetragonioides*

New Zealand spinach (*Tetragonia tetragonioides* (Pall.) Kuntze) is a leafy green wild plant endemic to Australasia. It grows along New Zealand's North and South Islands' coast in localized pockets and is used as a vegetable. It is considered New Zealand's only indigenous vegetable (Roskruge, 2009).

The germination of *T. tetragonioides* fruits however is slow and erratic due to their hard fruit coverings (Grubben, 2004). The ISTA Rules (2010) specifies that fruits of *T. tetragonioides* should be washed for two hours as for *Beta* sp. or have the pulp surrounding the fruit removed prior to sowing in order to alleviate the dormancy. However, in 2011 the washing specification was changed to soaking the fruits for 24 hours (ISTA, 2011).

The objective of this study was to:

1) compare the effectiveness of soaking and washing for two hours in breaking dormancy in *T. tetragonioides* fruits
2) compare the dormancy-breaking treatments in 1) with the new 24 hour pre-sowing treatment together with the assessment of the effect of cutting (depulping) the fruits on breaking dormancy.

CHAPTER 2

LITERATURE REVIEW

BETA VULGARIS

2.1. Introduction

The species *B. vulgaris* contains a range of beets including sugar beet, vegetable beets and fodder beets. All are commercially important. The vegetable beets include garden red or table beets (beetroot) which are cultivated for their edible swollen taproots, and also spinach beets and Swiss chard which are grown for their leaves. Fodder beets or mangels are cultivated for animal feed as a forage crop. Sugar beet is cultivated for its sugar and contributes about 30% of the 125 million tonnes annual total world consumption of raw sugar (Jaggard and Halmer, 2006).

The successful production of any crop depends on seedling emergence and stand establishment, and in *B. vulgaris*, emergence both in the field and under laboratory conditions has been a problem. In the field, monogerm sugar beet varieties were reported to have poor germination resulting in irregular stands (Tekrony and Hardin, 1968). Habib (2010) also reported that a major limiting factor for satisfactory stand establishment of sugar beet is the emergence of seedlings, and even under ideal environmental conditions, table beet seed lots have non-synchronised germination (Taylor, *et al.*, 2003). Tekrony and Hardin (1968) reported that in laboratory germination tests of sugar beet fruits, obtaining full germination potential has been a constant problem. For example, fruits of X/90, a sugar beet variety, reached only 25% germination under laboratory conditions after seven day's incubation (Coumans, Come, and Gaspar, 1976). Habib (2010) reported that among other factors affecting the germination of sugar beet i.e. fruit hardness and water and oxygen impermeability (Hadi, 2001), and lack of adequate food reserves due to fruit immaturity and underdevelopment (Hadi, 2001; Habib, 2010), is the presence of chemical inhibitors in the pericarp tissue. Evenari (1949) described germination inhibitors as substances that plants produce, which delay or inhibit the germination of the same or other species. These germination inhibitors are reported to be produced by all types of *B. vulgaris* (Evenari, 1949). Inoue and Yamamoto (1975) and Stout and Tolman (1941), demonstrated that the water leached from sugar beet fruits inhibited seed germination in at least 29 other species. For example, common corncockle *(Agrostemma githago)* seeds cannot germinate in *B. vulgaris*

fields because *B. vulgaris* excretes a substance which inhibits their germination (Evanari, 1949). Although most of the research done on germination inhibitors in *B. vulgaris* has mainly focused on sugar beet, the problem is common across all of the beets (i.e. vegetable beets and fodder beets). Chiji, Tanaka, and Izawa (1980), reported that germination of seeds is inhibited by water extracts from fruit clusters and leaves of sugar, vegetable and fodder beets. Silva, Vieira, and Cecilio Filho (2005) also reported that reduced crop stand in beetroot can be a result of the presence of germination inhibitors in the fruits (although the extent of the role of germination inhibitors was not discussed), while a study by Juntilla (1976) found that methanol and water extracts of beetroot fruits (*B. vulgaris* subsp. *vulgaris*) contained germination inhibitors. The methanol extracts inhibited germination of lettuce seeds but did not affect the germination of red beet fruits, while the water extracts inhibited the germination of both red beet fruit clusters and lettuce seeds.

Dormancy is also a problem in testing fruit germination of *B. vulgaris* (Tekrony and Hardin, 1968). This is because dormancy may not allow seed analysts to measure the full germination potential of a seed lot (Tekrony and Hardin, 1968).

The International Seed Testing Association (ISTA) has recommended dormancy-breaking procedures for *B. vulgaris* fruits. ISTA (2009) specifies that *B. vulgaris* fruits should be washed with running water at a temperature of 25 ˚C for two hours (multigerm fruits) and four hours (monogerm fruits) and dried back for two hours prior to sowing.

2.2. The *B. vulgaris* fruit

2.2.1. Multigerm and monogerm *B. vulgaris* fruit

Byford (1963) described sugar beet fruit as consisting of several dry fruits forming a cluster as a result of fusion of flower parts. Because of this phenomenon, unprocessed or natural sugar beet fruit may contain a number of true seeds, most often two or three (Byford, 1963), but not uncommonly four or five (Byford, 1963; Jaggard and Halmer, 2006). These fruits are therefore known as “multigerm”, i.e. more than one seedling may emerge from each fruit (Figure 1). Other *B. vulgaris* fruit are called “monogerm fruits”. Coumans, *et al*. (1976) described a monogerm sugar beet fruit as a one seeded fruit consisting of a single seed

surrounded by a fruit wall. Varieties that have been bred to have monogerm fruits are referred to as having "genetic monogerm" fruits (McGrath, 2010; Savitsky, 1952).

Figure 1. A multigerm *B. vulgaris* fruit cluster (external features showing two fruits with opercula)

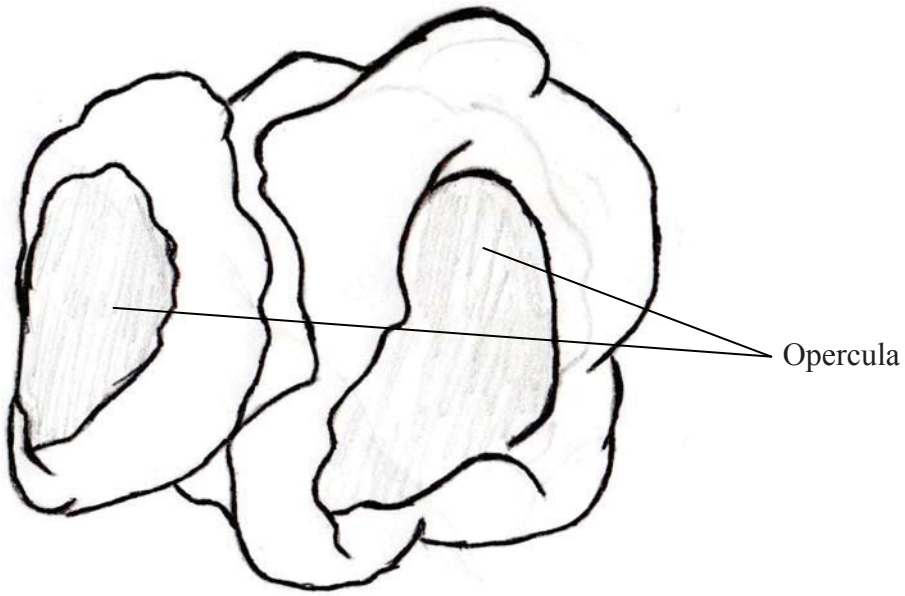
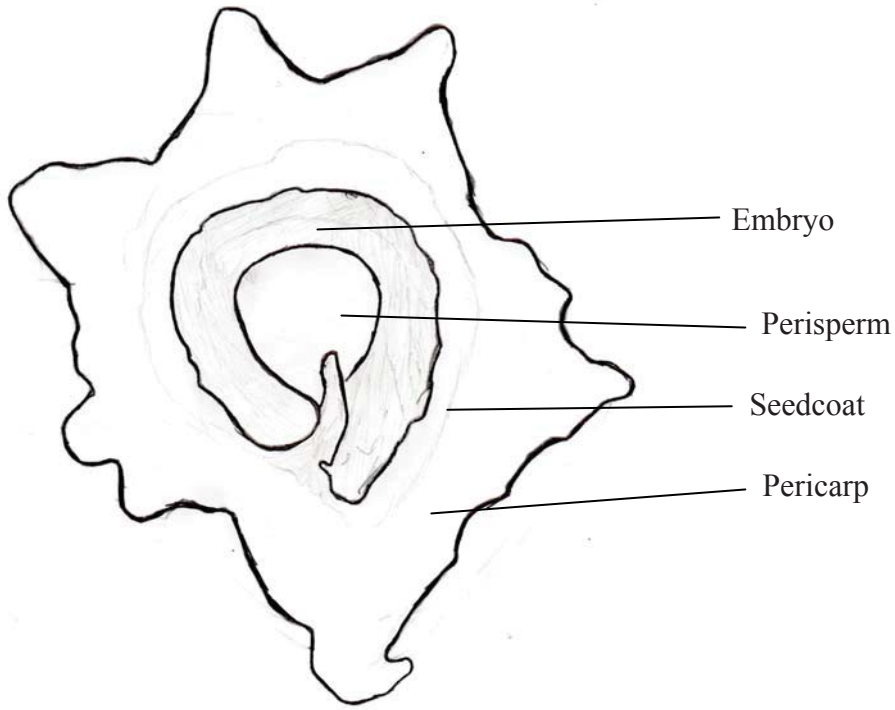


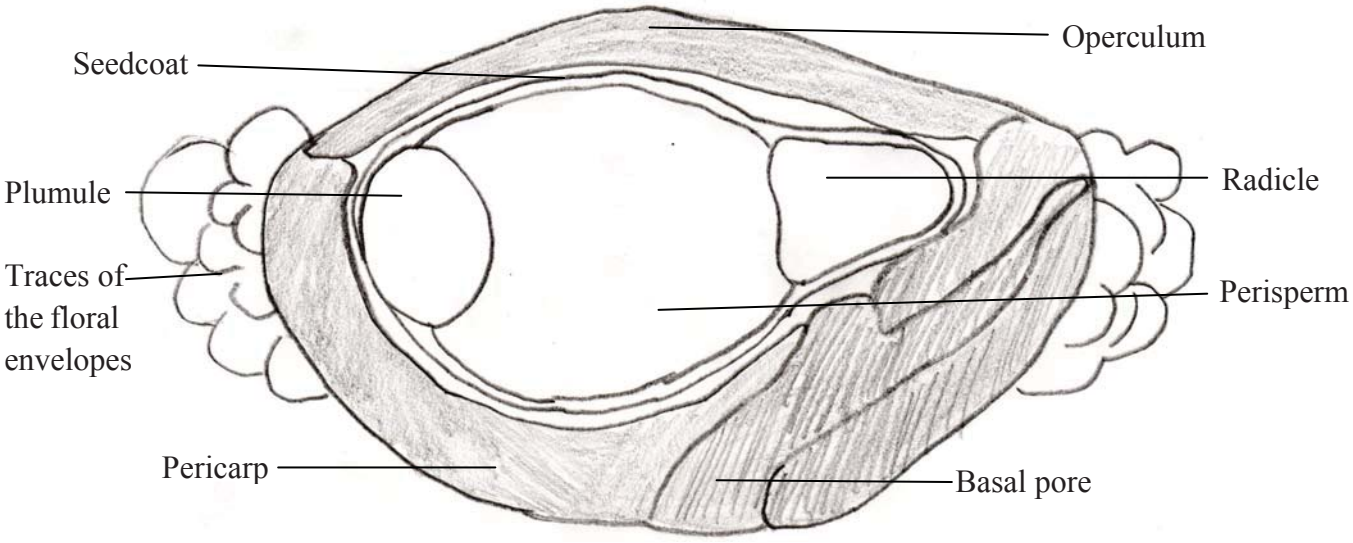
Figure 2. Internal features of a *B. vulgaris* fruit showing one seed



2.2.2. Structure of the individual *B. vulgaris* fruit

The true seed in *B. vulgaris* lies within dead maternal perianth tissue (Perry and Harrison (1974). According to Hermann, *et al.,* (2007) and Orzeszko-Rywka and Podlaski (2003) the perianth tissue (pericarp) in the sugar beet fruit is made up of dense layers of sclerenchyma cells. The upper part of the pericarp contains an ovary cap, which is termed the operculum (Figure 1). The bottom part of the pericarp (Figure 2) has a basal pore (Figure 3), this is essentially a pole like pericarp structure consisting of loose cells. Both the operculum and the basal pore have been proposed as major pathways for water and oxygen to access the germinating embryo in *B. vulgaris* fruit (Hermann, *et al.*, 2007).

Figure 3. Structure of a monogerm *B. vulgaris* fruit in longitudinal section



2.3. Seed dormancy in *B. vulgaris*

2.3.1. What is seed dormancy?

Seed dormancy is the failure of an intact viable seed to germinate under favourable environmental conditions (Finch-Savage and Leubner–Metzger, 2006). Foley (2001) pointed out that this failure to germinate in dormant seeds is only temporary.

The germination process starts with water uptake by the dry seed (imbibition). This leads to rehydration of the seed tissue and the expansion of the embryo. As water uptake progresses, germination metabolism begins, leading to the axis of the embryo elongating and breaking through the pericarp. This represents the completion of germination in the strictest sense (*sensu stricto*) (Debeaujon, Leon–Kloosterziel and Koornneef, 2000; Finch-Savage and Leubner–Metzger, 2006). The seed requires water, oxygen and appropriate temperature in order to germinate. In dormant seeds, however, the process of germination does not occur despite the presence of the three requirements. Dormant seeds may require other factors such as light and/or nitrate to be available before germination can proceed (Finch-Savage and Leubner-Metzger, 2006).

2.3.2. Types of seed dormancy

Kelly, Van Staden, and Bell (1992) categorised dormancy into two types: embryo imposed dormancy and coat imposed dormancy.

2.3.2.1. Coat imposed dormancy

The tissues surrounding the embryo, in particular the seed and/or fruit coats, can impose dormancy in a number of ways. The seed or fruit coat may be impermeable to water and/or gases, thereby preventing rehydration of the seed and/or respiration. The seed or fruit coat may also physically constrain embryo growth thereby preventing radicle emergence. The pericarp may also prevent germination inhibitors from leaving the embryo, or when located in the pericarp or surrounding tissues, be a source of compounds that inhibit germination of the embryo (Kelly, *et al.*, 1992). Hadi (2001) suggested that dormancy found in *B. vulgaris* is coat imposed dormancy.

2.3.2.1.1. Pericarp impermeability

Rolston (1978) stated that even in the presence of favourable germination conditions, viable seeds will not always imbibe water thus leading to germination failure. Seeds that fail to imbibe remain hard and are therefore commonly called impermeable or hard seeds, in contrast with imbibed seed which soften during germination. According to Thanos, Georghiou, Kadis, and Pantazi (1992), hard-seededness is a specific type of seed dormancy which is widespread among flowering plants, especially in the Fabaceae, Malvaceae, Cannaceae and Geraniaceae families (Meisert, Schulz and Lehmann, 1999) and is caused by the tissues surrounding the embryo, which can include the pericarp, preventing the seed from taking up water.

Thanos, *et al.* (1992) stated that the water impermeability of the pericarp can be due to the structure of the pericarp. Here the presence of one or more water impermeable layers of palisade cells in the pericarp (Baskin and Baskin, 2004; Rolston, 1978; Thanos, *et al.*, 1992), results in pericarp impermeability (Baskin and Baskin, 2004). These impermeable palisade layers are made out of typically non-living sclerid cells with thick lignified secondary walls (Baskin and Baskin, 2004; Geneve, 2010; Rolston, 1978). Deposits of water repellent materials are made within and on the macrosclerid cells surface during the later stages of seed development thus sealing the seed and making it water impermeable (Geneve, 2010).

Hard fruits have been reported in *B. vulgaris*. Stewart (1950) found that apart from producing fruits of poor quality, hard-seeded species of *Beta* spp. often have empty pericarps or contain non-viable seeds or seeds that are not fully developed. A study by Stewart (1950), identified fruits of *Beta procumbens*, *Beta patellar, Beta webianna* and *Beta lomatogona* and the small fruit clusters of *Beta trigyna* as falling into this category and demonstrates that the trait of poor fruit germination is common across fruits of the genus *Beta*.

2.3.2.1.2. The pericarp as physical constraint of radicle protrusion

According to Adkins, Belairs and Loch (2002), the process of embryo expansion and emergence of the radicle in some seeds is restricted by the pericarp which consists of toughened tissues that prevent both germination and growth.

For these seeds to germinate, mechanical weakening of the tissues restraining the embryo needs to occur over time. The influence of the embryo or physical parameters such as temperature fluctuations may produce enzymes which may help weaken the pericarp.

Saprophytic fungal action, ingestion of the fruits by animals, and heat and smoke from fire may also weaken the pericarp (Adkins, *et al.*, 2002).

2.3.2.1.3. Germination inhibitors in the pericarp

According to Adkins, *et al.* (2002), several examples exist where isolation of chemical inhibitors from various outer pericarp tissues of seeds has been reported and the most frequently isolated chemical inhibitors are abscisic acid and coumarin.

Adkins, *et al.* (2002) also reported that when seeds with pericarps that contain germination inhibitors are exposed to prolonged rainfall, it may result in the leaching out of the inhibitors and may also cause pericarp decay which sometimes results in the loss of this type of dormancy. Physical features of the environment like fluctuations of temperature or wetting and drying of the seed may also alter the nature of the chemicals in the pericarp (Adkins, *et al.*, 2002)

Hadi (2001) reported that sugar beet fruit germination and establishment can be influenced by chemical inhibitors in the pericarp, and water and oxygen impermeability of the fruit coat.

2.3.2.2. Embryo imposed dormancy

In some species germination is not improved after removal of the pericarp or other tissues surrounding the embryo. This observation indicates that there is a dormancy mechanism within the embryo itself and is called embryo-imposed dormancy (Adkins, *et al.*, 2002; Kelly, *et al.*, 1992). In this type of dormancy, changes in the balance of germination promoters and inhibitors within the embryo may both cause the dormancy and also break the dormancy (Adkins, *et al.*, 2002). Another possible embryo dormancy mechanism is the inadequate metabolic control of the mobilisation of seed food reserves, whereby the germination process does not proceed because food reserves are unable to be accessed by the dormant embryo. Another factor that causes embryo dormancy is the immaturity of embryos i.e. seeds are under developed at the time of shedding, or seeds are at an earlier stage of development (Adkins, *et al.*, 2002).

Before germination can proceed, hormonal, temperature, and/or light treatment requirements must be met (Kelly, *et al.*, 1992). These changes or conditions may be satisfied during a period of after ripening once the seed has reached maturity (Kelly, *et al.*, 1992). After

ripening is the loss of dormancy in seed of many species after storage under dry and warm conditions (Amen, 1966; Kelly, *et al.*, 1992).

2.3.3. Importance of dormancy

The distribution of germination over time as well as the dispersal of the seeds in space is increased by seed dormancy. This encourages the survival of a plant species by establishing new populations in different environments (Foley, 2001). Moreover, physically dormant seeds are dry until dormancy is broken, this helps them remain viable for long periods of time (Geneve, 2010).

2.3.4. Problems caused by seed dormancy

Snyder (1963) explained that more timely cultural operations and greater yields may result from rapid and uniform germination and these traits are lacking in commercial varieties of sugar beets in varying degrees as a result of their dormancy. However, this issue of dormancy may not be as important now and this is discussed in detail in 2.5.2. Because of the importance of adequate stand establishment and difficulty in achieving this with seed or fruit of some species, scientists have developed laboratory tests in order to predict field establishment of seeds even in the presence of seed dormancy (Campbell and Enz, 1991). These tests involve breaking seed dormancy so the prediction of field establishment of the seed lot in the laboratory will be based on its actual germination potential.

2.3.5. Role of germination inhibitors in *B. vulgaris* fruits

2.3.5.1. Where are the inhibitors in the fruit?

The inhibitors in sugar beet fruit appear to be mainly located in the fruit coat. This was demonstrated in a study where 93% germination was reached with naked fruits, where the fruit coat was removed from dormant sugar beet fruits. In contrast, scarified fruits where only the operculum was removed, gave 73% germination and intact fruits only 25% germination (Coumans, *et al.*, 1976). The high germination observed when the whole fruit coat was removed suggests that there are inhibitors in the pericarp; the high germination with partial removal of the pericarp (operculum) suggests that the pericarp may also act as a barrier to oxygen essential for germination. The oxygen factor is discussed in 2.3.8 and 2.3.8.1. Regardless, dormancy in sugar beet fruit is non embryonic (Coumans, *et al.*, 1976). In another experiment by Abu-Shakra and Aquil (1968) water extracts of pericarp tissue extracted from crushed fruits of whole sugar beet and the true seed were tested to determine

their effect on germination of sugar beet seed extracted from the fruit, alfalfa and sorghum seed. The fresh weight of 10 radicles of each seed lot tested, after a germination period of 72 hours, was 20.71 mg, 19.39 mg, 2.35 mg, and 1.40 mg for sugar beet, 55.69 mg, 49.69 mg, 13.75 mg, and 10.69 mg for alfalfa, and 118.50 mg, 118.19 mg, 38.13 mg, and 33.75 mg for sorghum, for control (distilled water), true seed, pericarp tissue, and crushed fruit extracts. The results from the study again indicated an inhibitor located in the fruit coat imposes dormancy.

While in sugar beet the pericarp harbours the inhibitors it also appears to be the main location of germination inhibitors in the fruits of other *Beta* species and types although some variations exist. In a list of *Beta* species that produce germination inhibitors, Evenari (1949) reported that the inhibitor for *Beta saccharifera* is contained in the fruit coat, *B. vulgaris* (beetroot) contained inhibitors in the leaf sap and all parts of the fruit, and *B. vulgaris* (mangel and red beet) also have inhibitors in the leaf sap.

2.3.5.2. What are the inhibitors?

Although a number of compounds in *B. vulgaris* fruits have been identified as being responsible for inhibition of germination, a single principle inhibitor has not been identified. Evenari (1949) reported that the common inhibitors in *B. vulgaris* include oxalic acids, phenolic acids, vanillin, cis-4-cyclohexene-1, 2-dicarboximide and potassium nitrate. This was also reported by Hoover and Goodin (1966) who commented that the inhibitors found in *B. vulgaris* fruits may not be specific i.e. it is not one compound that is solely responsible for inhibiting germination.

The germination inhibitors in *B. vulgaris* appear to be mainly, if not totally, water soluble. Inoue and Yamamoto (1974) for example, found that when two day old sugar beet seedlings were exposed to water extracted from sugar beet fruits, root growth was inhibited and root tips turned black which suggested toxicity. The inhibitory compound was identified as mono-sodium oxalate, a compound previously reported to elicit this response in *B. vulgaris* by De Kock and Hunter (1950). Chiji, *et al.* (1980) reported that inhibitors were also found in water extracts of not only sugar beet, but also fodder and vegetable beets.

De Kock, Hunter and MacDonald (1953) identified another potential germination inhibitor as an unsaturated yellow oil that is found in sugar beet water extracts. The oil, in addition to inhibiting germination of sugar beet, also inhibited germination of other seeds, for example cress seeds (De Kock and Hunter, 1950). Mitchell and Tolbert (1968) identified cis-4-

cyclohexane-1,2-dicarboximide as one of several germination inhibitors from sugar beet fruits. This was found to inhibit lettuce seed germination but the authors did not report whether the compound inhibited the germination of sugar beet itself. Chiji, *et al.* (1980) isolated and identified 8 phenolic germination inhibitors from red beet (*B. vulgaris* L. subsp. *vulgaris*) fruit clusters. These were identified as aldehydes (vanillin, p-hydroxybenzaldehyde, syringaldehyde and protocatechualdehyde), and aromatic carboxylic acids (vanilic acid, p-hydroxybenzoic acid, syringic acid, and protocatechuic acid). Some or all of the above inhibitors may be present in the *Beta* fruit at any one point but differ in their concentrations. Concentration appears to determine whether a compound is the principle inhibitor of a particular *B. vulgaris* seed lot. An interesting example is from the study by Hoover and Goodin (1966), where oxalic acid, which is also one of the germination inhibitors identified by Morris, *et al.* (1984), was added to the germination medium of four sugar beet varieties (HHS, US75, HC-1, and HH4) at concentrations of 0.001% to 1.0%. Seed germination was carried out without prior treatment of the fruits before sowing. This is because no significant differences were observed between the germination of fruits washed in running water for two hours and unwashed fruits. Germination was carried out at 20 $^{\circ}$C and 45 $^{\circ}$C in order to find the optimum germination temperature. At the optimum germination temperature of 20 $^{\circ}$C, germination in all the four varieties did not decrease until concentrations of the oxalic acid reached 1.0%. The control fruit produced 96%, 79%, 83%, and 53% germination respectively while at a 1.0% oxalic acid concentration, germination obtained was 51%, 42%, 44%, and 17% respectively. The study did not report whether remaining fruits were fresh ungerminated. The decrease in germination suggests that oxalic acid was at least partly responsible for inhibiting germination, and yet it appeared not to be present in sufficient concentration to inhibit germination. The authors argued that if the germination inhibitor present was oxalic acid, physiological concentrations would be expected to inhibit germination, and yet germination did not decrease in the four varieties tested until oxalic acid concentrations reached 1.0% (Hoover and Goodin, 1966). However, one noted limitation of the study is that the presence and concentration of oxalic acid in the sugar beet fruits was not determined.

It appears that the inhibitors discussed in this section are still the best candidates as there is no recent literature that states otherwise. One of the reasons for this lack of literature is because there has been no research on dormancy in *B. vulgaris* in recent years. This may be a result of dormancy being bred out *B. vulgaris* in recent years. This is discussed in detail in 2.5.2.

2.3.6. Role of the operculum in germination inhibition

Apart from inhibitors in the sugar beet fruit coat, the operculum, located in the fruit coat, also plays a role in germination inhibition. Coumans, *et al.* (1976) defined the operculum as the upper toughened part of the fruit coat originating from the ovary, and found that germination improved in fruits with removed opercula. Coumans, Ceulmans, and Gasper (1979) also found that 81 - 98% of the fruits from which the operculum was removed germinated, and in comparison to whole fruits, this represented an increase in germination of 18 - 49%. These studies indicate that the operculum plays a key role in the prevention of germination of sugar beet. Coumans, *et al.* (1976) also reported that water soluble compounds, presumably, found in the operculum, combine with oxygen and as a result, deprive the embryo of oxygen, oxygen that might have otherwise been used by the embryo for germination. It is unclear in the literature whether the previously mentioned inhibitory chemicals are also found in the operculum, no reported studies have assessed the operculum separately from other fruit tissues. Heydecker and Chetram (1971) reported that the operculum has been found to prevent oxygen access to the embryo due to its imperviousness to gas. In the presence of excess water the operculum also produces mucilage around its rim which further prevents diffusion of oxygen (Heydecker and Chetram, 1971).

2.3.7. The physical structure of *B. vulgaris* fruits and its role in germination inhibition

Snyder (1959) pointed out that it is not clear whether improved germination after pre-soaking fruits is due to inhibitory substances being removed from the fruit cluster or operculum being loosened as a result of wetting and subsequent drying of the fruit. Snyder (1959) explained that the physical structure of the fruit cluster of many sugar beet varieties delay germination. Again when sugar beet fruit clusters were notched i.e. a small hole was made in order to expose the true seed's embryo, germination was faster than when fruits remained intact. This is because when the fruit cluster is notched, water uptake is accelerated and the imbibitional force that is required for the loosening of the operculum is decreased (Snyder, 1959).

Peto (1964) found that multigerm fruit clusters of the sugar beet seed lot CS 36 had higher germination (45%) than monogerm fruits of the same seed lot (33%). Seven to ten days after sowing, non-germinating monogerm fruits appeared sound and plump and because of this, the possibility of abnormally thick opercula in the monogerm fruit inhibiting germination was investigated. When the opercula were measured, it was found that monogerm opercula were 23.5% thicker than the multigerm.

Chipping off part of the operculum of the fruits increased germination, but seedlings that emerged were frequently deformed due to the constricting action of the remaining part of the operculum. This suggests that the thicker opercula increased the tightness with which the operculum was attached to the pericarp and led to the conclusion that tightness of the operculum is an important limiting factor in the germination of some monogerm *B. vulgaris* fruits.

In the Peto (1964) study, hemicelluloses and pectins were suspected as the cementing substances in the monogerm fruits. So hemicellulase and pectinase enzymes were used in an attempt to loosen the opercula of the same monogerm seed lot (CS 36). Hemicellulase was found to be more effective at a range of concentrations. Water treatment (control) gave 54% germination, hemicellulase treatments at 1%, 0.1%, and 0.01% concentrations gave 73%, 71%, and 61% germination while pectinase gave 68%, 59%, and 51% germination at the same respective concentrations. All treatments (i.e. exposure of fruits to the two enzymes and water) were for a duration of two hours.

The role of soaking and drying in loosening the operculum was also investigated and it was found that three and four one hour soakings in water followed by drying improved germination by 17% and 15% respectively (Peto, 1964). Although tightness of the fruit was measured (by estimating the force required to pry the operculum loose (Snyder, 1959)), the fact that inhibitors were also likely leached during the same treatments may have confounded results thus limiting the study's usefulness.

2.3.8. The role of the basal pore of the sugar beet fruit

The basal pore, located at the opposite end to the operculum in *B. vulgaris* fruits (Hermann, *et al.*, 2007) also plays a role in germination.

In a study by Coumans, *et al.* (1979), sugar beet fruits were germinated at two positions and water contents. In one treatment, 3.5 ml of water, positioned with the operculum facing downwards, the germination of four lots was 86%, 67%, 90% and 41% while for the same lots positioned with the operculum facing upwards (i.e. the basal pore was in contact with water as the basal pore is located opposite to the operculum), germination was lower at 18%, 65%, 66%, and 29% respectively. In contrast, in 1.5 ml of water, when the same fruits were positioned with the operculum facing downwards germination was 81%, 79%, 91% and 71%, and when positioned with the operculum facing upwards, germination was 67%, 83%, 84%

and 76%. This demonstrates the importance of both oxygen and water for the germinating embryo and the role of both the basal pore and the operculum as the main entrances for water and oxygen into the *B. vulgaris* fruit. It was also concluded that limiting the amount of water to 1.5ml was optimal for germination because of better oxygen diffusion (Coumans, *et al.*, 1979). Moisture content of germinated fruits at the two positions was not done in the study. This would have identified whether the amount of water taken up by fruits in different positions differed.

2.3.8.1. Importance of oxygen

Perry and Harrison (1974) demonstrated the importance of oxygen in *B. vulgaris* fruit germination. In monogerm sugar beet fruits, excess water (8ml) in the substrate inhibited germination (18%) compared to the control amount (3ml) which gave a germination of 89% seven days after sowing. Replacing the air with pure oxygen for both treatments eliminated the initial effect of germination inhibition due to excess water. In air, germination was 93% and 30% respectively for the 3ml and 8ml treatments while in oxygen, germination was 91% and 83% respectively.

2.3.8.2. Summary

Thus from the discussion, the main factors inhibiting germination in *B. vulgaris* are:

1) the pericarp containing germination inhibitors, and

2) the operculum depriving the seed of oxygen during germination under wet conditions and restricting embryo growth.

2.4. Differences in *B. vulgaris*

Although the inhibition of germination caused by chemical germination inhibitors located in the fruit coat is reported to be a common phenomenon across sugar, vegetable and fodder beets, differences in the level of dormancy exists between different types of *B. vulgaris*, different cultivars of the same type of *B. vulgaris*, and even different seed lots of the same cultivar harvested in different years.

Wood, Brewbaker and Bush (1950) for example reported that, in a study where 16 varieties of sugar beets were germinated at temperatures of 35 to 37 ˚C, germination percentages

ranging from 7 to 38% were obtained. These results demonstrate that there is variability between varieties of sugar beet in their ability to germinate at a particular temperature. Sester, Durr, Darmency, and Colbach (2006) observed that in weed beet, seed dormancy varied with season. There was an increase in the proportion of dormant fruits in winter (i.e. secondary dormancy which occurs when particular environmental conditions cause dormancy to be induced in previously non-dormant seeds that have been already shed from the parent plant) and a decrease during the rest of the seasons (loss of dormancy).

B. vulgaris fruits of the same type, but different varieties, will respond differently to the same environment. For example Hoover and Goodin (1966) found that when sugar beet fruit from four different varieties produced in the same place were germinated at 20 ˚C, the highest germinating variety (HH3) had 96% germination 14 days after sowing, and the lowest germination was 50% (HH4) after the same length of time. The other two varieties, (US75 and HC-1) were intermediate (85 and 80% respectively). All the varieties were multigerm except HH4 which was a monogerm variety.

Heide, Juntilla and Samuelsen (1976) found that the parent plant environment affected subsequent fruit germination of red beet (*B. vulgaris* subsp. *vulgaris*). In their study, red beet was exposed to low temperatures and long days during seed development and maturation. As a result of this treatment, the proportion of empty fruit clusters increased and the germination levels of normally developed fruits were reduced. It was not reported in the study whether this reduction in germination was due to increased fresh ungerminated (dormant) or dead fruits.

Battle and Whittington (1969) found that inhibition of germination by extracts from sugar beet fruit clusters was higher in extracts obtained from sugar beet fruits that had poor germination compared with those that had good germination. Extracts were obtained from immature fruits or from fruits that had received overhead irrigation. This is because in high germinating fruits and immature fruits, insufficient inhibitors are present to inhibit germination, while in fruits harvested from plants that had been irrigated overhead, some of the inhibitors are likely to have been leached out.

Therefore, it can be concluded that for fruit of the same cultivar, there are differences in dormancy levels, depending on when and where the fruits were grown and the growing conditions experienced at different stages of seed development. Different agronomic practices, overhead irrigation for example, may also affect dormancy levels. As a result

dormancy levels within the same cultivar may vary from year to year. It is clear that pre-sowing treatment methods are important for maximising germination in *B. vulgaris* fruit which display dormancy. These pre-sowing treatments and breeding of *B. vulgaris* and the role it may play in dormancy alleviation are discussed in sections 2.5. and 2.6.

2.5. Breeding in *B. vulgaris*

2. 5.1. General *B. vulgaris* breeding

Van Geyt, Lange, Oleo, Bock (1990) suggested that some morphological and physiological characteristics important in agriculture be included in a *B. vulgaris* breeding programme. For example monogerm fruit clusters and fruit roundness are important for mechanical sowing. Monogermity is also important to give a plant population which can be both pre-determined and uniformly spaced by precision sowing. This is not possible with multigerm *B. vulgaris* fruits due to multiple seedlings produced (Jaggard and Halmer, 2006). Savitsky (1952) reported breeding of sugar beet fruits for monogermity, the genetic monogerm fruits, as early as 1948, and according to Evans and Weir (1981), they were widely introduced in the 1950's. By 1977, Frankel (1977) reported that monogerm sugar beet fruits had almost completely displaced older multigerm varieties.

Van Geyt, *et al.* (1990) reported that the development of varieties capable of surviving in saline soils was also considered to be important as this would increase areas where sugar beet could be cultivated. A further priority has been to increase yields in combination with an elevated content of sucrose (Van Geyt, *et al.*, 1990).

B. vulgaris is extensively genetically variable. This has allowed the development of morphologically diverse cultivars like Swiss chard (*B. vulgaris* subsp. *cicla*), beetroot (*B. vulgaris* subsp. *vulgaris*), fodder beet (*B. vulgaris* subsp. *vulgaris*) and sugar beet (*B. vulgaris* subsp. *vulgaris*) to be selected for breeding (Schmidt, *et al.*, 1993). For example red table beet provides the possibility for root shape improvement since round shaped roots can be harvested more easily (Pillen, Steinrucken, Wricke, Herrmann and Jung, 1992).

In addition, wild *B. vulgaris* species such as *B. lomatogona*, which possesses traits like monogermity, drought resistance and tolerance to some virus's, have contributed to the development of cultivated *B. vulgaris* (Cleij, Debock and Lekkerkerker, 1976; Frese, 2010).

2.5.2. What is the current state of *B. vulgaris* seed dormancy?

2.5.2.1. Dormancy and selection

Many improvements to *B. vulgaris* varieties achieved through breeding have been reported in the literature but there are few reports of breeding to reduce fruit dormancy. Campbell and Enz (1991) commented that up until that time no effort has been made specifically to develop rapidly emerging *B. vulgaris* genotypes. However, this situation appears to have changed over the last two decades because Wagmann, *et al.* (2010) reported that rapid and synchronised germination has been selected for in cultivated *B. vulgaris*, and has led to the total elimination of the dormancy problem. Also, Frese (2010) listed *B. vulgaris* (leaf beet group, garden beet group, fodder beet group, and sugar beet group) as having a normal fruit type, while *B. vulgaris* subsp. *maritima*, *B. vulgaris* subsp. *adanensis*, and *Beta macrocarpa,* which are wild species, were listed as having a fruit type with varying degrees of dormancy. The reports of both Frese (2010) and Wagmann, *et al.* (2010) seem to indicate that dormancy in cultivated *B. vulgaris* fruits has been bred out, but remains in the wild species. This is supported by Sester, *et al.* (2006) who stated that the presence of primary dormancy in weed (wild) *B. vulgaris* fruits and the absence of it in cultivated *B. vulgaris* is the difference between the two types of *B. vulgaris*.

Even in the absence of direct selection of non-dormant *B. vulgaris* fruits, dormancy may be inadvertently lost during the selection of other traits. Frese (2010) reported that gene banks collect, reproduce and preserve sugar beet fruit samples under cold storage conditions away from their habitat (*ex situ*) in order to conserve the genetic information which can be used in subsequent breeding programmes. During the process of multiplying seed (fruit) in gene bank seed production *ex situ* to replenish genebank stocks, late germinating genotypes are excluded and lost from the first *ex situ* reproduction cycle, thus encouraging the loss of dormancy genes. Also, genotypes with no or limited dormancy tend to be selected as they do not require the time consuming step of pericarp removal.

It is important therefore to confirm whether this historically reported dormancy continues to exist in modern cultivars, and if still present, at what levels. This will determine whether pre-sowing treatments in both the field and the laboratory are still needed to alleviate dormancy and if still needed what pre-sowing treatment or treatments are best.

2.6. Treatments for improved germination prior to sowing

In order to achieve high germination percentages in dormant *B. vulgaris* fruit, the growth inhibitors present in the fruit coat have to be removed and the ability of the operculum to inhibit water and/or oxygen uptake and thus subsequent germination should be reduced. There are several pre-treatment methods that are commonly used for *B. vulgaris* fruits. The following are the common ones:

i. Washing fruit in running water to wash out inhibitors
ii. Soaking fruit in water in order to leach out inhibitors
iii. Steeping, whereby fruits are immersed in dilute aqueous solutions (Durrant and Mash, 1991)
iv. Priming, whereby fruit is exposed to moisture levels that are sufficient to enable the early seed germination processes to take place but too low for radicle emergence (Orzesko-Rywka and Podlaski, 2003)
v. Rubbing to partially remove the fruit coat layer causing a decrease in the pericarp thickness and removal of some of the chemicals that inhibit germination (Orzesko-Rywka and Podlaski, 2003), or scarification: i.e. forcing the operculum off the rest of the fruit (Coumans, *et al.*, 1976).

There are numerous modifications that can be made to these methods. These methods can be used singly or in different combinations. Different amounts of water can be used on the germination substrate followed by variations of temperatures and times that fruits are left to germinate. Similar variations can be used for the washing, soaking or steeping treatments, including whether the fruits are dried at the end of the treatments or not (Longden, 1976; Orzesko-Rywka and Podlaski, 2003).

2.6.1. Effects of the pre-sowing treatments on *B. vulgaris* seed germination

In a study on how sugar beet seed vigour is affected by pre-sowing treatments, Orzeszko-Rywka and Podlaski (2003) found that all fruit treatments individually, i.e. rubbing, washing, priming, and a combination of rubbing and priming (where fruits were placed on blotting paper with water capacities of 40% (shortage), 65% (optimum) and 100% (excess) for 24 hours followed by air drying), improved germination after 14 days although improvement was more under stressful conditions (excess and shortage of water). Mean germination of three sugar beet varieties tested at 40% water capacity was 49% 71%, 85%, 85%, and 86%

for control, rubbed, primed, washed, and rubbed plus primed treatments respectively, and at 65%% water capacity mean germination was 76%, 82%, 93%, 93%, and 90% while at 100% water capacity mean germination was 60%, 71%, 85%, 73%, and 86% for the same respective treatments. However, the authors did not discuss whether the 65% water capacity was sufficient to leach out any inhibitors but not to impede oxygen uptake, while the 40% water capacity was insufficient to leach out inhibitors and the 100% water capacity inhibited oxygen uptake.

While the pre-sowing treatments have the ability to increase total germination, they can also improve the rate of germination. Longden (1971) found that the optimum treatment for speeding the germination of monogerm sugar beet fruits was priming the fruit by wetting the fruit with its own weight of water. The fruit with its equal weight of water was placed in sealed airtight containers and stored for 24 hours. This was done in four cycles with a 48 hour drying interval after each soaking. The result of the treatment was that the number of cells per embryo was doubled. As a result, seedling emergence occurred approximately 2.5 days sooner and seedling shoot weight increased by 31 to 53%. Meikle (1981) suggested that this increased speed of germination occurred because priming allowed the early stages of the germination process to occur. Using the same priming procedure as Longden (1971), Meikle (1981) found that sugar beet seed lots that reached 98%, 84%, and 65% germination in a standard test (i.e. fruits soaked in tap water for 1.5 hours and dried back at 20 ˚C for 16 hours and 25 ˚C for eight hours per day) had 95%, 89%, and 82% germination respectively. Mean germination times for the same seed lots were 3.06 days, 3.17 days and 6.23 days in the standard test and 2.15 days, 2.79 days and 4.94 days respectively. These results showed that while total germination of only one lot was improved by priming, mean germination time was improved in all of them.

2.6.2. How pre-treatment methods achieve improved germination

The mechanisms by which pre-sowing treatments work to achieve higher germination differ between treatments, but a common mechanism is changing the structure of the fruit coat either physically resulting in improved moisture and oxygen intake, or chemically by leaching chemical inhibitors.

Rubbing *B. vulgaris* fruits, for example, changes the structure of the pericarp in favour of improved germination. The structure changes because the most porous outer layer of parenchyma cells is removed by rubbing. Orzeszko-Rywka and Podlaski (2003) found that

when three varieties of sugar beet fruits were rubbed, primed and washed, the characteristics of the pericarp changed, including the density, moisture and chemical content of the fruits. The water leached from the fruits with and without treatment was measured by electrical conductivity. The water leached from control fruits had significantly higher electrical conductivity (4.5 mS/cm) than treated fruits (2.10 mS/cm, 1.61 mS/cm, 1.82 mS/cm, and 1.75 mS/cm for rubbing, washing, priming, and rubbing plus priming respectively). Rubbing and washing especially removed chemicals from the pericarp and all treatments caused an accelerated rate of germination time (3.92, 3.41, 2.90, 3.01, and 2.64 days for control, rubbed, primed, washed, and rubbed plus primed fruits respectively). In contrast, priming alone and priming in combination with rubbing caused an increase in seed respiration intensity after two days which resulted from easier water access to the fruits following the treatment (0.34 and 0.25 mg CO_2/h/g of fresh matter for priming and rubbing plus priming respectively, 0.18 mg CO_2/h/g of fresh matter for both washing and rubbing, and 0.1 mg CO_2/h/g of fresh matter for control). Overall, the treatments improved the ability and the rate of sugar beet fruit germination. Orzeszko-Rywka and Podlaski (2003) suggested that when the most porous outer layer of parenchyma is removed by rubbing, chemicals are also removed reducing the number of chemicals present. Similar removal of chemicals can be obtained by fruit washing. Rubbing also decreases the thickness of the pericarp and also provides better exposure of the basal pore (Orzeszko-Rywka and Podlaski, 2003) the pathway for water uptake and oxygen diffusion.

In a study where different pre-treatments were applied to sugar beet fruits, Rush (1991) found that solid matrix priming (SMP), produced better results than the other methods used. Germination for solid matrix priming, NaCl, Polyethylene glycol (PEG), washing and control treatments was 56%, 12%, 6%, 5% and 2% respectively three days after sowing. It was suggested that the superiority of SMP over the other pre-sowing treatment methods could be a result of improved aeration that is attained with this treatment. As previously discussed, the operculum may prevent oxygen access (essential for germination) to the embryo, the improved aeration attained by SMP should be alleviating this.

According to Rush (1991), the solid material used for the SMP was a clay mineral, a friable mixture readily permeable to oxygen. This is in contrast to the viscous PEG solution also used but which causes major aeration problems.

2.6.3. Standard pre-sowing treatments

Although several methods can be used to alleviate dormancy in *B. vulgaris* fruits prior to sowing, not all of them have been standardised. In 1965, Hoover and Goodin (1965) commented that standard testing procedures recommend pre-soaking the fruit in water for at least two hours in order to leach out endogenous inhibitors but no comments were made whether to soak the fruits in the same water for two hours or change the water at certain intervals. Inoue and Yamamoto (1974) on the other hand suggested that since the growth inhibitory substances present are water soluble, they can easily be removed from the fruit clusters by washing with water before sowing.

These two methods, pre-soaking and pre-washing are the most used pre-sowing methods in *B. vulgaris*. Both have factors that affect their effectiveness.

2.6.3.1. Factors affecting soaking or washing

2.6.3.1.1. Time as a factor affecting pre-sowing treatments

A number of factors can determine the effectiveness of pre-soaking and pre-washing treatments in improving *B. vulgaris* fruit germination. For instance there are issues around how much time is needed for pre-soaking or pre-washing to achieve maximum germination of sugar beet. In a study by Silva, *et al.* (2005), where fruits were washed from zero (control) to six hours, washing fruits in running water for two hours was sufficient to increase germination. When fruits were immersed in running water for 0, 1, 1.5, 2, 3, 4, 5, and 6 hours, germination obtained was 79, 81, 83, 92, 85, 80, 77, and 75% respectively, and the germination obtained after two hours was significantly higher than the rest. Both shorter and longer washings significantly reduced germination compared to the two hour washing. Two hour washing for multigerm fruits is also the dormancy-breaking technique recommended in the ISTA Rules. Four hour washing at 25 ˚C is recommended for monogerm fruits (ISTA, 2010). Habib (2010) found that soaking fruit for six hours with diluted acid or water (without changing the soaking water every half-hour) improved seed germination, the mean time of germination (T_{50}), and rate of germination. The rate of germination for 0.03 N dilute hydrochloric acid soaking treatment for six hours and untreated fruits was 6 germs per day and 3 germs per day respectively. Germination was 85, 88, 89, and 90% for untreated, 2, 4, and 6 hours water soaking treatments respectively. Rate of germination was 13, 14, 16, and 21 germs per day, and T_{50} was 7.76, 6.34, 5.97, and 4.43 days for the four respective treatments. The water treatment rather than dilute acid was recommended as it was of lower

cost and more effective. The data also suggests that at least six hours soaking is best for improving the rate of germination. It may also suggest that the two hour soaking practiced in New Zealand may be insufficient to improve germination.

2.6.3.1.2. Water sensitivity in *B. vulgaris* fruit

Although washing or soaking are the commonly used pre-sowing treatments, *B. vulgaris* fruit germination will sometimes react negatively to water pre-treatments. The germination of some *B. vulgaris* fruit is inhibited by water treatments. Beetroot germination for example has been found to be negatively affected by excess water. This was demonstrated by Chetram and Heydecker (1967) who found that washed beetroot fruits immediately placed on a filter paper for germination will rarely germinate. Eliminating the drying process after a washing treatment means that the fruits have excess water when they are placed for germination. According to Heydecker, Chetram, and Heydecker (1971), fruits fail to germinate because under wet conditions, the operculum produces mucilage around its rim and also prevents access of oxygen to the embryo due to its imperviousness to gas.

In comparison, if the fruits are washed then dried before being placed on the filter paper, germination is improved. Moreover, germination in beetroot is depressed or inhibited when fruits are placed for germination on an excessively wet substrate. The effect on germination is much more obvious in fruits that have not been washed since the possible effect of the water (i.e. inhibiting/depressing germination) obtained from washing the fruits is eliminated leaving the effect of the excess water in the substrate clearer. Heydecker and Chetram (1971) also found similar results when 25 two-seeded clusters of two seed lots of the beetroot cultivar Detroit Globe, either un-treated or rubbed, germinated poorly in 8 ml of water in a 9 cm diameter petri dish, but germinated well in 3 ml of water. Similarly, Perry and Harrison (1974) found that excessive water in the substrate inhibited monogerm sugar beet fruit germination, but did not cause lethal results. The germination of sugar beet on substrate with 3ml of water was 89% after seven days while germination on substrate with 8ml of water was only 18%. Seed of other species also show some sensitivity to water where germination is delayed in the presence of excess water in the substrate. Perry and Harrison (1974) reported that not only was this phenomenon common in many *B. vulgaris* cultivars (both monogerm and multigerm), *B. vulgaris* was also more sensitive to excess water than the other species tested. Carrot, cabbage, tomato, parsnip, onion and the monogerm sugar beet were tested. In 3 ml of water, germination was 83%, 91%, 94%, 69%, 94%, and 87% respectively 23 days after

sowing. In contrast with 8ml of water in the germination substrate, fruit had 84%, 91%, 100%, 65%, 84%, and 29% germination respectively after 23 days.

Orzeszko-Rywka and Podlaski (2003) also found that both excess water and water stress resulted in a significant decrease in germination four days after sowing i.e. 75% in excess water and 75% under water stress compared with 87% under optimum conditions. In washed (73% germination) fruits, excess water caused a decrease in germination four days after sowing from 12% to 13% in comparison with primed and primed fruits after rubbing (85% and 86% germination respectively). The authors suggested that the reason for the germination decrease in washed fruit compared to that of the other treatments was because of higher porosity in the washed fruits where water filled pores restricted oxygen access to the fruits.

NEW ZEALAND SPINACH (*TETRAGONIA TETRAGONIOIDES)*

2.7. Introduction

Tetragonia tetragonioides belongs to the Aizoaceae, a large plant family with 150 genera and 2600 species of succulents (Prakash, 1967). Within the family Aizoaceae, the genus *Tetragonia* is characterised by its very hard indehiscent-like fruits but detailed studies have never been carried out on the genus (Taylor, 1994).

Taylor (1994) reported that 50-60 species of *Tetragonia* are known. These are found in the regions of the southern hemisphere including the subtropical Pacific coasts, temperate coasts of both South American oceans and other regions such as Africa. Species are also found in Australia (five species) and New Zealand (two species) (Taylor, 1994). In New Zealand, *T. tetragonioides* is rarely found growing naturally in inland areas. The plant's natural habitat is localised pockets on the coasts of both the North and South Islands (Roskruge, 2009).

2.7.1. Uses

While *T. tetragonioides* is mostly grown in rock gardens as an ornamental plant, it is sometimes used as a source of table greens (Prakash, 1967). Apart from being used as a vegetable (Prakash, 1967; Yousif, *et al.*, 2010) it is also cultivated throughout the world for use as groundcover and a medicinal plant (Yousif, *et al.*, 2010). In New Zealand, many New Zealanders consider the plant to be an indigenous vegetable (Roskruge, 2009). Although often considered endemic, it is also found in Australia (Webb and Simpson, 2001).

The wild and cultivated plants of *T. tetragonioides* differ in that the cultivated plant has much larger leaves than the wild plant (Allan, 1961). This suggests that there has been some limited selection in the cultivated seed lines, although there are no named varieties.

Despite *T. tetragonioides* having a number of uses, it is not a major crop (Newstrom, *et al*., 2003). Grubben (2004) explained that one of the reasons why *T. tetragonioides* is not usually grown on a commercial scale is because it cannot compete with regular spinach in yield and ease of cultivation. Seed germination is erratic, and germination may take from two weeks to more than three months. *T. tetragonioides* difficult seed germination is a limitation to it becoming a significant and important vegetable crop.

2.7.2. The *T. tetragonioides* fruit

T. tetragonioides fruits are hard, dry and indehiscent (Taylor, 1994). According to Webb and Simpson (2001) *T. tetragonioides* fruits have a usually compressed obtriangular 8 – 13 mm long endocarp which is sometimes 2 – 4 angled, ridged or veined. The fruits have an irregular flattened apex which is characterised by 3 – 5 marginal horns (1 - 2 mm long). The endocarp of the only other *Tetragonia* species found naturally in New Zealand, *Tetragonia implexicoma*, is not horned and the fruit is smaller being only 3 – 5 mm long. The *T. tetragonioides* endocarp surface is grey to nut brown with medium brown buff and covered in transparent papillae. The number of seeds found within each fruit ranges from 3 to 10 (Webb and Simpson, 2001).

Figure 4. *T. tetragonioides* fruit (external features)

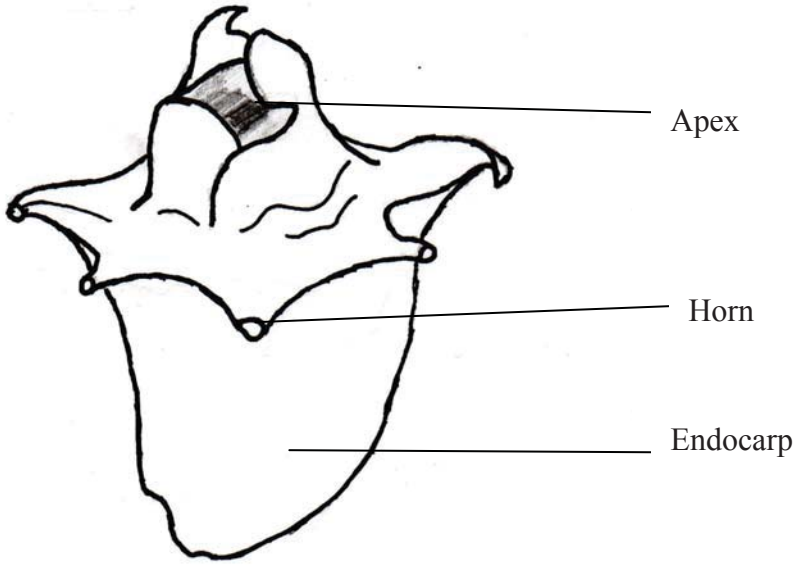
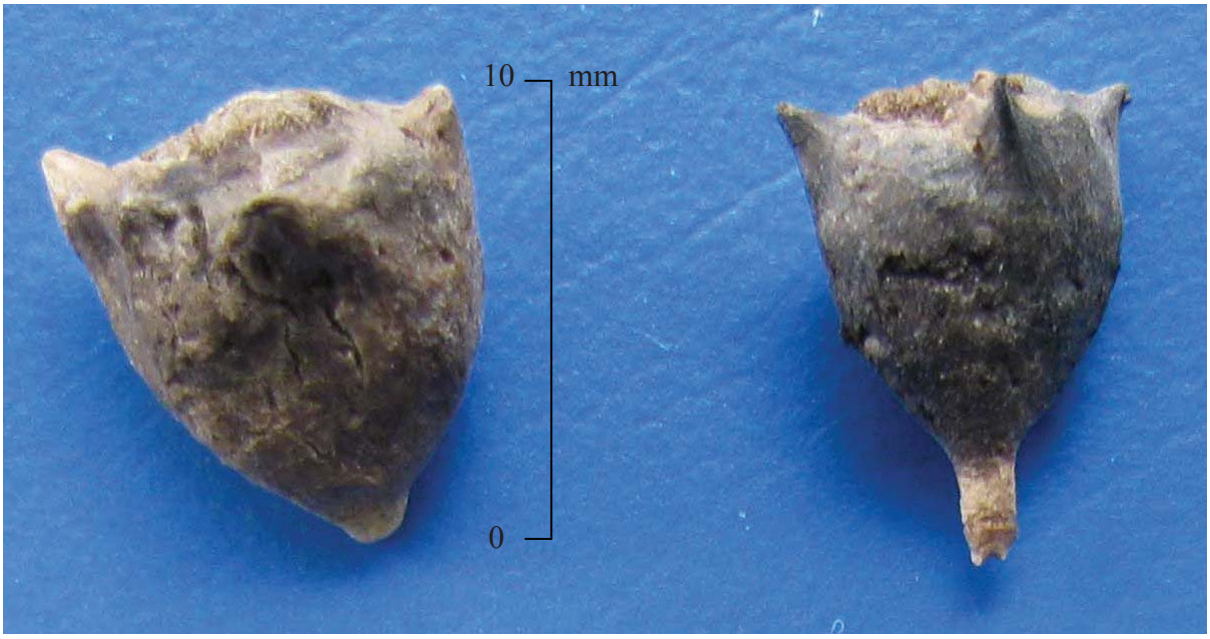


Figure 5. *T. tetragonioides* fruit



2.8. Germination issues and pre-sowing treatments

T. tetragonioides fruits have a very hard coat and need to be soaked preferably in water before sowing (Grubben, 2004). Soaking should be for a day in order to soften the pericarp and enable faster germination. This implies that the difficulty in *T. tetragonioides* fruit germination is due to its hard fruit coat. Grubben (1978) also stated that for fruits which are hard coated like okra, peas and beans including *T. tetragonioides*, dipping in water of over 90 ˚C for a few seconds, or for larger quantities, in water of 55 ˚C for a duration of 20 minutes, or scarification, may be required to obtain good germination.

The 2009 edition of the ISTA Rules (ISTA, 2009), specified that fruits of *T. tetragonioides* should be pre-washed in water before sowing, the same recommendation as for *B. vulgaris* fruit. *T. tetragonioides* was the only other species apart from *B. vulgaris* in the ISTA International Rules for Seed Testing (2009) whose fruits required pre-washing prior to sowing. In the 2011 edition the recommendation for *T. tetragonioides* was changed to 24 hours soaking (ISTA, 2011).

2.8.1. Breeding of *T. tetragonioides*

Grubben (2004) stated that no genetic improvement of *T. tetragonioides* has been undertaken and using breeding and seed technology to solve the germination problems in *T. tetragonioides* should be the focus of research.

CHAPTER 3

MATERIALS AND METHODS

3.1. Preliminary experiment

The quality of each *B. vulgaris* and *T. tetragonioides* seed lot was assessed on receipt at Massey University. The purpose of this assessment was 1) to find out if dormancy was present in the seed lots and if present, the level of any dormancy, and also, 2) based on the germination and dormancy of the seed lots, identify seed lots for the main study.

Nineteen seed lots were screened for the presence of dormancy and to determine the approximate level of dormancy of individual seed lots. The level of dormancy sought in each seed lot was a minimum of 40%.

To determine the level of dormancy in the seed lots, germination tests were conducted.

3.1.1. Seed lots used

i) *T. tetragonioides* seed lot

The *T. tetragonioides* seed lot was purchased from Kings Seeds (NZ) Limited (Bay of Plenty, New Zealand). The seeds (fruits) are an open pollinated variety purchased fresh each year from Italian growers. Seed was purchased in September, 2010 but had been in storage for approximately 12 months following harvest in 2009.

ii) *B. vulgaris* seed lots (sugar, vegetable, and fodder beets)

1. Sugar beet from Kings Seeds Limited, New Zealand

One seed lot of sugar beet was purchased from Kings Seeds (NZ) Limited (Bay of Plenty, New Zealand). The seed (fruit) was an unnamed open pollinated genetic monogerm variety that was grown in the United States of America and harvested in 2009. It was in storage for about 12 months before being purchased in June, 2010 (Gerard Martin, pers. comm., 2010).

2. Fodder beet and sugar beet from Maribo Seed, Denmark

Two sugar beet seed lots (cvs. ‘Flores’ and ‘Palace’) and three fodder beet seed lots (cvs. ‘Nestor’, ‘Magnum’ and ‘Kyros’) were kindly provided by Maribo Seed (Holeby, Denmark). These were all hybrid genetic monogerm varieties. The seed was multiplied in Southern Europe (northern Italy and south west France) and was harvested in 2009 (Hillary Snow, pers. comm., 2010).

3. Fodder beet from Seed Force Limited, New Zealand

The multigerm fodder beet cultivar 'Brigadier' was kindly provided by Seed Force Limited (Christchurch, New Zealand). This seed lot was pelleted.

4. Vegetable beet from Midlands Seed Limited and Canterbury Seeds Limited, New Zealand

Cultivar 'Ruby', a red beet, was obtained from Canterbury Seeds Limited (Christchurch, New Zealand). It is a multigerm, open pollinated variety, harvested in 2006 and grown in Canterbury, New Zealand.

Ten seed lots of red beets were obtained from Midlands Seed Limited (Canterbury, New Zealand). They were all multigerm hybrid seeds (fruits) from different growers and were size graded as firsts (bigger fruits) or seconds (smaller fruits) from different growers. They were harvested in 2010 (Joanne Townshend, pers. comm., 2011).

The seed lots were numbered as follows:

Table 1: **Numbered seed lots used, *B. vulgaris* types, fruit types, year harvested and source of the seed lots. SL = seed lot, FB, SB, and VB = fodder beet, sugar beet and vegetable beet. N/A = not applicable**

No.	Seed lot or cultivar name	*B. vulgaris* type	Grade	Fruit type	Harvest year	Source
SL1	Blain	VB	Firsts	Multigerm	2010	Midlands Seed
SL2	Blain	VB	Firsts	Multigerm	2010	Midlands Seed
SL3	Blain	VB	Seconds	Multigerm	2010	Midlands Seed
SL4	Taylor	VB	Firsts	Multigerm	2010	Midlands Seed
SL5	Taylor	VB	Seconds	Multigerm	2010	Midlands Seed
SL6	Taylor	VB	Seconds	Multigerm	2010	Midlands Seed
SL7	Watson	VB	Firsts	Multigerm	2010	Midlands Seed
SL8	Watson	VB	Seconds	Multigerm	2010	Midlands Seed
SL9	Le Poutre	VB	Firsts	Multigerm	2010	Midlands Seed
SL10	Le Poutre	VB	Seconds	Multigerm	2010	Midlands Seed
SL11	Ruby	VB	N/A	Multigerm	2006	Canterbury Seeds
SL12	Brigadier	FB	N/A	Multigerm	2009	Seedforce
SL13	Sugar beet	SB	N/A	Monogerm	2009	Kings Seeds
SL14	Flores	SB	N/A	Monogerm	2009	Maribo Seed
SL15	Nestor	FB	N/A	Monogerm	2009	Maribo Seed
SL16	Palace	SB	N/A	Monogerm	2009	Maribo Seed
SL17	Kyros	FB	N/A	Monogerm	2009	Maribo Seed
SL18	Magnum	FB	N/A	Monogerm	2009	Maribo Seed
SL19	NZ spinach	N/A	N/A	Multigerm	2009	Kings Seeds

3.1.2. Seed characteristics

3.1.2.1. Thousand seed weight

All seed lots were split into four replicates using the hand halving method where the seed lot was placed on a flat table, mixed with a spatula and divided into quarters and discarding opposite quarters. This process was followed until the size required for analysis was reached.

The thousand seed (fruit) weight in grams was determined by counting 100 fruits four times and weighing them on a four decimal place electronic balance (ISTA, 2010).

3.1.2.2. Seed moisture content

For each replicate 4 - 5 g of fruit was used to measure the seed moisture content (SMC) as specified by the ISTA Rules (2010).

B. vulgaris fruits were oven dried for 1 hour at 130 - 133 ˚C. The one hour period began when the oven had returned to 130 – 133 ˚C (around 5 – 10 minutes). *T. tetragonioides* fruits were oven dried for 17 ± 1 hours at 103 ± 2 ˚C.

3.1.3. Germination tests

3.1.3.1. *B. vulgaris*

All *B. vulgaris* fruits were germinated using the pleated paper method (ISTA, 2010). The germination paper used was 38 lb regular weight seed germination paper (Anchor Paper Company, St. Paul, Minnesota). Four replicates of 50 fruits were sown on pleated paper ten fruits per row in five rows. The ends of the pleated paper were fastened with rubber bands in order to prevent loss of fruits. The pleated paper was placed in labelled covered plastic boxes and then into a germinator set at alternating temperatures of 20 - 30 ˚C (16:8 hours) with alternating temperatures and light (8 hours at 30 ˚C) and dark (16 hours at 20 ˚C). Interim and final germination counts were done at 4 and 14 days respectively (ISTA Rules, 2010).

The *B. vulgaris* fruits were not washed for two hours as specified by ISTA (2010) because the purpose of the experiment was to find out if dormancy existed in the seed lots and also assess the levels of dormancy.

3.1.3.2. *T. tetragonioides*

T. tetragonioides fruits were germinated using the between paper method as specified by ISTA Rules (2010). Four replicates of 50 fruits were sown using the top of paper method and placed in a germinator set at 20 - 30 ºC (16:8 hours) with alternating temperatures and light (8 hours at 30 ˚C) and dark (16 hours at 20 ˚C). Interim and final germination counts were at 7 and 35 days respectively.

T. tetragonioides fruits were not pre-washed as specified by the ISTA Rules (2010) because the level of dormancy was being assessed.

Seedlings of both *B. vulgaris* and *T. Tetragonioides* were classified as “normal” if they were intact with well developed essential structures i.e. primary root, hypocotyl and cotyledons,

healthy and in proportion showing potential continued development according to ISTA Rules (2010). In contrast seedlings were classified as "abnormal" if they were damaged, deformed, out of proportion, or showing signs of decay that prevented normal development (ISTA, 2010).

The classification of dead and empty fruits is outlined in detail in section 3.2.5.

3.2. Main experiment

The main experiment was carried out at the AsureQuality Palmerston North Seed Laboratory as they have pre-washing equipment that meets ISTA specifications. The selected seed lots ('Brigadier' FB, 'Kyros' FB, 'Flores' SB, Kings Seeds unnamed SB, and unnamed *T. tetragonioides*) were selected for the main experiment as they had higher levels of dormancy compared to the other seed lots. Treatments were the following:

3.2.1. Pre-washing treatment

According to the ISTA Rules (2010), *B. vulgaris* multigerm fruits must be pre-washed for two hours in running water at 25 ºC and genetic monogerm fruits for four hours. The fruits should then be dried at a maximum of 25 ºC. The ISTA Rules (2010) also specify that *T. tetragonioides* fruit can be pre-washed.

The unnamed *T. tetragonioides* variety and 'Brigadier' FB were multigerm and were therefore pre-washed for two hours and 'Kyros' FB, 'Flores' SB, and the Kings Seeds SB (unnamed open pollinated variety) were all monogerms and were therefore pre-washed for four hours.

500 fruits of each seed lot were counted for each replicate i.e. 50 fruits for germination after drying, half the remaining fruits for the determination of moisture content before drying and the other half for the determination of moisture content after drying. Each treatment was replicated four times.

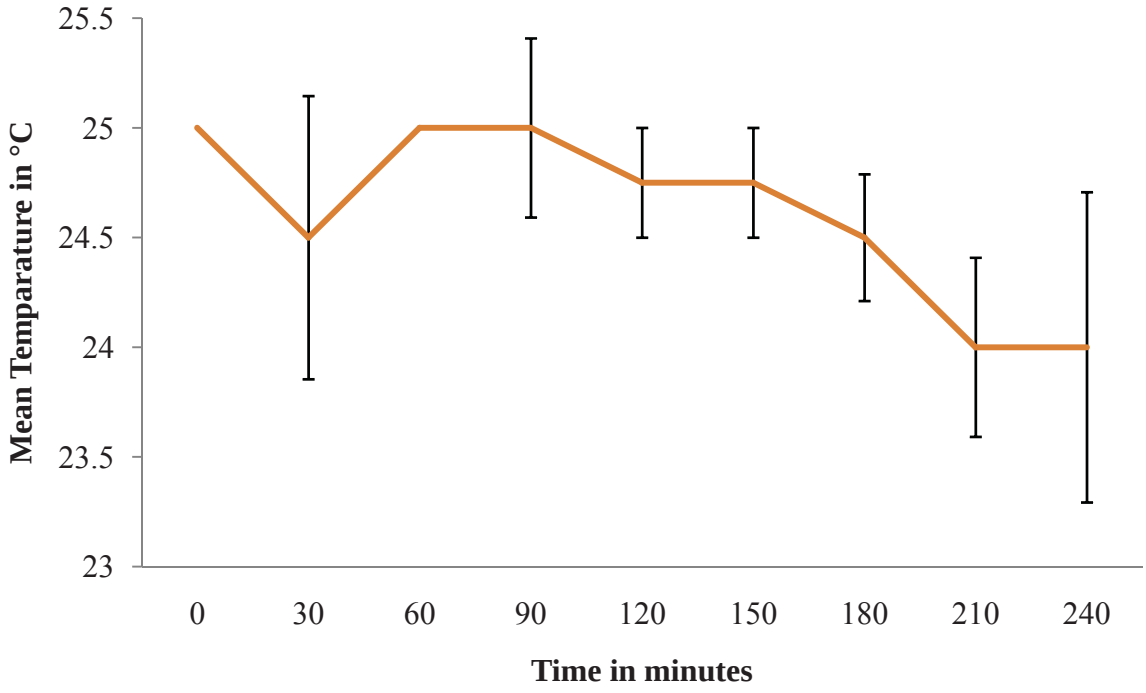
Each replicate of 500 fruits was placed in a small organza bag which was tied firmly at the opening to prevent fruit spillage. There was enough room in the bags for the fruits to move freely in order for them to be fully exposed to the water. The bags containing the fruits were then placed in a large plastic container within a sink which had continuously flowing water from a pipe attached to the water tap (Figure 6). The mouth of the pipe was placed at the

bottom of the container in order to let the water flow continuously. The bags were weighed down by use of metal clippers and sieves in order to keep the fruits submerged in the running water as much as possible (Figure 7). A thermometer was positioned close to the mouth of the pipe so that it was submerged in the running water in the plastic container to allow the monitoring of temperature. The temperature of the water was set at 25 ± 2 ºC i.e. the pre-equilibrated temperature, and the thermometer was checked every 30 minutes according to the documented AsureQuality quality system procedure (Figure 8) (AsureQuality 2008; ISTA, 2010) One replicate from each seed lot was completed each day meaning five bags were washed in the container at any one time.

Figure 6. Washing fruits in organza bags with running water. Thermometer for monitoring temperature is attached to the plastic tubing.

Figure 7. The washed fruits weighed down by sieves to ensure complete submersion in water

Figure 8. Mean temperature of running water used for pre-washing every 30 minutes with mean standard error (MSE) bars.

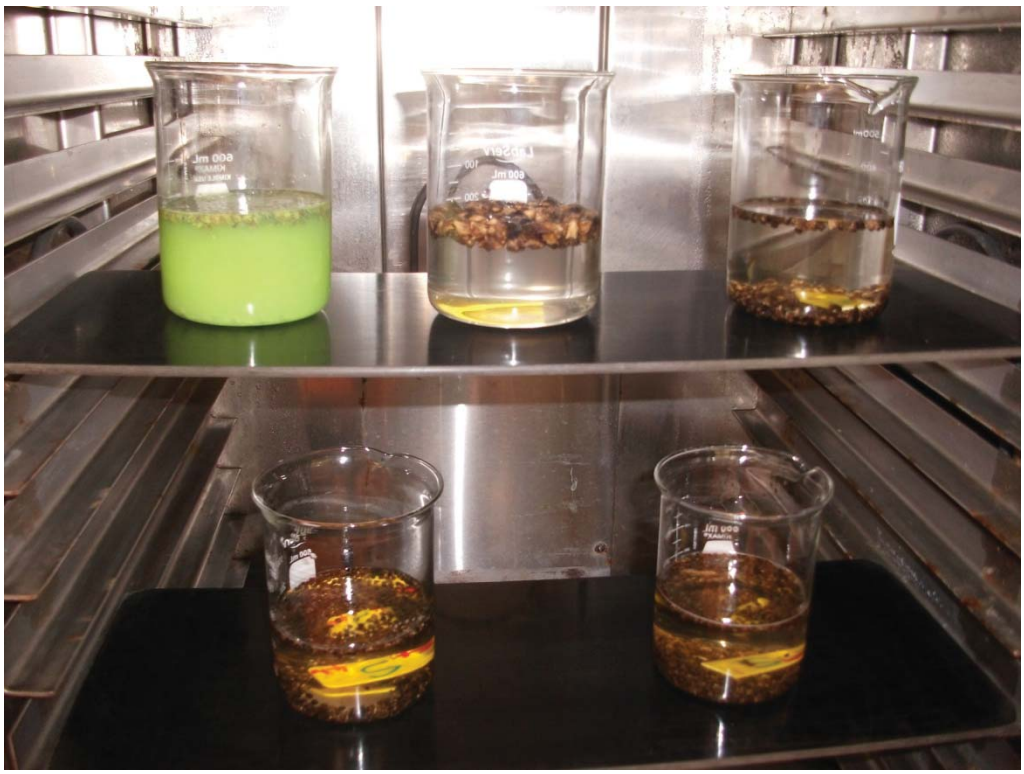


3.2.2. Pre-soaking treatment

The fruits for the pre-soaking treatment were placed in a beaker and soaked in 250 ml of water that had been pre-equilibrated to 25 ± 2 ºC. The beakers were placed in a germinator that was pre-set to 25 ºC in order to keep the water at the required temperature (Figure 9). The water was changed every 30 minutes to remove any germination inhibitors (AsureQuality, 2008). To change the water, water and fruit from the beaker were poured into a strainer and the remaining fruit then placed in the beaker again with a fresh 250 ml water pre-equilibrated to 25 ˚C.

T. tetragonioides and Brigadier FB were pre-soaked for two hours as they are multigerm and 'Kyros' FB, 'Flores' SB, and the Kings Seeds SB (the unnamed open pollinated variety) are monogerm and were pre-soaked for four hours.

Figure 9. Pre-soaking the five seed lots in a germinator set at 25 ˚C. The seed lot on the top left is the pelleted 'Brigadier' fodder beet.



3.2.3. Seed moisture content

The seed moisture content (SMC) was measured for all seed lots before and after drying the fruits following the washing and soaking treatments.

Following the treatments, at the end of two hours or four hours depending on the seed lot, water was drained off the fruits and 50 fruits were counted for germination and placed on a labelled blotting paper for drying. Half of the remaining fruits were immediately used to determine the SMC before drying (MCBD). The fruits were left to dry on the blotting paper, under ambient conditions for two hours, the 50 fruits for germination were removed and the SMC of the remaining fruit was determined (MCAD) (AsureQuality, 2008; ISTA, 2010). *T. tetragonioides* fruit was always cut in half for all moisture content determinations in order to increase the surface area as they are bigger fruits than the *B. vulgaris*.

The remaining 50 fruits were germinated as was described in the preliminary experiment.

3.2.4. Final germination, mean germination time and uniformity of germination

In order to evaluate the effects of the treatments on speed of germination i.e. mean germination time as measured by the time taken for 50% of the germinating population to germinate (T_{50}) and uniformity, radicle emergence was scored twice daily, then daily as germination slowed for a total of 14 days for *B. vulgaris* fruits and 35 days for *T. tetragonioides* fruits (ISTA, 2010). Radicle emergence was scored as visual emergence of the radicle, but the fruits were left on the blotter so normal seedling development could be assessed. The viability of any seed that remained ungerminated at the end of the germination trial was assessed using the topographical tetrazolium test (Leist, Kramer and Jonitz, 2003).

Mean germination time (T_{50}) for individual replicates was calculated by using the following formula:

$$T_{50} = t_i + \frac{\frac{(N+1)}{2} - n_a}{(n_j - n_i)} \times (t_j - t_i)$$

Where n_i and n_j are cumulative emergence counts at adjacent counting times t_i and t_j, where $n_i < (N + 1)/2 < n_j$. N being the total number of fruits that germinate, not the total number sown (Coolbear, Francis, and Grierson, 1984).

Uniformity ($T_{90} - T_{10}$) which is the interval between the germination of 10% and 90% of germinated fruit was calculated by using the following formula for individual replicates:

$$(T_{90} - T_{10}) = \left[t_a + \frac{\frac{9(N+1)}{10} - n_a}{n_b - n_a} \times (t_b - t_a) \right] - \left[t_c + \frac{\frac{(N+1)}{10} - n_c}{n_d - n_c} \times (t_d - t_c) \right]$$

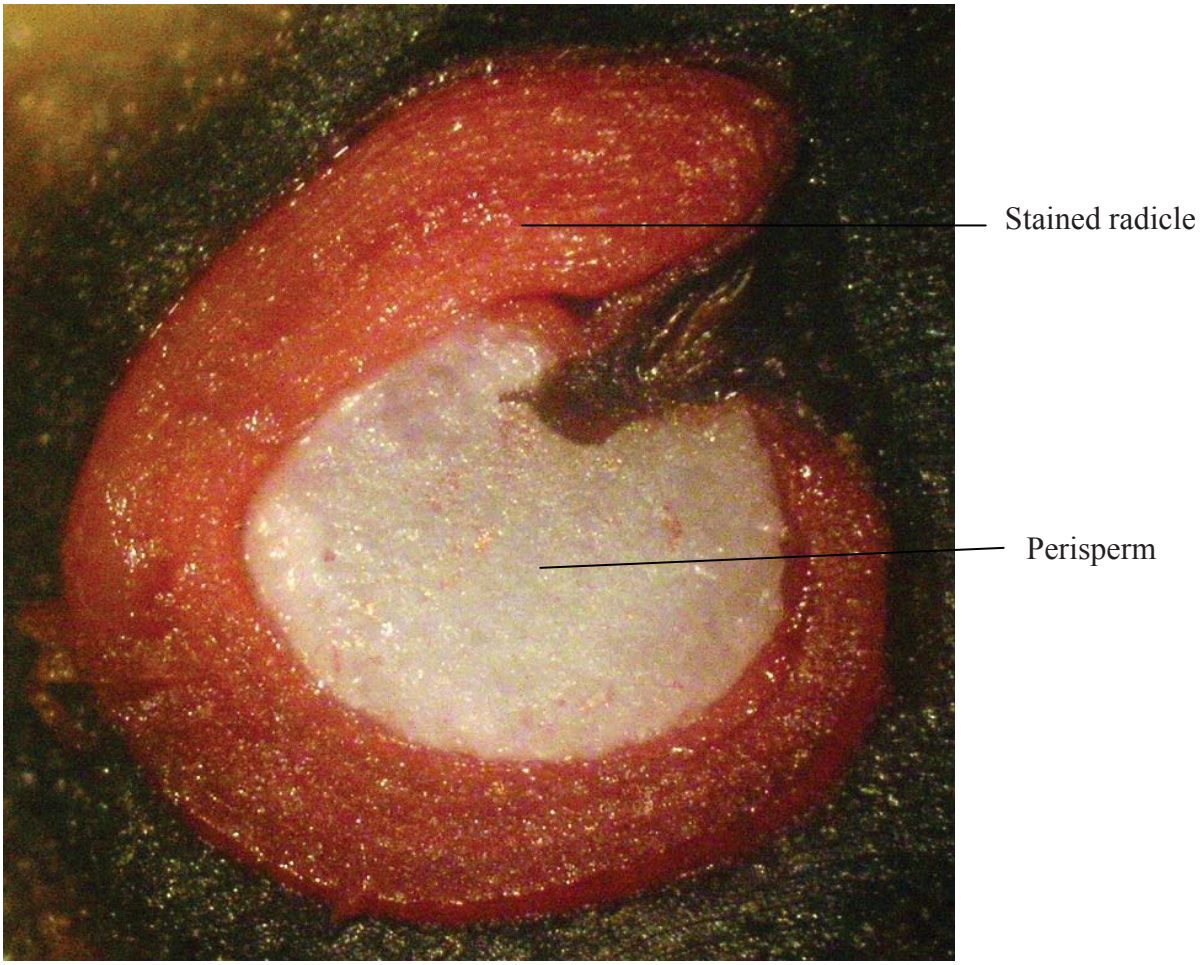
Where n_a and n_b are cumulative emergence counts at consecutive times t_a and t_b, where $n_a < 9(N+1)/10 < n_b$ and N is the final number of fruits emerged and, similarly, n_c and n_d are cumulative germination counts at consecutive times t_c and t_d, where $n_c < (N+1)/10 < n_d$ (Coolbear, *et al*., 1984).

3.2.5. Tetrazolium (TZ) test

3.2.5.1. *B. vulgaris*

Fruits that showed no evidence of seed germination after 14 days were carefully examined. The opercula/operculum was removed from the fruit in order to expose each seed. As specified by ISTA (2009), after removing each operculum, fruits were classified as "dead" if seeds were soft or flaccid and discoloured, and they were classified as "empty" if there was no seed present or if they only contained some residual tissue from underdeveloped seed. If the remaining fruits of multigerm clusters had at least one seed which appeared clean and firm, these were tested for viability. Fruits were soaked for 24 hours in a 1% tetrazolium chloride (TZ) solution at 30 ºC (Leist, *et al.*, 2003) (Figure 10). Fruit were classified as "viable" (dormant) if at least 2/3 of the seed's radicle was stained (ISTA, 2003). The perisperm is not expected to stain in *B. vulgaris* because it is non-living tissue. Unstained fruits or seeds which had more than 1/3 of their radicles unstained were classified as "non-viable". If fruits were found to be viable by the TZ test, they were considered to be dormant.

Figure 10. Tetrazolium stained embryo of a *B. vulgaris* seed indicating viability



3.2.5.2. *T. tetragonioides*

Ungerminated *T. tetragonioides* fruits were prepared by removing the spongy mesocarp tissue and then cutting the fruit to expose the multiple seeds within the fruit. The fruits were then soaked in a 1% TZ solution at 20 ºC for 24 hours (Figures 11 and 12). Fruits were classified as viable, non-viable, dead or empty as described for the *B. vulgaris* fruits. Fruits were classified as viable if at least one embryo was stained. This was assessed by pushing the individual seeds out from the fruit. The endosperm is not expected to stain in *T. tetragonioides* because it is non-living tissue.

Figure 11. A diagonally cut *T. tetragonioides* fruit showing stained (tetrazolium) embryos and a single unstained (non-viable) embryo.

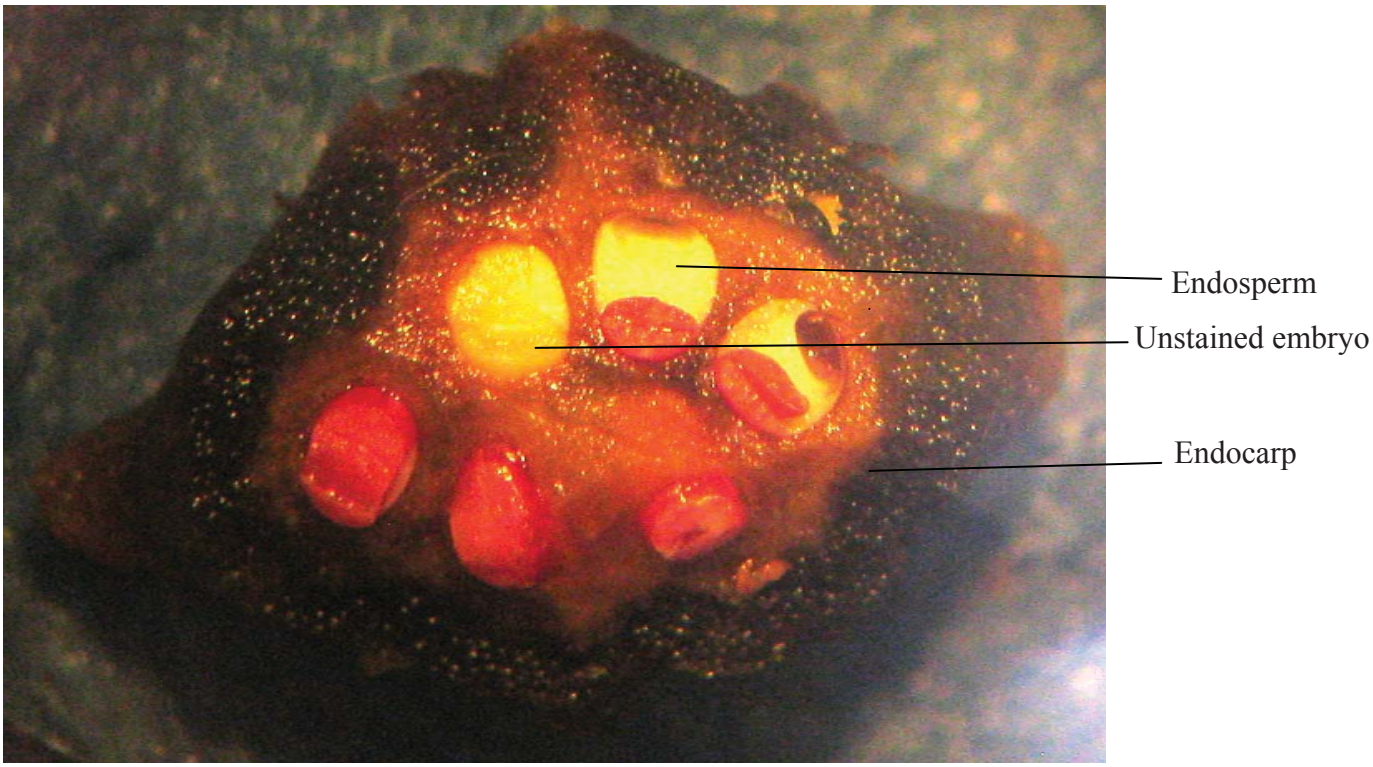
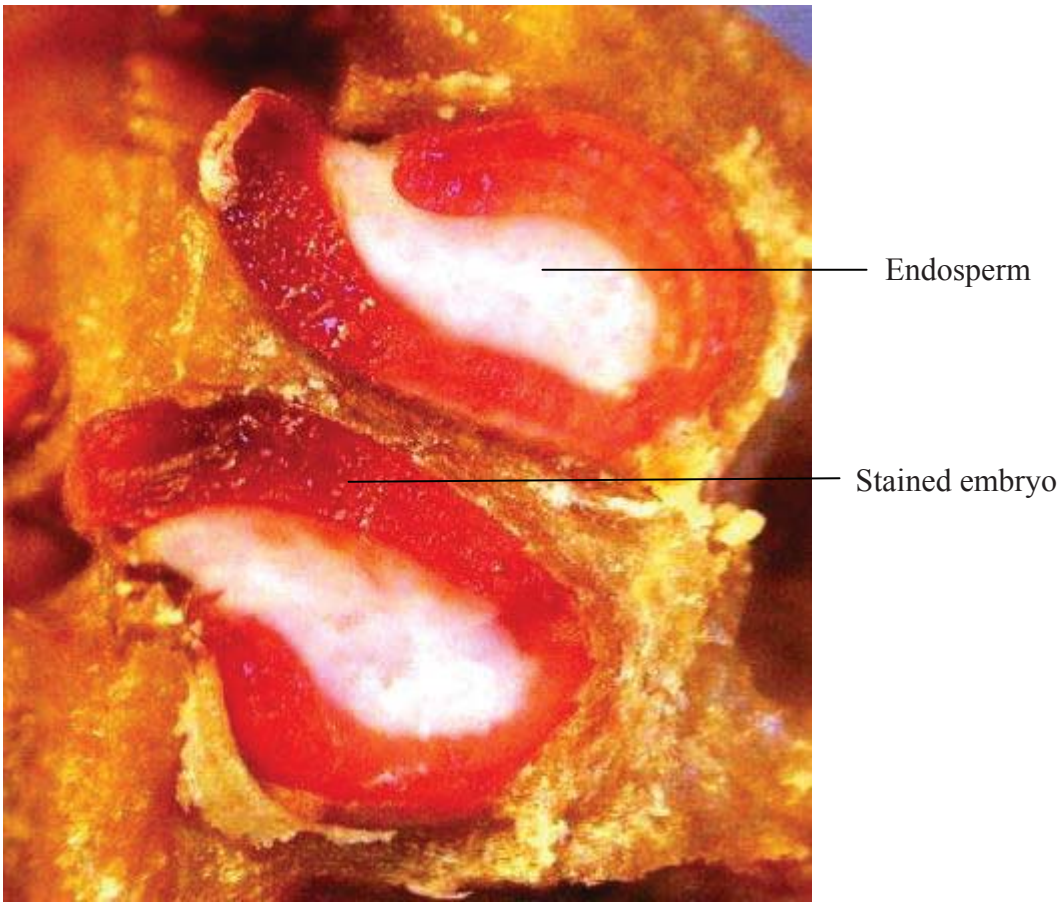


Figure 12. *T. tetragonioides* seeds showing stained (tetrazolium) embryos and non-stained endosperm.



3.3. Experiment 3: *T. tetragonioides* follow up experiment

A follow up experiment on *T. tetragonioides* was conducted in 2011 because the dormancy-breaking recommendations in the 2011 edition of the ISTA Rules (ISTA, 2011) for *T. tetragonioides* germination changed. The 2011 edition of the ISTA Rules (ISTA, 2011) specified that the fruits should be soaked for 24 hours before sowing as opposed to the 2010 edition (ISTA, 2010) which stated that *T. tetragonioides* fruits should be pre-washed for two hours.

The same seed lot that was used in the main experiment was used for the follow up experiment.

The treatments were as follows:

i) Fruits soaked for 24 hours in water at 25 ˚C

ii) Fruits soaked for two hours in water at 25 ˚C

iii) Fruits washed for two hours at 25 ˚C and dried back for two hours at 20 ˚C

iv) Dry fruit control

The washed fruits were dried back and the soaked fruits were not dried back because the 2010 edition of the ISTA Rules (ISTA, 2010) specify that washed fruits should be dried back while the 2011 edition (ISTA, 2011) stated that soaked fruits should be sown immediately after the soaking treatment without drying back.

As before, treatments were replicated four times and the water used was pre-equilibrated at 25 ˚C. Each replicate had 105 fruits. 50 fruits were sown for germination after the treatments and the remaining 55 fruits were used for measuring SMC following the treatments in order to assess the effects of the different treatments on elevating the initial SMC and perhaps contribute to explaining the effects on subsequent germination.

SMC, germination, time taken for fruits to reach 50% of the final germination percentage (T_{50}) and germination uniformity were all determined as in the main experiment.

After the germination period of 35 days, all the germinated fruits were removed and categorised as “abnormals” and “normals” for abnormal and normal seedlings respectively. The combined abnormal and normal germination numbers were named “total final germination 35 DAS”. To determine the effect of cutting on dormancy-breaking, any ungerminated fruits (“ungerminated fruits 35 DAS”) were cut to remove the hard dry endocarp where the horns are located (away from the pith tissue) and to expose the seed embryos. The cut fruits were left to germinate for a further six days and then assessed again. At the end of the six days, fruit which had germinated was classified as “germinated fruits after cutting based on the fruits sown”. This value was then expressed as a percentage of the total number of fruits that germinated after cutting, and this was named “germinated fruits based on cut fruits”. Fruits were classified this way in order to remove any bias that would be caused by variability in germination from the earlier dormancy-breaking treatments.

A TZ test was then carried out on remaining ungerminated fruits as in the main experiment. Dead and empty fruits were named “dead fruits based on fruits sown” and “empty fruits based on fruits sown”. Dead and empty fruits were then expressed as a percentage of the number of cut fruits and these were named “dead fruits based on cut fruits” and “empty fruits based on cut fruits”.

3.4. Data analysis

SAS Statistical Software package was used for statistical analysis of data (SAS 9.2 for Windows). Data was tested for normality using the Proc Univariate procedure. The data was not normally distributed. Where the arcsine square-root transformation was able to normalise the data, (Kolmogorov-Smirnov tests were $P>0.05$), the General Linear Model (GLM) procedure was used to perform the analysis of variance (ANOVA) on transformed data. Mean comparison was determined by using the least significant difference (LSD) procedure.

All data that could not be normalised (Kolmogorov-Smirnov tests were $P< 0.05$) i.e. 'dead' and 'empty' fruits data for the preliminary experiment, 'non-viable' fruits data for the main experiment, and all data in the *T. tetragonioides* follow up experiment except 'normal', 'abnormal', 'germinated fruits after cutting based on fruits sown', 'ungerminated fruits 35 DAS', and MC data was analysed using the Kruskal-Wallis test and the Bonferroni Multiple Comparisons test was used to determine differences among means. All differences declared for the non-normal data for all experiments are based on the Kruskal-Wallis test. Untransformed data is presented in the Tables and Figures, but where significant differences are identified these are based on analysis transformed data or the Kruskal-Wallis test as appropriate.

The Proc Reg procedure was used to determine the relationship between TSW and germination of multigerm vegetable beet seed lots.

CHAPTER 4

RESULTS

4.1. Preliminary experiment: Screening seed lots for dormancy

4.1.1. *B. vulgaris*

The level of dormancy in the *B. vulgaris* seed lots was low. Out of the 18 seed lots screened, 12 seed lots had more than 80% germination in the absence of any dormancy-breaking treatments (Figure 13). Half of these 12 were in the 90% plus germination range i.e. SL2 Blain VB (firsts), SL9 Le Poutre VB (firsts), SL1 Blain VB (firsts), SL16 Palace SB, SL7 Watson VB (firsts) and SL18 Magnum FB with 98%, 95%, 95%, 95%, 93%, and 90% germination respectively. The other half had germination of 80 - 90% range i.e. SL11 Ruby VB, SL4 Taylor VB (firsts), SL15 Nestor FB, SL10 Le Poutre VB (seconds), SL3 Blain VB (seconds), and SL17 Kyros FB. Of the remaining seed lots, four were in the 70% range i.e. SL6 Taylor VB (seconds) , SL12 Brigadier FB, SL13 Kings Seeds SB, and SL14 Flores SB, and two seed lots had the lowest germination (less than 70%) with SL5 Taylor VB (seconds) having a germination of 67% and SL8 Watson VB (seconds) a germination of 52%.

For those seed lots that had less than 90% germination, only SL13 Kings Seeds SB, SL14 Flores SB, SL12 Brigadier FB and SL17 Kyros FB had moderate to minor percentages of ungerminated viable fruit (Figure 14). SL13 Kings Seeds SB and SL14 Flores SB had 14% and 16% ungerminated viable fruit respectively and the other two seed lots had 5% ungerminated viable fruit each suggesting that there was some residual dormancy in these seed lots.

SL13 Kings Seeds SB and SL14 Flores SB were selected for the main experiment because the lower germination in these seed lots was mainly due to higher percentages of viable fruits i.e. 14% and 16% respectively. The Brigadier FB and Kyros FB seed lots were also selected because they had some residual dormancy (5% viable fruits remaining ungerminated). This was lower than that of SL13 Kings Seeds SB and SL14 Flores SB, but SL12 Brigadier FB and SL17 Kyros FB were included in the trial to determine if the dormancy-breaking treatments would alleviate this residual dormancy.

The remaining seed lots which also had germination of less that 90% had higher percentages of non-viable, dead, or empty ungerminated fruit than viable non-germinated fruit. The actual lowest germinating seed lots from the initial screening (SL5 Taylor VB (seconds) (67%) and SL8 Watson VB (seconds) (52%)) were not selected for the main experiment. SL8 Watson VB (seconds) was not selected because of a high percentage of empty fruits (32%) rather than seed dormancy. Seed lots 5 (67%) and 6 (79%), both Taylor VB (seconds) had significantly lower germination than SL4 Taylor VB (firsts) (88%). Seed lot 5 (Taylor VB, seconds) had low germination mainly due to a high percentage of empty fruits (23%). In contrast the low germination of the second seed lot of SL6 Taylor VB (seconds) was a result of a combination of non-viable (7%), dead (6%), and empty (7%) fruits.

Seed lots graded as “firsts” and “seconds” differed in their thousand seed weights (TSWs) with “firsts” always having a higher TSW than the same seed lot graded as “seconds” (Figure 15). The two significantly lowest germinating seed lots were the poorer grade (seconds) of the seed lots. SL8 Watson VB (seconds) (TSW = 11.7g) had a germination result of just 52% compared to SL7 Watson VB (firsts) (TSW = 15.0g) with 93% germination. Similarly, SL5 Taylor VB (seconds) with a TSW of 9.5g, compared with a TSW of 10.2g for the firsts also had a much poorer germination. The lower germination in the seconds was due to the presence of high levels of dead and empty fruits.

A correlation analysis of TSW and germination of the graded (“firsts” and “seconds”) multigerm vegetable beets and SL11 Ruby VB, the only other vegetable beet (also multigerm), found that $R^2 = 0.14$ i.e. TSW caused little of the variation in germination.

Figure 13: Germination (%) of 18 *B. vulgaris* seed lots screened for dormancy with MSE bars

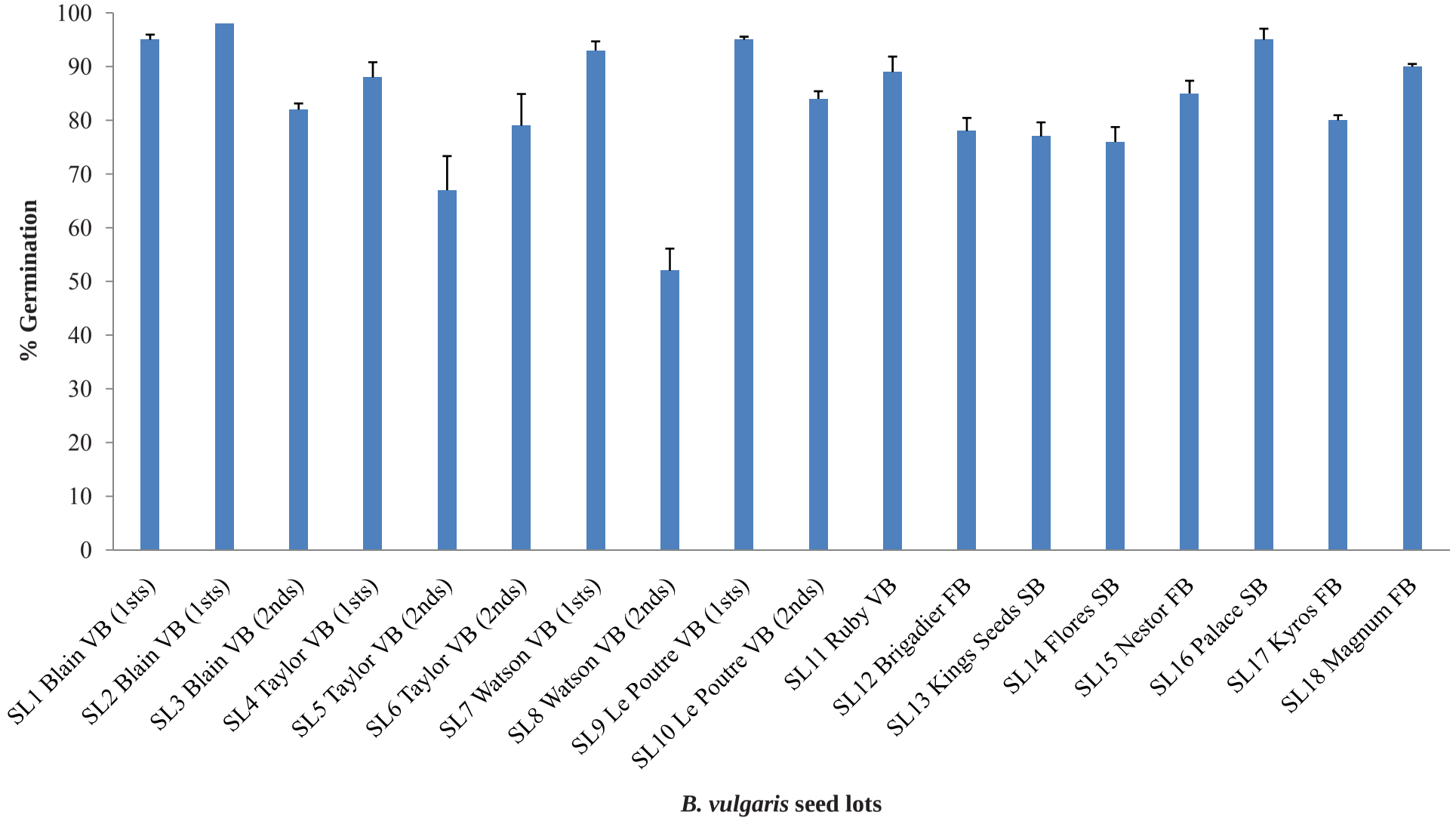


Figure 14: Dead and empty fruits (%) and tetrazolium (TZ) test results for remaining apparently healthy but non-germinated fruits (viable and non-viable fruits) of 18 *B. vulgaris* seed lots screened for dormancy with MSE bars

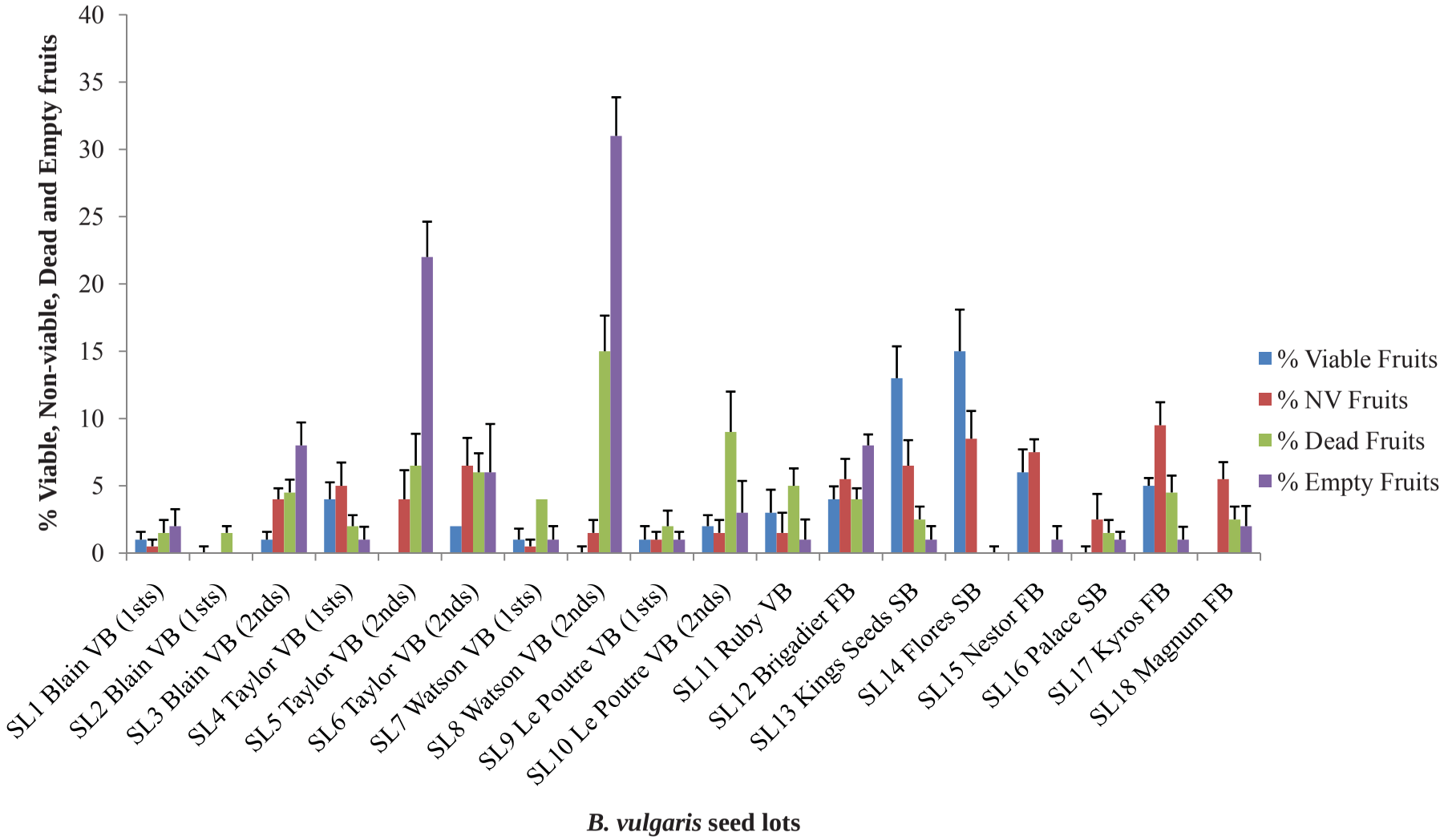
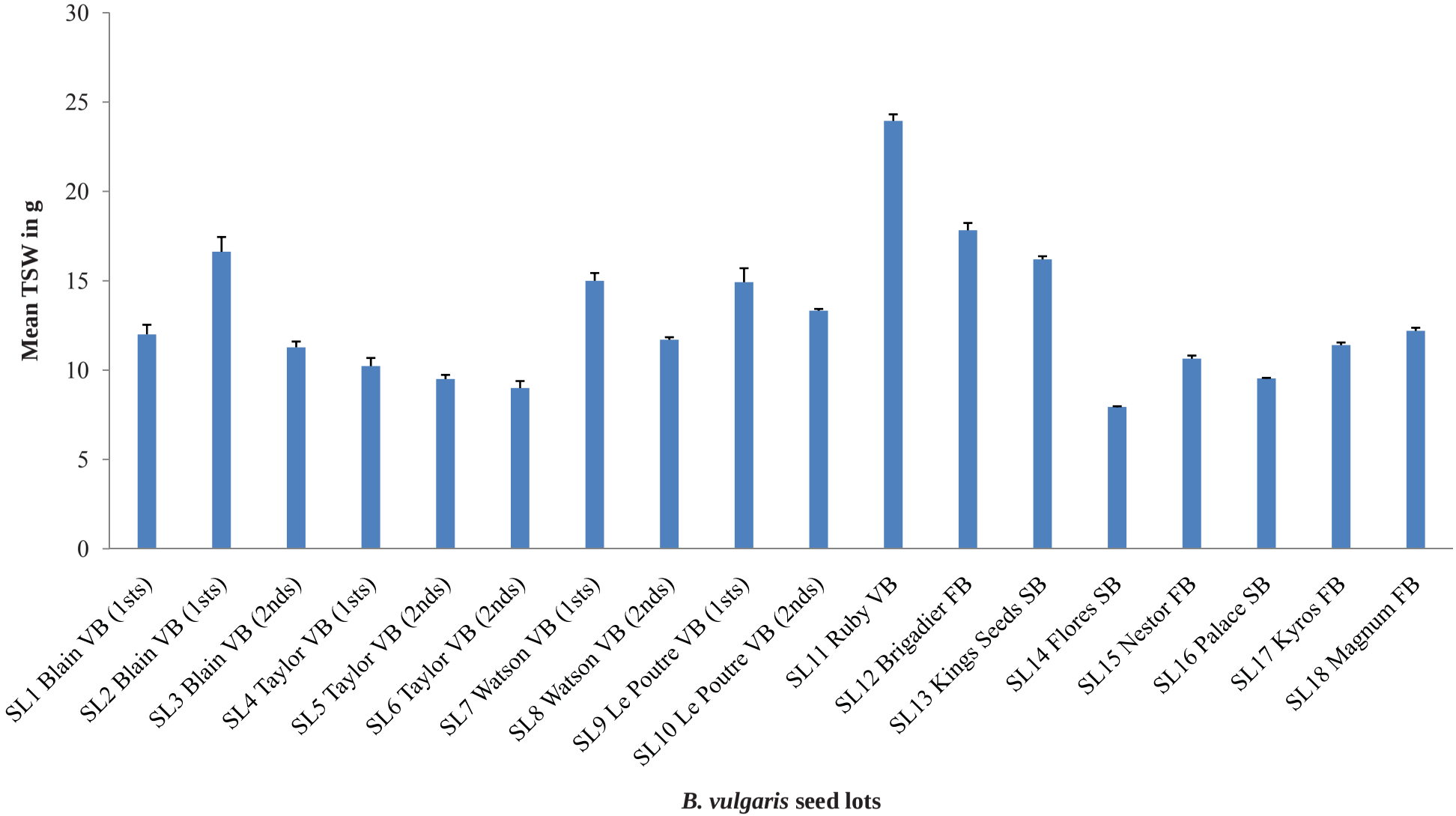


Figure 15: Thousand seed weight (TSW) (g) of the initially screened *B. vulgaris* seed lots with MSE bars



4.1.2. *T. tetragonioides*

A two hour washing treatment was also recommended for *T. tetragonioides* in the 2010 edition of the ISTA Rules (ISTA, 2010). *T. tetragonioides* as a different species from *B. vulgaris*, was analysed separately.

The seed lot of *T. tetragonioides*, assessed had poor germination (63%) but had a high percentage of viable fruit (34%) indicating higher levels of dormancy in *T. tetragonioides* than was observed in *B. vulgaris* seed lots (Table 2).

Table 2: TSW (g), mean germination (%), and tetrazolium (TZ) test results for the *T. tetragonioides* seed lot. SE of the mean is given after the means.

Name	Mean TSW (g)	%Mean Germination	% Viable Fruits	% NV Fruits	% Dead Fruits	% Empty Fruits
T. tetragonioides	64.48±1.92	63±5.85	34±5.51	1±0.5	0	2±1.15

4.2. Main experiment

4.2.1. *B. vulgaris*

For the main experiment the four seed lots that showed the highest percentage of viable but ungerminated (dormant) fruits in the screening experiment were used. These were two sugar beet (SB) cultivars: Flores SB and Kings Seeds SB, and two fodder beet (FB) cultivars, Brigadier FB and Kyros FB. There were significant differences amongst the four *B. vulgaris* seed lots in all the variables except in dead fruits (Table 3). However, moisture content differences which occurred, either before drying (MCBD) or after drying (MCAD) as a consequence of the two dormancy-breaking treatments (soaking and washing) did not affect germination compared with that of control fruit. Nor was there an interaction effect between the four *B. vulgaris* seed lots and the dormancy-breaking treatments (Table 4).

4.2.1.1. Selection of seed lots with the highest possible dormancy

The *B. vulgaris* seed lots selected were Brigadier FB, Kings Seeds SB, Flores SB, and Kyros FB with 78%, 76%, 75%, and 80% germination and 5%, 16%, 14%, and 5% dormancy

(ungerminated viable fruits) respectively. These seed lots were among the lowest germinated seed lots from the initial screening.

4.2.1.2. Moisture content

There were significant differences in the moisture contents within the seed lots following the washing and soaking treatments (Table 4). In all the *B. vulgaris* seed lots, the untreated dry fruits (control), as expected had significantly lower moisture content (9.67%) than the soaked (37.51%) or washed fruits (37.24%) before drying.

Within the four *B. vulgaris* seed lots, there was no significant difference between the moisture content of soaked compared with washed indicating that washing and soaking had allowed the same amounts of water to be absorbed by the fruits.

While among the different seed lots, the untreated dry fruits did not have significantly different moisture contents, the MCBD and the MCAD of the treated fruits, again as expected were different. Brigadier FB had the highest MCBD for pre-wash and pre-soak treatments, 41.8% and 40.57% respectively. The washing treatment for Flores SB had the second highest MCBD while the soaking was lower but similar to both MCBD of its washing treatment and both washing and soaking treatments of Brigadier FB. The moisture content of the Kyros FB washed and soaked fruits were not significantly different from each other but significantly lower than MCBD of both Flores SB treated fruits. Kings Seeds SB had the lowest MCBD of the treated fruits, 32.91% for the soaked fruits and 31.59% for the pre-washed fruits but these two were not significantly different from each other (Table 5A).

Table 3: Relative seed quality of the *B. vulgaris* four seed lots that showed the highest percentage of dormant fruits in the screening experiment

Seed Lot	MCBD[1]	MCAD[2]	Germination		TG[3]	Dead Fruits	Empty Fruits	Fresh Ungerminated Fruits		TV[5]	Germ. Based on TV[5]	T_{50} [6]	Uniformity[7]
			Normal	Abnormal				Viable	NV[4]				
N=12	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(hours)	(hours)
Flores SB	29.68b	23.04a	85a	2b	87a	3a	1b	7a	2a	94a	92b	64.45a	169.22a
Kings Seeds SB	24.52d	18.15b	74b	2b	76b	5a	3b	9a	7a	85b	89b	48.77b	98.36b
Brigadier FB	30.89a	23.91a	68b	8a	76b	7a	5a	8a	4ab	84b	90b	46.76b	73.17b
Kyros FB	27.47c	20.82ab	88a	4b	92a	4a	0c	3b	1b	94a	97a	43.80b	58.20b
Significance	<0.0001	0.0253	<0.0001	0.0038	<0.0001	NS	<0.0001	0.009	0.0628	<0.0001	0.0051	0.0022	0.0007

Values followed by the same letter are not significantly different at P=0.05

1= Moisture Content Before Drying (but after soaking or washing)

2=Moisture Content After Drying for two hours (but after soaking or washing)

3=Total germination (normal+abnormal germination)

4=Non-viable (as assessed by tetrazolium test)

5=Total Viability (normal+abnormal+tetrazolium stained seeds judged viable)

6=Mean Germination Time (T_{50} = time to 50% germination of all eventual germinants)

7=Spread of germination (T_{90} - T_{10} = time for 80% of all eventual germinants to emerge)

Table 4: Effect of soaking and washing dormancy-breaking treatments on the four *B. vulgaris* seed lots that showed the highest percentage of dormant fruits in the screening experiment

Treatment	MCBD[1]	MCAD[2]	Germination		TG[3]	Dead Fruits	Empty Fruits	Fresh Ungerminated Fruits		TV[5]	Germ. Based on TV[5]	T_{50}[6]	Uniformity[7]
			Normal	Abnormal				Viable	NV[4]				
N=16	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(hours)	(hours)
Control	9.67b	9.67b	79a	3a	82a	4a	3a	6a	5a	89a	93a	50.06a	106.10a
Soak	37.51a	28.10a	81a	4a	85a	4a	2a	6a	3a	91a	93a	50.74a	94.31a
Wash	37.24a	26.67a	77a	4a	81a	6a	2a	8a	3a	88a	92a	52.03a	98.80a
Significance	<0.0001	<0.0001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TRT X SEED LOT	<0.0001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values followed by the same letter are not significantly different at P=0.05

1= Moisture Content Before Drying (but after soaking or washing)

2=Moisture Content After Drying for two hours (but after soaking or washing)

3=Total germination (normal+abnormal germination)

4=Non-viable (as assessed by tetrazolium test)

5=Total Viability (normal+abnormal+tetrazolium stained seeds judged viable)

6=Mean Germination Time (T_{50} = time to 50% germination of all eventual germinants)

7=Spread of germination (T_{90} - T_{10} = time for 80% of all eventual germinants to emerge)

Table 5A: Changes in the moisture content of the four selected *B. vulgaris* seed lots as a result of washing or soaking treatments

Variety or Source of the Seed Lot	Treatment	MCBD[1] (%)	MCAD[2] (%)
Flores SB	Control	9.56	9.56
Flores SB	Soak	39.64	30.21
Flores SB	Wash	39.84	29.34
Kings Seeds SB	Control	9.07	9.07
Kings Seeds SB	Soak	32.91	23.57
Kings Seeds SB	Wash	31.59	21.86
Brigadier FB	Control	10.30	10.30
Brigadier FB	Soak	40.57	30.94
Brigadier FB	Wash	41.80	30.49
Kyros FB	Control	9.73	9.73
Kyros FB	Soak	36.93	27.69
Kyros FB	Wash	35.75	25.03
	$LSD_{0.05}$	1.52	6.84

Table 5B: Changes in the moisture content of the single *T. tetragonioides* seed lot as a result of washing or soaking treatments

Name of the Seed Lot	Treatment	MCBD[1] (%)	MCAD[2] (%)
T. tetragonioides	Control	10.46	10.46
T. tetragonioides	Soak	45.15	37.82
T. tetragonioides	Wash	44.07	30.78
	$LSD_{0.05}$	1.88	8.38

1=Moisture Content Before Drying (but after soaking or washing treatment)

2=Moisture Content After Drying for two hours (but after soaking or washing)

Table 6A: Effect of soaking and washing dormancy-breaking treatments on each *B. vulgaris* seed lot

Variety or Source of the Seed Lot	Treatment	Germination					Fresh Ungerminated Fruits			
		Normal (%)	Abnormal (%)	TG[1] (%)	Dead Fruits (%)	Empty Fruits (%)	Viable (%)	NV[2] (%)	TV[3] (%)	Germ. Based on TV[3] (%)
Flores SB	Control	83	1	84	3	2	9	2	94	90
	Soak	86	3	89	2	0	7	2	96	92
	Wash	87	2	89	4	1	5	1	94	95
Kings Seeds SB	Control	76	1	77	4	2	9	8	86	89
	Soak	78	2	80	3	2	7	8	87	92
	Wash	70	1	71	7	4	11	7	82	87
Brigadier FB	Control	69	6	75	5	7	5	8	80	94
	Soak	73	7	80	7	4	9	0	89	90
	Wash	64	9	73	8	4	11	4	84	87
Kyros FB	Control	90	3	93	3	0	2	2	95	98
	Soak	86	5	91	3	0	3	3	94	97
	Wash	88	3	91	6	0	3	0	94	97
	$LSD_{0.05}$	13.14	5.78	10.67	7.57	3.1	6.8	NS	8.73	7.86

NS according to the Benferroni Mutiple Comparisons test.

Table 6B: Effect of soaking and washing dormancy-breaking treatments on the single *T. tetragonioides* seed lot

Name of the Seed Lot	Treatment	Germination		TG[1]	Dead Fruits	Empty Fruits	Fresh Ungerminated Fruits		TV[3]	Germ. Based on
		Normal (%)	Abnormal (%)	(%)	(%)	(%)	Viable (%)	NV[2] (%)	(%)	TV[3] (%)
T. tetragonioides	Control	72	0	72	2	0	26	0	98	73
T. tetragonioides	Soak	63	0	63	1	1	32	3	95	67
T. tetragonioides	Wash	70	0	70	2	0	28	0	98	71
	$LSD_{0.05}$	13.08	0	13.08	3.84	2	15.01	NS	5.06	14.5

NS according to the Benferroni Mutiple Comparisons test.

1=Total germination (normal+abnormal germination)

2=Non-viable (as assessed by tetrazolium test)

3=Total Viability (normal+abnormal+tetrazolium stained seeds judged viable)

4.2.1.3. Germination and tetrazolium test results

There were no significant differences in total germination (i.e. the percentage of normal and abnormal seedlings) for any of the *B. vulgaris* seed lots or the single *T. tetragonioides* seed lot (Tables 6A and 6B) as a result of the washing and soaking treatments applied, indicating that the dormancy-breaking treatments were unable to alleviate residual dormancy in any of the seed lots.

For the *B. vulgaris* seed lots the total germination was corrected to a percentage of the total viability (TV) of the seed lots. TV is the total number of normal and abnormal seedlings, plus ungerminated viable fruit (as assessed by the Tetrazolium test). This correction was done in order to remove any bias caused by different seed lots having different levels of empty or dead fruit. There were no significant differences in the germination of the seed lots after this correction.

4.2.1.4. Mean germination time and uniformity

The four *B. vulgaris* seed lots had different responses to the pre-sowing treatments in terms of the mean germination time (T_{50}) and germination uniformity (Table 7A).

While there was no significant difference between the T_{50} of soaked and washed fruits of Flores SB, these two T_{50}'s were significantly higher than that of the control (49.9 hours). However, there was no significant difference in the uniformity of germination of the treated and untreated Flores SB seed lot.

Pre-soaking and pre-washing treatments in both Kings Seeds SB and Kyros FB in contrast produced no significant differences in either T_{50} or uniformity compared with the control.

However while there was no significant difference between the T_{50} of the two dormancy-breaking treatments for the Brigadier FB seed lot, the control T_{50} took significantly longer than that of the soaking but not the washing treatment. This shows that unlike in Flores SB, where soaking slowed down germination, in Brigadier FB soaking sped up germination.

There were no significant differences in the uniformity of germination between the control and the dormancy-breaking treatments for Brigadier FB. Thus, as in the other three *B. vulgaris* seed lots, there was no effect of the dormancy-breaking treatments on uniformity of germination.

4.2.2. T. *tetragonioides*

4.2.2.1. Moisture content

As for the *B. vulgaris* fruits, the MCBD and MCAD of the control *T. tetragonioides* seed lot were significantly lower than the MCBD and MCAD of the treated fruits (Table 5B)

There was, however, no significant difference between the MCBD for the soaking and washing treatments (45.15% and 44.07%), nor between the MCAD of the soaking and washing treatments (37.82% and 30.78% respectively).

4.2.2.2. Germination and tetrazolium test results

There was no significant difference in the percentages of TG, abnormal fruits, dead and empty fruits, viable and non-viable fruits, between either the soaked and washed fruits or the treated and untreated fruits.

As observed in the preliminary experiment, the main reason for lower germination was dormant fruits, with high percentages of fresh ungerminated fruits remaining at the end of the germinating trial (26%, 32%, and 28% for untreated, soaked, and washed fruits respectively). As there was no difference in the TG of soaked and washed fruits compared with the control, nor any reduction in the number of fresh ungerminated fruits after treatment, the washing and soaking treatments did not help to alleviate the dormancy in *T. tetragonioides*.

4.2.2.3. Mean germination time and uniformity

There were no significant difference in the T_{50} and uniformity of the untreated, washed, and soaked fruits of *T. tetragonioides* (Table 7B).

Table 7A: Mean germination time (T_{50}) and spread of germination (uniformity) of the four *B. vulgaris* seed lots without treatment and after washing and soaking

Variety or Source of the Seed Lot	Treatment	T_{50} (hours)	Uniformity (hours)
Flores SB	Control	49.9	176.53
Flores SB	Soak	72.26	172.36
Flores SB	Wash	71.17	158.77
Kings Seeds SB	Control	50.29	106.68
Kings Seeds SB	Soak	48.38	98.83
Kings Seeds SB	Wash	47.65	89.56
Brigadier FB	Control	56.79	72.75
Brigadier FB	Soak	37.76	47.49
Brigadier FB	Wash	45.72	99.27
Kyros FB	Control	43.28	68.45
Kyros FB	Soak	44.55	58.55
Kyros FB	Wash	43.58	47.6
	$LSD_{0.05}$	18.9	91.49

Table 7B: Mean germination time (T_{50}) and spread of germination (uniformity) of the single *T. tetragonioides* seed lot without treatment and after washing and soaking

Name of the Seed Lot	Treatment	T_{50} (hours)	Uniformity (hours)
T. tetragonioides	Control	93.75	251.66
T. tetragonioides	Soak	101.33	320.1
T. tetragonioides	Wash	93.35	303.62
	$LSD_{0.05}$	18.56	205.39

4.3. Experiment 2: *T. tetragonioides*

Due to a change in the seed testing specifications of *T. tetragonioides* between the 2010 and 2011 editions of the ISTA Rules, a second experiment was carried out. Instead of washing the fruits before sowing (ISTA, 2010), the 2011 edition specified that *T. tetragonioides* fruits should be soaked for 24 hours prior to being sown (ISTA, 2011). The reason for this change was not explained, but it was deemed to be useful that the two methods should be compared to determine any differences.

4.3.1. Moisture content

The 24 hour soaking treatment resulted in significantly higher MC being reached by the fruit (56.06%), than the two hour soaking (44.81%) or washing treatments (36.97%) (Table 8). This reflects the longer soaking time of the 24 hour treatment allowing the fruits to imbibe more water.

The two hour soaking treatment had a significantly higher moisture content at sowing than the two hour washing treatment. This is because the two hour soaked fruits had been dried for two hours before sowing. The control (dry) fruits had a moisture content of 10.86%.

4.3.2. Germination and tetrazolium test results

The results of final germination, 35 days after sowing, show that the 24 hour soak treatment had significantly lower germination (40%) compared with other treatments (av. 60%). There was no significant difference in germination between the control germination and the other two two-hour dormancy-breaking treatments.

At the end of the germination trial i.e. 35 days, the 24 hour soaking treatment had a significantly high percentage of ungerminated fruits (60%) compared with the control and the other two dormancy-breaking treatments. To determine the reason (e.g. dormancy or lack of viability), ungerminated fruits from all treatments were cut at the end of 35 days i.e. removing the hard tissue at the top of the fruit towards the apex. This allowed germination to proceed particularly in the 24 hour soaking treatment, which had a higher percentage of fresh ungerminated fruit.

As a result of the cutting, further germination proceeded across all treatments (av. 75%), especially in the 24 hour soak treatment, such that there was no significant difference between any treatments including the control.

There were no fresh ungerminated fruits remaining after cutting indicating the cutting was able to alleviate any dormancy remaining in the fruits. Any fruit that did not germinate was either empty (0 - 3%) or dead (8 - 15%).

4.3.3. Mean germination time and uniformity

There was no significant difference in the time it took the fruits to reach 50% germination between treatments and this is shown in Table 9. There were differences however in the uniformity of germination. The two hour wash and two hour soak treatments improved the uniformity of germination (479.20 and 414.39 hours respectively) compared to the control (596.96 hours). The 24 hour washing treatment increased the spread of germination (723.08 hours).

Table 8: The effect on seed quality of two soaking and one washing dormancy-breaking treatments on a single *T. tetragonioides* seed lot

Treatment	MC (%)	Final Germination		Total Final Germ 35 DAS (%)	Ungerminated Fruits 35 DAS (%)	Germinated Fruits After Cutting Based on Fruits Sown (%)	Dead Fruits Based on Fruits Sown (%)	Empty Fruits Based on Fruits Sown (%)	Germ. Based on Cut Fruits (%)	Dead Fruits Based on Cut Fruits (%)	Empty Fruits Based on Cut Fruits (%)	Total Germinati of Fruits After Eac Treatmer (%)
		Normal (%)	Abnormal (%)									
24 Hr Soak	56.06	39	1	40	60	45	15	0b	75	25	0b	85
2 Hr Soak	44.81	59	1	60	40	31	9	0b	74	26	0b	91
2 Hr Wash	36.97	63	1	64	36	25	8	3a	70	23	7a	89
Control	10.86	54	3	57	43	34	9	0b	79	21	0b	91
$LSD_{0.05}$	1.18	10.93	2.11	10.85	10.85	14.51	NS	-	NS	NS	-	NS

NS according to the Benferroni Multiple Comparisons test

Table 9: Mean germination time (T_{50}) and spread of germination (uniformity) of the single *T. tetragonioides* seed lot

Treatment	T_{50} (hours)	Uniformity (hours)
24 Hr Soak	350.6	723.08a
2 Hr Soak	151.1	414.39b
2 Hr Wash	136.0	479.20b
Control	133.6	596.96ab
	NS	-

The Benferroni Mutiple Comparisons test was used

CHAPTER 5

DISCUSSION

5.1. Preliminary experiment: Screening seed lots for dormancy

The preliminary experiment was carried out to determine the dormancy levels in the *B. vulgaris* seed lots and identify which had the highest level of dormancy for the main experiment.

T. tetragonioides was included in the study as it was the only other species in the ISTA Rules (ISTA, 2010) that required washing to alleviate dormancy and may therefore have similar germination requirements to *B. vulgaris*. As a different species, the *T. tetragonioides* data was analysed separately to that of the *B. vulgaris* and will therefore be discussed separately.

5.1.1. *B. vulgaris*

In this study, a range of *B. vulgaris* seed lots were assessed. Different types i.e. sugar beet, fodder beet, and vegetable beet were assessed, as well as different cultivars.

5.1.1.1. TSW and germination

The fruits with higher TSW had higher germination than fruits with lower TSW. In the graded seed lots, the "Firsts" had higher TSW and also germination than the "Seconds" of the same seed lot. These higher grade seed lots are the seed lots which would be sold, which means that the buyer would have access to the seeds (fruits) with better germination. The results showed that effective seed processing or postharvest handling will play a role in the availability of higher germinating *B. vulgaris* seed lots.

TSW reflects fruit size which influences germination. The greater the TSW, the bigger the size of the fruit. Scott, Harper, Wood, and Jaggard (1974), reported that in monogerm *B. vulgaris*, percentage emergence increased progressively as fruit size increased. One reason for the increased germination was identified through x-ray radiography. In larger fruits, more locules (positions for fruits) were filled with seeds while fewer locules were empty or had shrivelled seeds (Scott, *et al.*, 1974). This positive correlation was also reported by Snyder and Filban (1969) who found that in *B. vulgaris*, a greater percentage of larger sized fruits usually germinate than those in smaller sized fruits. In contrast a correlation analysis of TSW and germination for the multigerm vegetable beets (SL1 to SL11 (Table 1)) in this study (R^2 = 0.14) found that little of the variation in germination was caused by the different TSWs.

The poor correlation in the vegetable seed lots assessed in this study was due to high percentages of empty fruits especially in the graded vegetable multigerm seed lots i.e. the "firsts" and "seconds". Not unexpectedly, seed lots graded as "firsts" had higher TSW and germination than seed lots of the same cultivar graded as "seconds".

5.1.1.2. The question of dormancy in *B. vulgaris* fruits: Does dormancy exist?

In the preliminary experiment, only two of the lowest germinating *B. vulgaris* seed lots, Kings Seeds SB and Flores SB had reasonably high percentages of viable fruits (16% and 14% respectively), indicating at least some residual dormancy in these seed lots. In contrast, the other low germinating seed lots, which were all lower grade (seconds), had lower germination due to empty and dead fruits rather than residual dormancy.

The high germination of most of the *B. vulgaris* seed lots indicates little dormancy was present. In addition, the absence of high percentages of non-germinated viable fruits in those seed lots with low germination confirmed the absence of dormancy in *B. vulgaris* fruits. This adds to the literature evidence that dormancy is no longer a significant issue in many, if not all, of the commercial *B. vulgaris* cultivars used for sugar, fodder or vegetable beet production.

5.1.1.3. Why is dormancy no longer a problem in *B. vulgaris* fruits?

Recent literature (McGrath, 2010; Biancardi, McGrath, Panella, Lewellen, and Stevanato, 2010) does not directly discuss either dormancy in *B. vulgaris* fruits, or the direct selection of cultivars displaying little dormancy. Instead they discuss the availability of *B. vulgaris* cultivars with improved germination (i.e. improved speed and uniformity of germination). It therefore appears that the historically reported dormancy in *B. vulgaris* fruits has been effectively eliminated or at least reduced to such an extent that it is now only a minor issue. This appears to have been a result of breeding for other traits which has led to both the need for improved germination of *B. vulgaris* fruits and the indirect elimination/reduction of dormancy. In addition, *B. vulgaris* seed (fruit) production and seed (fruit) processing techniques have increasingly eliminated empty or immature fruits which would have no or low germination (Biancardi, *et al.,* 2010). This has also contributed to the availability of fruit with improved germination. Also, *B. vulgaris* seed multiplication has created the need for *B. vulgaris* cultivars with improved germination (McGrath, Saccomani, Stevanato, and Biancardi, 2007).

5.1.1.3.1. Seed multiplication purposes

The involvement of *B. vulgaris* breeders in breeding for traits related to *B. vulgaris* seed multiplication i.e. rapid emergence and high germination percentage (Biancardi, *et al.*, 2010) made the production of fruit with high germination ability important (McGrath, *et al.,* 2007). According to McGrath, *et al.* (2007), this is because not only does this trait influence field population uniformity, but in sugar beet cultivars it also affects sugar yield significantly. Improved yields are achieved when plantlets develop quickly, and cover inter-rows quickly for the best interception of light.

5.1.1.3.2. Breeding for other traits

Breeding has been able to produce significant results in *B. vulgaris* yield trait enhancement and also genetic disease resistance (Biancardi, *et al*., 2010). Some of these achievements in *B. vulgaris* breeding e.g. monogerm fruits and polyploidy, have led to the realisation of the importance of improved germination, and in some cases some dormancy has been indirectly bred out (Biancardi, *et al*., 2010).

Low germination, which was a result of breeding for polyploidy i.e. where efforts have been made to modify the number of *B. vulgaris* chromosomes (Biancardi, *et al.*, 2010), led to the indirect breeding of varieties with improved germination. The first tetraploid *B. vulgaris* families (2n = 36 i.e. with twice the number of chromosomes) that were bred had better root shape, few larger leaves, and larger flowers, fruit clusters and pollen grains in comparison to the diploid *B. vulgari*s (2n = 18 i.e. normal number of chromosomes). However Biancardi, *et al.* (2010), report that in Europe as well as elsewhere, the use of diploid hybrid varieties has been becoming more prevalent in the last 25 years. This is because in comparison with the diploid varieties, the tetraploid families had slower germination and root development. Apart from its simpler and less expensive breeding process, easier resistance traits integration and higher seed processing quality, improved germination was also listed as one of the reasons that diploid varieties are becoming prevalent.

McGrath, *et al.* (2007) and Biancardi, *et al.* (2010) reported that the development of genetically monogerm fruits is also one of the exceptional advances in *B. vulgaris* fruit breeding.

Although Wagmann, *et al.* (2010) earlier reported that rapid and synchronous germination has been selected for in cultivated *B. vulgaris* leading to dormancy elimination, it is not clear from the literature if this breeding has been a direct or indirect breeding out of dormancy.

5.1.1.3.3. Seed processing

Kockelmann, Tilcher, and Fischer (2010) stated that in commercial practice, specific pre-sowing treatments (or priming) are frequently carried out to improve seed (fruit) germination characteristics further particularly the homogeneity and speed of germination. Since primed fruits have already gone through part of the germination process, their germination and emergence are accelerated, and they are also more homogenous in comparison to untreated fruits. Pre-treated *B. vulgaris* fruits are well established in all markets and all seed producers in Europe offer varieties that have been pre-treated in some way (Kockelmann, *et al.*, 2010).

According to Joanne Townshend (pers. comm., 2011), *B. vulgaris* fruits (seeds) from the field are pre-cleaned and then put on a gravity table to sort them into different sizes, remove small light fruits, and remove contaminants. It is normally found that germination is highest in the heaviest (biggest) fruits (Joanne Townshend, pers. comm., 2011). Results from the preliminary assessment agree with this report as the graded seed lots (firsts and seconds) always differed in their germination, with the "firsts" always having higher germination that the "seconds" (Figure 13). This finding implies that seed processing plays a role in the germination ability of *B. vulgaris* seed lots.

Kockelmann, *et al.* (2010) stated that the future needs and development of *B. vulgaris* fruits (seeds) include the requirement for seed suppliers to maintain or increase the high level of fruit quality. This is characterized by improved germination and emergence which results in high levels of final field emergence and homogenous plant stands even under stressful environment conditions.

The results from this work i.e. the presence of little or no dormancy in the *B. vulgaris* seed lots assessed, agree with this discussion and suggest that dormancy has been bred out of many commercial *B. vulgaris* cultivars to meet production requirements for the crop produced from the seed. Based on this, it appears that the application of dormancy-breaking requirements as specified by ISTA may not be required for the majority of seed.

5.1.1.4. Possible dormancy mechanisms in *B. vulgaris*

In the preliminary study, two *B. vulgaris* varieties were identified, that still had relatively high percentage of viable fruit that did not germinate (14% and 16% ungerminated viable fruits for Flores SB and Kings Seeds SB respectively). This suggests that there could be factors that may still inhibit germination in some *B. vulgaris* varieties or seed lots.

Three factors that may inhibit *B. vulgaris* germination are 1) a mucilaginous layer that may surround the fruit ii) the tightness of the operculum and 3) the presence of phenolic inhibitors (Taylor, *et al.,* 2003). It is the combined effect of these factors that inhibits *B. vulgaris* seed germination (Taylor, *et al.,* 2003).

5.1.1.4.1. The mucilaginous layer and germination inhibitors

Taylor, *et al.* (2003) conducted a study on Ruby Queen, a non hybrid multigerm vegetable beet cultivar in order to assess factors that affect its germination.

A stereo microscope (10X magnification) was used to observe the presence or absence of mucilage. While separation of fruits (i.e. with/without mucilage) was able to be carried out when fruits were dry, differences became more apparent when fruits were soaked in water for 10 minutes. Fruits with a mucilaginous layer floated, while fruits that did not sank. This is because air was trapped in the mucilage and made the fruits float. Using this method, i.e. density separation, it was found that 75% of the seed lot had significant amounts of mucilage. When fruits were germinated, it was found that rate and final germination were reduced and this reduction was profoundly influenced by the presence of a mucilaginous layer (Taylor, *et al.,* 2003).

When the operculum was removed it was also found that seed germination was enhanced. Removal of the operculum was done by first softening the fruits by soaking for 50 minutes in water, then lifting or decapping the fruits (Taylor, *et al.,* 2003). Both the germination of fruits with or without a mucilaginous layer had about 20% germination on day one and had about 70% germination by day 10 while fruits without a mucilaginous layer had 60% germination by day two and about 85% germination by day 10. When fruit leachate was assessed, it was also found that fruits which had a mucilaginous layer leaked more compounds than fruits without mucilage. This was attributed to a higher concentration of phenolic compounds present in fruits with a mucilaginous layer (Taylor, *et al.,* 2003). These phenolic compounds have previously been reported to inhibit germination in *B. vulgaris* fruits (Chiji, *et al.*, 1980;

Evenari, 1949). One of the cultivars used in the preliminary experiment was SL11 Ruby VB which had only 3% dormancy (ungerminated viable fruits). It is likely that this seed lot is the same as the cultivar "Ruby Queen" reported by Taylor, *et al.* (2003). Based on this assumption, the finding from the preliminary assessment does not agree with the report by Taylor, *et al.* (2003) as there was clearly no effect of the mucilaginous layer, or the presence of germination inhibitors in the total germination of SL11 Ruby VB (89%) without any treatment.

However, Nottingham (2004) in comparison reported that this mucilaginous layer observed in *B. vulgaris* fruit clusters is found in sugar beet in particular. Heydecker, *et al*. (1971) had also reported this phenomenon in beetroot fruits. This suggests that this phenomenon may be present in a range of *B. vulgaris* types. According to Nottingham (2004) cultivars that are prone to having a mucilage layer have a lower germination potential, and are slower to germinate as well as a lower final germination percentage.

5.1.1.4.2. The tightness of the operculum

According to Taylor, *et al.* (2003) other *B. vulgaris* fruits fail to germinate as cells at the operculum periphery fail to separate as a result of tight bonding between lignified cell walls in the region. This is a problem because in order for radicle emergence to occur in germinating fruits, the operculum must separate from the lower fruit wall. Nottingham (2004) explained that the operculum acts as a barrier to the exchange of gases between the environment and the growing embryo. The length of time the operculum stays attached to the rest of the embryo varies among cultivars. In the fruits of cultivars where the operculum takes longer to detach from the rest of the embryo, development is slow since the amount of oxygen reaching the embryo is limited. In instances where the operculum has been softened due to soaking, or has been physically lifted or removed, the rate of germination increases. Nottingham (2004) also pointed out that this effect is more pronounced in cultivars with a mucilaginous layer. The effect of the tightness of the operculum on germination has been discussed in section 2.3.6.

It is these three factors, together termed physico-chemical factors, that play a role in inhibiting *B. vulgaris* seed germination (Taylor, *et al.,* 2003). Any one or combination of these factors, i.e. the presence of chemical germination inhibitors, the tightness of the operculum, or the presence of a mucilaginous layer could have caused ungerminated viable (dormant) fruits of *B. vulgaris* to be present in the screening experiment.

The results from this study found no effect of soaking or washing on the germination of *B. vulgaris* fruits and do not support the presence of germination inhibitors because the residual dormancy present in two of the seed lots was not alleviated by washing or soaking. This implies that the residual dormancy present was a result of the presence of tight opercula in the fruit or the presence of a mucilaginous layer, or the interaction of both factors. However, the purpose of the ISTA dormancy-breaking recommendation (washing) for fruits of *B. vulgaris* is to leach out germination inhibitors. The literature and results from this study i.e. tight opercula and presence of a mucilaginous layer as possible causes of dormancy, imply that washing fruits prior to sowing is not an effective method of alleviating this kind of dormancy. The two and four hour washing does not loosen the opercula or the water used for washing increases the mucilaginous layer which inhibits germination by trapping oxygen.

5.1.2. *T. tetragonioides* germination and its possible dormancy mechanisms

In *T. tetragonioides*, the low germination was due to a high percentage of viable fruits which did not germinate (35%), indicating higher levels of dormancy in this species than observed in *B. vulgaris*. Dormancy is high in *T. tetragonioides* due to the thick endocarp present in the dry fruits of this species. This is evident in the high TSW of *T. tetragonioides* fruits. Also, as the larger part of the fruit is made out of the pith (endocarp) in *T. tetragonioides* and the apex of the fruit is very hard, this may be playing a role in the higher dormancy level observed in *T. tetragonioides*.

5.2. Main experiment

5.2.1. *B. vulgaris*

5.2.1.1. Moisture content

The washing and soaking treatments resulted in the same amount of water being absorbed by the fruits within each seed lot (Table 5A). Therefore there should be no advance in germination due to fruits either being soaked or washed having to rehydrate further than the other treatment. The amount of water absorbed by fruits differed between seed lots. As expected, Brigadier FB absorbed the highest amount of water (MCBD = 40.57 and 41.80% for soaked and washed fruits respectively) because the seed lot had bigger multigerm seeds (TSW = 17.83g) compared to the other two monogerm seed lots i.e. Flores SB (7.94g) and

Kyros FB (11.40g). However, although fruits of Flores SB had the lowest TSW, they absorbed more water (MCBD = 39.64 and 39.84% for soaked and washed fruits respectively) than fruits of Kyros FB (MCBD = 36.93 and 35.75% for soaked and washed fruits respectively) which had a higher TSW. Fruits of Kings Seeds SB (TSW = 16.20g) absorbed the least amount of water although their TSW was not significantly different to that of Brigadier FB. It is not clear why TSW of the three monogerm seed lots appeared not to influence water uptake.

Also, the two hour drying-back step after the washing or soaking treatments permitted the seed moisture content to decline but to a MC (av. MCAD = 28.10 and 26.67% for soaked and washed fruits respectively) which was still significantly above the initial MC (av. 9.67%) i.e. control (Table 5A). Although the untreated fruits (control) were sown with significantly lower MC than the treated (soaked or washed) fruits, this did not appear to have any effect on subsequent germination as there were no significant differences in the untreated fruits and the treated (washed and soaked) fruits.

5.2.1.2. Germination and tetrazolium test results

Total viability (TV) and germination based on TV were assessed in order to ensure that any dead and empty fruits would not bias germination results, i.e. a seed lot that had a higher germination percentage of empty fruits than other seed lots, such as Brigadier FB, would have a lower germination because of the higher percentage of dead and empty fruits. Correcting germination percentages to that based on viable fruit ensured that differences in germination observed when comparing seed lots were the result of different dormancy levels rather than empty or dead fruits. The results corrected to germination as a percentage of viable seed show that there was no effect from washing or soaking treatments on germination compared with that of the control. Neither dormancy-breaking treatment was able to alleviate the residual dormancy (5 - 16%) in the seed lots studied.

A limitation of the experiment was that the levels of dormancy in the seed lots used were relatively low. This was compounded by the Flores SB control having a lower percentage of ungerminated viable fruits (9% for the untreated fruits) than in the preliminary experiment (16% of untreated fruits). A t-test was done to compare the two percentages of ungerminated viable fruits for the untreated fruits (16% and 9%) and it was found that they were significantly different. A t-test was also done for ungerminated viable fruits of Kings Seeds SB, whose dormancy also declined from 14% (for untreated fruits) in the preliminary

experiment to 9% (for untreated fruits) in the main experiment. It was found that 14% was significantly different but similar to 9%. The reasons for this decline of dormancy are not clear. The time between when this seed lot was first tested (28 October, 2010) for the preliminary experiment and the time it was next tested in the main experiment (12 November, 2010) was approximately two weeks.

5.2.1.2.1. The effect of initial moisture content on *B. vulgaris* germination

Drying the fruits back for two hours, as specified by ISTA (2010), caused the fruits to decline to average MC of 28.10 and 26.67% for soaked and washed fruits respectively but as expected did not allow the fruits to reach the original moisture content before washing or soaking (av. 9.67%) (Table 5A). Although the control fruits began imbibition at a lower moisture content than the washed or soaked fruits, there was no effect on either final germination or germination rate. It is therefore unclear why the two hour drying-back step is recommended by ISTA and there are no reports in the literature to indicate that drying back is required. The results from this study suggest that the drying back step may be unnecessary.

5.2.1.2.2. The effect of washing and soaking fruits prior to sowing on germination

There is evidence in the literature to support the findings in this study that washing and/or soaking is not necessary to alleviate dormancy in *B. vulgaris* fruits. Snyder (1959) found that in comparison to unsoaked fruits, there was no alteration in the germination of pre-soaked sugar beet fruits (varieties US400 and US410) germinated at room temperature. According to Snyder (1959), it appears that only in instances where fruits contain high amounts of inhibitors, is soaking beneficial as it dilutes the chemicals to concentrations that allow germination. In a later study by Hoover and Goodin (1966), unwashed sugar beet fruits were used because of the absence of significant differences in germination between fruit washed for two hours in running water and unwashed fruits. The temperature at which fruits were germinated was not reported.

Snyder (1959) reported that fruits of certain *B. vulgaris* varieties may be nearly free of germination inhibitors and soaking of fruit clusters of different varieties will either have no effect, may be beneficial or even be detrimental to germination. In this study, neither washing nor soaking had any effect on subsequent germination. This finding suggests that the fruits were free of germination inhibitors or if germination inhibitors were present, they were present at concentrations too low to have an effect. This is consistent with the suggestion of

Heydecker and Chetram (1971) that water soluble germination inhibitors like phenolic acids, may inhibit germination only at higher concentrations. Moreover this suggests that there have always been varieties at least from the late 1950's that have little or no dormancy and that selection for this trait either directly or indirectly has been used in the development of modern commercial cultivars.

The focus of this study was a comparison between the ISTA recommended dormancy-breaking technique of washing for two hours at 25 °C and the preferred method in New Zealand of soaking for two hours. This has meant that the dormancy-breaking techniques have focussed on removal of water insoluble inhibitors from the fruit. However the failure of the washing and soaking treatments to remove the albeit low levels of residual dormancy in the four seed lots in the main experiment suggest that there may be other dormancy mechanisms present other than the presence of germination inhibitors, such as the operculum physically constraining embryo growth (Heydecker and Chetram, 1971; Snyder, 1959; and Rochalska and Orzeszko-Rywka, 2008). Snyder (1959) also concluded that apart from leaching out germination inhibitors, soaking and drying may also weaken the operculum. There is a possibility therefore that the dormant fruits in this study may have benefitted from additional alternate soaking/washing and drying episodes in order to weaken the operculum but this was outside the scope of this study.

Also, in this study, the preliminary assessment had a higher number of vegetable beet seed lots than the other types of *B. vulgaris*. This study has found that vegetable beet fruits have lower levels of dormancy compared to fodder beets and sugar beets (Figure 14).

In this study, germination was neither improved nor reduced by soaking or washing treatments in either the pelleted (Brigadier FB) or the unpelleted seed lots. This suggests that the pelleting is not inhibiting germination.

In general the results from the main experiment again suggest that there was no effect on germination by germination inhibitors that may have been present or that germination inhibitors were largely absent from the seed lots studied. It seems likely that in the seed lots tested the dormancy present in some fruits is imposed by another mechanism other than germination inhibitors. The mechanism is most likely the tightness of the operculum (Nottingham, 2004; Taylor, *et al*., 2003).

5.2.1.2.3. What does this mean for the use in the ISTA Rules of the washing procedure to alleviate dormancy?

The results of this study suggest that there is little dormancy in commercial *B. vulgaris* cultivars and where there is a small amount of dormancy present, the washing treatment is ineffective in alleviating it. This suggests that the ISTA Rules (2010) pre-sowing specifications for this species may not be required. This is worthy of further investigation as there is considerable cost both in time, specialised equipment, and considerable volumes of 25 °C water required to carrying out these pre-sowing treatments.

After further investigation, e.g. assessing dormancy in a larger number of *B. vulgaris* seed lots of each type of *B. vulgaris* i.e. fodder, sugar and vegetable beets, and perhaps finding that the dormancy-breaking treatments should be retained, the soaking treatment should be considered as an alternative mainly because it is less costly as it does not require the installation of specialised washing equipment, nor large volumes of 25 °C water. Changing the soaking water every 30 minutes is only a minor inconvenience compared with the expense and monitoring involved in setting up and operating a pre-washing system which this study appears to find unnecessary.

Furthermore after washing, the fruits are required to be dried back for another two hours before sowing (ISTA, 2010). This total time of at least four hours may delay the commencement of the germination test by one day (if there is insufficient time to pre-treat the fruit on the day of receipt) and by adding extra steps, the costs associated with germination testing of *B. vulgaris* fruits are increased.

5.2.1.2.4. Why the different responses in T_{50} of *B. vulgaris* seed lots?

In this study, different *B. vulgaris* seed lots responded differently to washing and soaking before sowing. This is in agreement with Snyder (1963) who reported that *B. vulgaris* varieties lack rapid germination and uniformity of emergence varied. Also, germination speed markedly varies even amongst fruits from the same plants within a given variety.

The speed of germination in Flores SB was slow after the washing and soaking treatments compared with both the Flores SB control and the other three *B. vulgaris* seed lots (Table 7A). The lack of any significant difference in the speed of germination between control and washing or soaking treatments is further evidence that germination inhibitors are not present or only present in low (non-inhibitory) concentrations in the seed lots.

Heydecker, *et al.* (1971) found that in imbibing *B. vulgaris* fruits, depressed germination is usually correlated with depressed oxygen uptake. This is probably a result of tight opercula, caused by a mucilaginous layer that surrounds the fruit reported by Nottingham (2004). In some cultivars, the operculum remains in position for a longer time period limiting oxygen reaching the embryo and thus slowing germination. According to Nottingham (2004) this happens more commonly in cultivars with a mucilaginous layer. This may mean that the mucilaginous layer also acts as a cementing agent firmly holding the operculum in place. Thus Flores SB may have a mucilaginous layer and/or tighter opercula which restricted oxygen from reaching the embryo and/or physically restricted the emergence of the embryo which subsequently caused slower germination. Also, if Flores SB does have a mucilaginous layer, washing and soaking may have increased the ability to tighten the operculum.

5.2.2. *T. tetragonioides*

5.2.2.1. Moisture content

As with *B. vulgaris* fruits, drying back for two hours did not reduce the MC back to the initial MC (control) of the *T. tetragonioides* fruits but again this did not appear to have any effect on subsequent germination as there were no significant differences between the untreated fruits and the treated (washed and soaked) fruits.

5.2.2.2. Germination and tetrazolium test results

There was no significant difference in total germination (TG) for any of the three treatments (control, soak and wash), also showing that the washing and soaking treatments did not alleviate dormancy in the *T. tetragonioides* fruits. Nottingham (2004) suggested that the soaking as a pre-sowing treatment would likely be beneficial in seeds that are traditional unimproved or heritage varieties. In contrast with *B. vulgaris* fruits studied, *T. tetragonioides* is a mainly unimproved species with no currently recognised cultivars. However, neither soaking nor washing improved germination in *T. tetragonioides*. While *T. tetragonioides* fruits may benefit from longer soaking by giving more time to soften the endocarp that surrounds the embryo and allow radicle emergence.

5.2.2.2.1. Possible ways of breaking *T. tetragonioides* dormancy

The ISTA Rules (2010) however specified that fruits of *T. tetragonioides* should be washed or have the ‘pulp’ removed to alleviate dormancy before sowing. The assumption is that the ISTA term ‘pulp’ is referring to the endocarp. Thus removal of the pulp may be more

beneficial than washing. The failure of both washing and soaking treatments to alleviate dormancy may be because the main cause of dormancy is by mechanical restriction of the radicle or possibly oxygen restriction by the *T. tetragonioides* fruit outer coverings.

Kaye (1997) stated that an effective way of breaking dormancy is in most cases suggested by the seed morphology and behaviour. Scarification in legumes for example may break the dormancy as they have water impermeable seed coats. The morphology of the *T. tetragonioides* fruit suggests that seed dormancy may be due to the fruit layers, in particular the endocarp, preventing radicle emergence. Removal of these outer seed/fruit coverings may alleviate dormancy. The recommendations for breaking dormancy in *T. tetragonioides* changed in the 2011 edition of the ISTA Rules (ISTA, 2011) to remove the pulp, soak in water for 24 hours prior to sowing. Although the reasons for this change from washing (ISTA, 2010) to soaking (ISTA, 2011) have not been explained, this study has demonstrated that the washing treatment by itself was not successful in breaking dormancy.

Although a limitation of this study is that only one seed lot of *T. tetragonioides* was tested, dormancy is none-the-less more likely imposed by the tissues surrounding the embryo and therefore subsequent results with more seed lots are likely to give similar results.

The washing and soaking treatments also had no effect on the mean germination time (T_{50}) and uniformity of *T. tetragonioides*. This implies the purpose of the ISTA washing recommendation (ISTA, 2010) for fruits of *T. tetragonioides* may be to soften the endocarp for easy depulping.

The effectiveness of the ISTA (2011) dormancy-breaking recommendations on germination and dormancy-breaking, as well as on depulping, speed of germination and its uniformity was assessed in a follow up experiment which looked at soaking fruits for the longer period of 24 hours.

5.3. Experiment 2: *T. tetragonioides*

The aim of this experiment was to compare the 24 hour soaking treatment recommended by ISTA in 2011 with the previous 2010 two hour pre-washing ISTA recommendation. Another aim of the experiment was to assess the effectiveness of cutting/depulping *T. tetragonioides* fruits on subsequent germination.

5.3.1. Moisture content

Two hour soaked (non-dried) and fruits washed for two hours and then dried for two hours after the treatment had the same germination. Thus, as with *B. vulgaris* this implies that drying back in *T. tetragonioides* may also not be a requirement.

5.3.2. Germination and tetrazolium test results

The germination data from the dormancy-breaking treatments show that soaking the fruit for 24 hours prior to cutting reduced germination (40% cf av. 60%). MC for the 24 hour soaked fruits was significantly higher (56%) than other treatments (two hour soak = 45%; two hour wash = 37%; control = 11%) (Table 8) and seems to be the major factor. There was no significant difference in the number of dead fruits, but the data is very variable because *T. tetragonioides* is a wild population.

5.3.2.1. The effect of water on germination decrease in *T. tetragonioides*

It is not clear why the 24 hour soaking treatment inhibited germination of *T. tetragonioides.* From the results, it is unlikely that *T. tetragonioides* is prone to soaking injury because the viable fruits remained viable after the soaking treatment and were able to germinate after the endocarp was cut. For the 24 hour soaking, two hour soaking, two hour washing, and control treatments, germination of cut fruits was 75%, 74%, 70% and 79% respectively. The remaining cut fruits were dead (25%, 26% 23% and 21% for the four respective treatments). The two hour washing treatment had 7% empty fruits and the other three treatments had no empty fruits (Table 8). There were no significant differences in the percentages of cut germinated fruits or dead fruits among the four treatments.

T. tetragonioides fruits did not die and germination reached that of the control fruits after fruits were cut. The percentage of abnormal seedlings did not increase compared to that of the control. These both imply that there was no permanent injury caused from the soaking. However, in the case of *T. tetragonioides*, water might have been trapped in the spongy endocarp tissue (mesocarp), perhaps between the endocarp and the seed, thereby restricting oxygen uptake by the embryo. Larson (1968) suggested that the 24 hour soaking period allowed for the fruits to imbibe excess moisture which resulted in reduced respiration, or was insufficient to cause damage to the imbibing tissues of the *T. tetragonioides* fruits. If this water remained trapped in the endocarp and was only released by subsequent cutting this may

explain the delay in germination in the 24 hour soaking treatment. There is indirect evidence to support this hypothesis from a study by Doneen and MacGillivray (1943) on the effects of different percentages of available soil moisture on the germination of vegetable seeds that used soil with different moisture contents for seed germination. While all the vegetable seeds assessed had low germination at the highest moisture content, the differences were only significant in two crops and one of them was *T. teragonioides*. *T. tetragonioides* had 4%, 42%, 64%, 76%, 81%, 83%, 80%, and 61% germination at 8%, 9%, 10%, 11%, 12%, 14%, 16%, and 18% soil moistures respectively. These results suggest that germination of *T. tetragonioides* is affected by high moisture content. Also, Allan (1961) stated that the native environment for *T. tetragonioides* is coastal sands, dunes and stony beaches. These are likely to be very free-draining situations, thus it is perhaps not surprising that this species did not respond well to the longer soaking period.

However, whether water remained trapped in the fruit (which was not dried back) and inhibited germination until the fruits were cut 35 days later is largely speculation. Firstly the 24 hour soaking treatment needs to be repeated with this and other seed lots to confirm that the delay is a real effect and if so further investigation into the mechanism by which soaking delays germination be undertaken. As the fruits were not killed due to the 24 hour soaking treatment but appeared to be adequately softened, it is possible that the main purpose of soaking the fruits prior to sowing is to make it easier to remove the pulp to break dormancy (Zita Ripka, 2011, pers. comm.), although this is not clear from the dormancy-breaking recommendation in the 2011 ISTA Rules (ISTA, 2011).

5.3.2.2. The effect of cutting *T. tetragonioides* fruits

Cutting the fruits alleviated all the dormancy in viable *T. tetragonioides* fruit that remained ungerminated after 35 days. All fruits germinated within six days of being cut. This suggests that cutting/depulping in combination with some pre-soaking prior to depulping is the best dormancy-breaking method for fruits of *T. tetragonioides.* This supports the earlier suggestion by Kaye (1997) that an effective way of breaking dormancy is in most cases suggested by seed (or fruit) morphology and germination behaviour. It also supports the 2010 edition of the ISTA Rules (ISTA, 2010) specification of ‘depulping’ as a dormancy-breaking method for *T. tetragonioides*. The new recommendation in the 2011 Rules (ISTA, 2011) may be valid as a treatment for softening the endocarp for subsequent cutting, but data from this work does not support 24 hours soaking alone as being able to break dormancy.

5.3.2.3. The effect of water on T_{50} and uniformity of germination

Uniformity of germination results in this study suggest that the 24 hour soaking treatment slows down the spread of germination.

5.4. Conclusion

5.4.1. *B. vulgaris*

The results from this study and other literature (Biancardi, *et al.*, 2010; Frese, 2010; Wagmann, *et al.*, 2010) suggest that dormancy is no longer a significant problem in commercial *B. vulgaris* seed lots. This is most likely a result of dormancy being bred out of commercial varieties in recent years, either deliberately or as a by-product of the selection of other traits.

From the study it can be concluded that germination inhibitors may not be present in *B. vulgaris* fruits, or if present, they are in concentrations that do not affect germination.

Because of this lack of germination inhibitors in *B. vulgaris* fruits, the soaking and washing treatments do not have any effect on *B. vulgaris* seed (fruit) germination as their function is primarily to leach out germination inhibitors (although they may also soften the operculum). This suggests there is no need to carry out pre-soaking treatments in modern *B. vulgaris* cultivars to reach maximum germination of the seed lot. Pre-sowing treatments with water at 25 ˚C neither increased nor decreased germination.

A limitation of the study was that only 18 *B. vulgaris* seed lots were used in the preliminary screening test. The main limitation in the main experiment was the lack of dormancy in the tested fodder beet and sugar beet seed lots which did not allow for a rigorous comparison of the dormancy-breaking techniques i.e. washing and soaking.

5.4.2. *T. tetragonioides*

T. tetragonioides fruits have considerable dormancy as around one third of the viable fruit from the single seed lot tested still had not germinated after 35 days.

However, cutting the fruit after the specified germination period of 35 days then allowed germination to proceed immediately and concluded within an additional six days. A limitation is that the control seed was also cut so there is a possibility, albeit very remote that

some other factor other than cutting allowed germination to proceed. Accepting that this possibility is very remote, the data from this study suggests that the cutting method has the potential of allowing better germination of *T. tetragonioides* fruits. It also supports the 2010 ISTA recommendation of 'depulping' the fruits prior to sowing.

An unexpected result was that soaking for 24 hours impeded germination. This requires further investigation to firstly confirm that this is a real effect and if so to determine why 24 hours soaking impedes germination.

5.5. Recommendation

In this study, 18 *B. vulgaris* seed lots were assessed and it was found that they had little or no dormancy. This was also supported by the literature. The screening of more *B. vulgaris* seed lots and the surveying of seed laboratories to determine if they still encounter dormant *B. vulgaris* seed lots would be beneficial to more definitively establish the presence or absence of dormancy in modern *B. vulgaris* varieties.

Further research is needed to explore the mechanisms by which dormancy is imposed in *T. tetragonioides* fruits and why 24 hours soaking appears to be reducing seed germination.

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APPENDICES

APPENDIX 1: Preliminary experiment data collected for four replicates of each *B. vulgaris* seed lot (Table 1A) and the *T. tetragonioides* seed lot (Table 1B) assessed i.e. TSW (g) of each replicate and the number of normal plus abnormal seedlings (total germination), ungerminated viable and non-viable fruits (determined by the tetrazolium test), and the number of dead and empty fruits out of the 50 fruits sown.

Table 1A. *B. vulgaris*

Seed Lot	Replicate	TSW(g)	Total Germination	Fresh Ungerminated Fruits		Dead Fruits	Empty Fruits
				Viable	NV		
S□1 □lain □□ □1sts□	1	1□.□	4□	1	0	2	1
S□1 □lain □□ □1sts□	2	12.□	47	0	0	0	□
S□1 □lain □□ □1sts□	□	11.7	48	0	0	1	1
S□1 □lain □□ □1sts□	4	10.7	48	1	1	0	0
S□2 □lain □□ □1sts□	1	17.0	4□	0	0	1	0
S□2 □lain □□ □1sts□	2	17.□	4□	0	0	1	0
S□2 □lain □□ □1sts□	□	14.2	4□	1	0	0	0
S□2 □lain □□ □1sts□	4	17.4	4□	0	0	1	0
S□□□lain □□ □2nds□	1	12.1	40	0	2	□	□
S□□□lain □□ □2nds□	2	10.8	42	0	1	1	□
S□□□lain □□ □2nds□	□	11.□	40	1	2	□	4
S□□□lain □□ □2nds□	4	10.7	42	1	□	2	2
S□4 Taylor □□ □1sts□	1	10.□	48	0	0	2	0
S□4 Taylor □□ □1sts□	2	10.8	42	2	□	1	2
S□4 Taylor □□ □1sts□	□	8.□	42	2	4	1	1
S□4 Taylor □□ □1sts□	4	10.□	44	□	□	0	0
S□□Taylor □□ □2nds□	1	□.0	□□	0	2	□	□
S□□Taylor □□ □2nds□	2	10.0	40	0	0	0	10
S□□Taylor □□ □2nds□	□	□.8	□□	0	1	□	11
S□□Taylor □□ □2nds□	4	□.2	2□	0	□	□	1□
S□□Taylor □□ □2nds□	1	8.□	□□	1	□	□	8
S□□Taylor □□ □2nds□	2	10	4□	1	1	2	0
S□□Taylor □□ □2nds□	□	□.2	□4	1	□	□	4
S□□Taylor □□ □2nds□	4	8.2	4□	1	□	2	1

Seed Lot	Replicate	TSW(g)	Total Germination	Fresh Ungerminated Fruits: Viable	Fresh Ungerminated Fruits: NV	Dead Fruits	Empty Fruits
S□7 □ atson □□ □1sts□	1	12.0	2□	0	2	11	14
S□7 □ atson □□ □1sts□	2	11.□	□1	0	0	□	14
S□7 □ atson □□ □1sts□	□	11.8	22	1	1	□	20
S□7 □ atson □□ □1sts□	4	11.7	27	0	0	8	1□
S□8 □ atson □□ □2nds□	1	1□.□	44	1	1	2	2
S□8 □ atson □□ □2nds□	2	1□.□	47	1	0	2	0
S□8 □ atson □□ □2nds□	□	14.□	4□	2	0	2	0
S□8 □ atson □□ □2nds□	4	14.2	48	0	0	2	0
S□□□e □outre □□ □1sts□	1	14.□	48	0	1	0	1
S□□□e □outre □□ □1sts□	2	1□2	47	2	1	0	0
S□□□e □outre □□ □1sts□	□	1□2	47	0	0	2	1
S□□□e □outre □□ □1sts□	4	1□0	48	0	0	2	0
S□10 □e □outre □□ □2nds□	1	1□1	4□	1	1	□	2
S□10 □e □outre □□ □2nds□	2	1□.□	42	1	0	7	0
S□10 □e □outre □□ □2nds□	□	1□2	40	2	2	1	□
S□10 □e □outre □□ □2nds□	4	1□.□	4□	0	0	7	0
S□11 □uby □□	1	24.□	4□	2	0	□	0
S□11 □uby □□	2	24	42	0	□	2	□
S□11 □uby □□	□	22.□	42	4	0	4	0
S□11 □uby □□	4	24.□	48	1	0	1	0
S□12 □rigadier F□	1	17.□	□□	2	4	1	4
S□12 □rigadier F□	2	18.□	□□	□	4	□	4
S□12 □rigadier F□	□	1□.□	42	1	2	2	□
S□12 □rigadier F□	4	17.□	□□	□	1	2	□
S□1□□ings Seeds S□	1	1□2	□7	7	□	0	0
S□1□□ings Seeds S□	2	1□4	□8	□	□	2	2
S□1□□ings Seeds S□	□	1□7	42	□	2	1	0
S□1□□ings Seeds S□	4	1□.□	□□	10	2	2	0
S□14 Flores S□	1	8.0	□□	□	4	0	1
S□14 Flores S□	2	7.□	□□	8	7	0	0
S□14 Flores S□	□	7.□	41	□	4	0	0
S□14 Flores S□	4	8.0	□□	12	2	0	0

Seed Lot	Replicate	TSW(g)	Total Germination	Fresh Ungerminated Fruits: Viable	Fresh Ungerminated Fruits: NV	Dead Fruits	Empty Fruits
S□1□□estor F□	1	10.□	44	□	□	0	0
S□1□□estor F□	2	10.8	40	□	□	0	0
S□1□□estor F□	□	10.7	41	4	□	0	2
S□1□□estor F□	4	10.1	4□	1	4	0	0
S□1□□alace S□	1	□.4	4□	0	4	0	1
S□1□□alace S□	2	□.□	4□	0	0	0	1
S□1□□alace S□	□	□.□	4□	0	0	1	0
S□1□□alace S□	4	□.□	4□	1	1	2	0
S□17 □yros F□	1	11.□	41	□	□	2	1
S□17 □yros F□	2	11.8	□□	2	□	4	0
S□17 □yros F□	□	11.1	□□	□	7	1	0
S□17 □yros F□	4	11.□	40	2	4	2	2
S□18 □agnum F□	1	12.□	4□	0	□	2	0
S□18 □agnum F□	2	12.1	4□	0	1	2	2
S□18 □agnum F□	□	11.8	4□	0	4	1	0
S□18 □agnum F□	4	12.4	44	0	□	0	□

Table 1B. ***T. tetragonioides***

Replicate	TSW (g)	Total Germination	Fresh Ungerminated Fruits: Viable	Fresh Ungerminated Fruits: NV	Dead Fruits	Empty Fruits
1	□4.2	□□	14	1	0	2
2	□□.□	□8	12	0	0	0
□	□□2	24	24	0	0	2
4	□8.0	□0	20	0	0	0

APPENDIX 2: Main experiment data collected for four replicates of the four *B. vulgaris* seed lots (Table 2A – 2D) and the *T. teragonioides* seed lot (Table 2E) i.e. moisture content before (MCBD) and after (MCAD) drying of the soaked, washed and control fruits, the number of abnormal and normal seedlings, dead and empty fruits, non-viable (NV) and ungerminated viable fruits determined by the tetrazolium test, and mean germination time (T_{50}) and uniformity of germination following the washing and soaking treatments and the control fruits.

Table 2A: Flores sugar beet

Seed lot	Treatment	Replicate	MCBD (%)	MCAD (%)	Germination		Dead Fruits	Empty Fruits	Fresh Ungerminated Fruits		T_{50} (hours)	Uniformity (hours)
					Normal	Abnormal			Viable	NV		
Flores S□	□ontrol	1	□47	□47	4□	0	2	0	2	0	42.10	117.4□
Flores S□	□ontrol	2	□2□	□2□	4□	0	0	0	□	2	□1.17	14□.□0
Flores S□	□ontrol	□	10.1□	10.1□	□4	2	4	2	8	0	□1.28	2□1.□0
Flores S□	□ontrol	4	□.□□	□.□□	41	0	0	2	□	1	4□07	210.4□
Flores S□	□ ash	1	□□78	18.71	4□	0	1	0	2	1	41.□□	42.81
Flores S□	□ ash	2	40.8□	27.0□	40	□	2	0	□	0	□4.□□	1□4.□8
Flores S□	□ ash	□	□□48	□□84	4□	1	□	1	0	0	77.84	178.□7
Flores S□	□ ash	4	□□24	□7.78	44	0	0	1	□	2	110.□0	2□8.7□
Flores S□	Soak	1	□□24	24.28	□0	0	0	0	0	0	□□7□	□4.□0
Flores S□	Soak	2	41.42	2□4□	4□	2	0	0	2	0	7□21	172.48
Flores S□	Soak	□	□□.□□	□□.□0	□8	2	4	0	□	0	□□08	2□1.70
Flores S□	Soak	4	□8.□8	□□7□	□7	2	0	0	□	□	108.00	2□0.7□

Table 2B: Kings Seeds sugar beet

			MCBD	MCAD	Germination		Dead	Empty	Fresh Ungerminated Fruits		T_{50}	Uniformity
Seed lot	Treatment	Replicate	(%)	(%)	Normal	Abnormal	Fruits	Fruits	Viable	NV	(hours)	(hours)
□ings Seeds S□	□ontrol	1	□22	□22	4□	0	1	0	□	□	□□12	18□22
□ings Seeds S□	□ontrol	2	8.□2	8.□2	40	1	0	0	4	□	□1.28	7□.0□
□ings Seeds S□	□ontrol	□	8.□4	8.□4	□1	0	8	□	8	0	□4.□4	8□.87
□ings Seeds S□	□ontrol	4	□2	□2	□7	1	0	1	4	7	□□42	78.□□
□ings Seeds S□	□ ash	1	□□.4□	1□22	□□	0	7	2	□	0	□1.□8	□2.□2
□ings Seeds S□	□ ash	2	2□.□7	17.□2	□□	1	1	1	4	4	4□	12.□
□ings Seeds S□	□ ash	□	□1.47	2□48	□1	0	□	□	11	0	□0.7□	107.8□
□ings Seeds S□	□ ash	4	□1.48	2□.□4	□4	0	1	2	2	11	□8.87	14□.□□
□ings Seeds S□	Soak	1	□□8	1□4□	42	0	0	0	2	□	44.□1	42.2
□ings Seeds S□	Soak	2	□1.□□	21.□4	41	0	□	1	□	0	□1.□4	44.41
□ings Seeds S□	Soak	□	□2.□	2□8□	40	1	4	2	□	0	□□2□	77.□□
□ings Seeds S□	Soak	4	□□.□7	2□02	□2	4	0	1	4	□	44.□8	2□0.7□

Table 2C: Brigadier fodder beet

			MCBD	MCAD	Germination		Dead	Empty	Fresh Ungerminated Fruits		T_{50}	Uniformity
Seed lot	Treatment	Replicate	(%)	(%)	Normal	Abnormal	Fruits	Fruits	Viable	NV	(hours)	(hours)
□rigadier F□	□ontrol	1	10.2	10.2	4□	1	0	□	1	0	4□.□4	□□.□
□rigadier F□	□ontrol	2	10.□	10.□	□0	□	□	1	4	7	□□.88	4□.□
□rigadier F□	□ontrol	□	10.2□	10.2□	□□	1	8	□	□	0	□7.8□	12□
□rigadier F□	□ontrol	4	10.41	10.41	2□	□	0	4	1	10	□□.88	87.4□
□rigadier F□	□ ash	1	41.□□	2□.□1	□1	□	□	□	0	□	4□.27	□7.□
□rigadier F□	□ ash	2	40.11	22.8□	2□	8	4	□	□	0	48.□□	2□.42
□rigadier F□	□ ash	□	4□.04	□□.8□	□4	0	8	0	8	0	4□.□□	7□.□2
□rigadier F□	□ ash	4	42.0□	□□.□□	□□	□	1	1	□	1	44.□	2□□.24
□rigadier F□	Soak	1	40.17	27.74	□□	1	□	2	□	0	42.0□	41.□7
□rigadier F□	Soak	2	41.□4	27.□1	□8	□	2	1	4	0	4□.□□	27.□8
□rigadier F□	Soak	□	□□.□□	□4.1□	□□	2	□	□	□	0	28.□□	□8.88
□rigadier F□	Soak	4	41.1□	□4.□7	□□	□	4	□	□	0	□□.□□	□1.84

Table 2D: Kyros fodder beet

Seed lot	Treatment	Replicate	MCBD (%)	MCAD (%)	Germination		Dead Fruits	Empty Fruits	Fresh Ungerminated Fruits		T_{50} (hours)	Uniformity (hours)
					Normal	Abnormal			Viable	NV		
□yros F□	□ontrol	1	□82	□82	4□	0	0	0	1	0	44.1□	42.□□
□yros F□	□ontrol	2	□.□□	□.□□	4□	1	□	0	1	0	48.1	2□.□8
□yros F□	□ontrol	□	□.□□	□.□□	4□	0	4	0	1	0	□□.1	□□.1□
□yros F□	□ontrol	4	□.□□	□.□□	41	□	0	0	1	□	44.7□	1□8.2□
□yros F□	□ ash	1	□□.7□	1□74	47	0	1	0	2	0	42.77	40.□7
□yros F□	□ ash	2	□□.□□	2□07	42	2	□	0	1	0	41.87	2□.7□
□yros F□	□ ash	□	□□.77	2□.□8	□7	□	□	1	□	0	4□.□4	4□.□□
□yros F□	□ ash	4	□□.12	□0.□□	4□	1	0	0	0	0	4□.04	77.04
□yros F□	Soak	1	□□.1□	1□81	4□	0	0	0	4	0	40.28	2□.42
□yros F□	Soak	2	□□.□7	28.□1	44	□	2	0	1	0	4□.0□	□□.□□
□yros F□	Soak	□	□□.47	□2.12	44	0	□	0	1	0	4□.□7	□1.08
□yros F□	Soak	4	□□.4	2□.□□	□8	7	0	0	0	□	4□.□1	7□.7□

Table 2E: *T. tetragonioides*

Treatment	Replicate	MCBD (%)	MCAD (%)	Germination		Dead Fruits	Empty Fruits	Fresh Ungerminated Fruits		T_{50} (hours)	Uniformity (hours)
				Normal	Abnormal			Viable	NV		
□ontrol	1	10.21	10.21	□8	0	1	0	11	0	□2.00	141.4
□ontrol	2	□.□0	□.□0	□□	0	□	0	11	0	□□.48	□□1.□7
□ontrol	□	10.48	10.48	□7	0	0	0	12	1	□□.7□	□□□.□7
□ontrol	4	11.2□	11.2□	□2	0	0	0	18	0	□1.7□	17□74
□ ash	1	44.04	24.4□	□□	0	□	0	11	0	108.0□	4□□.□1
□ ash	2	4□2□	22.□□	40	0	0	0	10	0	82.□0	10□2□
□ ash	□	4□01	□7.□□	□□	0	0	1	1□	0	8□1□	□77.□0
□ ash	4	4□.□7	□8.□□	□0	0	1	0	1□	0	□7.8□	2□0.70
Soak	1	44.22	□4.27	□8	0	1	1	10	0	11□.□□	□8□.□□
Soak	2	4□.□0	□□.□0	□2	0	0	0	1□	□	87.11	12□71
Soak	□	4□4□	40.□□	□0	0	1	2	17	0	114.78	□□7.1□
Soak	4	47.04	40.88	2□	0	0	0	24	0	87.7□	40□.□□

APPENDIX 3: *T. tetragonioides* follow up experiment data collected for four replicates of the four pre-sowing treatments applied to fruits i.e. % moisture content at sowing (MC%), the number of normal and abnormal seedlings after 35 days germination period, ungerminated fruits 35 days after sowing (DAS) that were cut, fruits that germinated after cutting, dead and empty fruits, total number of fruits that germinated after each treatment, germination uniformity and mean germination time (T_{50}) of fruits after each treatment.

			Final Germination								
Treatment	Replicate	MC (%)	Normal	Abnormal	Ungerminated Fruits 35 DAS	Germinated Fruits After Cutting	Dead Fruits	Empty Fruits	Total Germination of Fruits After Each Treatment	Uniformity (hours)	T_{50} (hours)
24 hour Soak	1	□□.41	14	0	□7	28	□	0	42	□84.0□	7□0.2□
24 hour Soak	2	□□.□□	2□	1	28	24	4	0	47	771.08	1□0.00
24 hour Soak	□	□□.□□	2□	1	27	18	8	1	41	777.04	17□00
24 hour Soak	4	□□.4□	20	0	□0	21	□	0	41	□□0.0□	27□2□
2 hour Soak	1	44.□0	2□	0	22	20	2	0	4□	□48.44	184.2□
2 hour Soak	2	44.2□	□0	1	20	1□	□	0	4□	□□8.□4	1□□.00
2 hour Soak	□	4□.80	□□	0	1□	□	□	0	41	□42.47	128.00
2 hour Soak	4	4□.8□	27	1	22	20	2	0	47	2□7.71	1□8.00
2 hour □ ash	1	□7.17	2□	0	20	1□	4	1	44	□□4.18	120.00
2 hour □ ash	2	□□.□□	□4	0	1□	□	7	□	40	4□□.04	11□00
2 hour □ ash	□	□□.□□	□2	1	18	1□	4	1	4□	□□8.87	172.0□
2 hour □ ash	4	□7.□□	□□	1	17	1□	1	0	4□	4□0.71	1□□.00
□ontrol	1	10.7□	27	0	24	18	□	0	4□	481.42	□□00
□ontrol	2	10.8□	□4	1	1□	14	2	0	48	□72.8□	110.40
□ontrol	□	11.07	2□	2	22	1□	□	0	4□	□1□2□	120.00
□ontrol	4	10.7□	2□	2	2□	21	□	0	4□	□18.28	208.00