A Survey of Winter-Hardy *Prunus* Species: Evaluation of Seed Germination, Seedling Establishment, and Pollen Viability.

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Dedication

In loving memory of my paternal grandfather and maternal grandmother,

Richard A. Kostick
1941-2009

Mary C. Iden
1926-2014

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Introduction

Prunus is a large, economically important genus in the Rosaceae, sub-family Amygdaloideae, which contains over 250 species including fruit crops such as almond, apricot, peach, plum as well as tart and sweet cherries (Das et al., 2011; Griffiths, 1994; Potter, 2012; Wen et al., 2008). *Prunus* species have drupes for fruit and flowers with five petals and five sepals (Potter, 2012; Lee and Wen, 2001). The genus *Prunus* is classified into the sub-family Amygdaloideae along with the genera *Maddenia* Hook. F. & Thomson, *Oemlerian* Reichb., and *Prinsepia* Royle because of characteristics like drupes for fruit and a base chromosome number of x = 8 (Lee and Wen, 2001; Potter, 2012). Species in this genus can be deciduous or evergreen with growth habits that range from shrubs to trees (Griffiths, 1994). Westwood (1993) as well as Das et al. (2011) divide *Prunus* into three sub-genera including *Amygdalus*, *Prunophora*, and *Cerasus.* However, a recent study by Shi et al. (2013) suggested three different subgenera *Padus, Cerasus* and *Prunus*, which contains the seven sections *Amygdalus, Armeniaca, Emplectocladus, Microcerasus, Persicae, Prunocerasus* and *Prunus*. The conflict in the area of classification indicates that the phylogeny of *Prunus* is not fully understood and is likely to change in the future.

Due to the size and extensive geographical distribution of this genus as well as its long history of human cultivation, there is not one specific point of origin (Potter, 2012). However, most *Prunus* species are indigenous to the Northern Hemisphere (Wen et al., 2008) with most species likely being Eurasian in orign (Bortiri et al. 2001). The Eurasian descent of *Prunus* is evidenced by the points of origin of some important crop species in

this genus. Contrary to the sound of its name, the peach (*P. persica* Batsch.*)*, originated in China not Persia, modern-day Iran (Das et al. 2011; Potter, 2012; Westwood, 1993). The apricot's (*P. armeniaca* L.*)* center of origin is Asia and the Caucasus region including Armenia (Das et al. 2011; Potter, 2012). Both sweet (*P. avium* L.) and tart (*P. cerasus* L.*)* cherries are native to Europe and Asia (Das et al., 2011; Westwood, 1993). Depending upon the species, the center of origin for plums ranges from Europe to North America to Asia (Das et al. 2011; Potter, 2012). The European plum (*P. domestica* L.) originated in western Asia to southern Europe whereas the Japanese plum (*P. salincia* Lindl.) is indigenous to China and the American plum (*P. americana* Marsh.) is native to North America (Das et al., 2011;Westwood, 1993).

Uses of Prunus species

Fruit production is one of the most prominent uses of species in this genus. *Prunus* fruits are often used for fresh eating as well as jam, jelly and dried fruit (Somogyi, 1996). As a result, this genus provides an expansive list of globally important fruit crops. Due to the large number of shapes and sizes as well as flower colors and bark types, *Prunus* species can be utilized in a wide variety of landscaping venues. From public gardens to homeowners, *Prunus* species provide a multitude of options for ornamental use. Some species are also valued for medicinal properties (e.g. *P. africana* Kalkman) or timber quality (Potter, 2012). Peaches, apricots, almonds and other *Prunus* species synthesize compounds called cyanogenic glycosides that, once hydrolyzed, can release hydrogen cyanide (Chaouali et al., 2013). Cyanide, a poison which can be deadly to both livestock and humans, is used for gold and silver extraction as well as the

manufacture of computer electronics, dyes, pharmaceuticals, paints, cosmetics, etc. (Mudder and Botz, 2004).

Apricots

The majority of cultivated apricot cultivars are *P. armeniaca*, which originated in northeastern China (Mehlenbacher et al. 1991). *Prunus armeniaca* cultivars vary in size as well as growth habit and vigor (Mehlenbacher et al. 1991). Apricot cultivars tend to bloom early and the flowers range in color from white to pink (Mehlenbacher et al. 1991). The leaves are usually elliptic or ovate, with serrate edges and long, red petioles (Mehlenbacher et al. 1991). Fruits are yellow to orange in color with uses ranging from jam to fresh/dry eating (Griffiths, 1994; Westwood, 1993).

Tart cherries

Prunus cerasus (tart cherry) and *P. avium* (sweet cherry) are indigenous to the same area of the world (Westwood, 1993). The tart cherry is hypothesized to have developed from the unreduced gametes (pollen) of a *P. fruticosa* Pall. x *P. avium* L. cross (Westwood, 1993; Iezzoni et al. 1991). Besides differences in fruit flavor between *P. avium* and *P. cerasus, P. cerasus* cultivars tend to bloom later and be more winter-hardy (Iezzoni et al., 1991). Tart cherry trees range in size and shape from large, spreading trees to small, round forms (Iezzoni et al. 1991). The fruit is depressed-globose in shape with red exocarps and flesh that ranges in color from colorless (amarella) to very dark red (morello; Brown et al. 1989; Iezzoni et al. 1991). The cultivars that are not as tart in flavor can be utilized for fresh eating but the majority of tart cherries are used for pies, specialty jams and distilled liqueur (Iezzoni et al. 1991). Most *P. cerasus* cultivars are

self-compatible meaning that a separate source of pollen is not necessarily required for fruit set (Westwood, 1993). Self-compatibility makes cultivation of tart cherries conducive to monocultures. The most commonly cultivated tart cherry cultivar in North America is 'Montmorency', which accounts for 96% of the trees in production (Brown et al. 1989).

Plums

Although there are many important plum species around the world, one of the most important and widespread is the European plum, *P. domestica* (Westwood, 1993). According to Westwood (1993), this species was the result of triploid hybrid cross between *P. cerasifera* Ehrh. x *P. spinosa* L. Late blooms, thick leaves and cultivars with high concentrations of soluble solids are characteristic of this species (Ramming and Cociu, 1991). The trees and fruits of *P. domestica* cultivars come in a wide variety of shapes, sizes and colors. For example, the fruit can range in size from very small to large with a oval to round shapes; blue, red, yellow or even green colors; free or clingstone pits, and high sugar content with usually good flavor (Ramming and Cociu, 1991).

Winter-hardy Prunus

Although the species of this genus serve many important ecological and economic functions throughout the world, damage to flowers due to spring freezes, and mid-winter low temperatures restrict the number of species that can survive in northern climates (Andersen and Weir, 1967; Taylor, 1965). Even *Prunus* species that can be successfully cultivated in USDA Zones 3-4 are often short lived and do not produce consistent crops (Andersen and Weir, 1967). The focus of early 1900s breeding programs in northern

climates, e.g. the University of Minnesota, was to release cultivars with good fruit quality that were winter-hardy and produced viable pollen (Andersen and Weir, 1967). Some cultivars were produced via the hybridization of species with superior fruit quality like *P. domestica* or *P. salicina* with native species like *P. americana*, which had relatively poor fruit quality but were able to withstand cold winter temperatures (Andersen and Weir, 1967). A number of apricot, tart cherry and plum cultivars were released by the University of Minnesota and affiliated breeding programs. The Minnesota Agricultural Experiment Station Fruit Breeding Farm released 'Moongold' and 'Sungold', two relatively winter-hardy apricots, in 1961 (Brooks and Olmo, 1997). In the early 1950s, *P. cerasus* 'Northstar' and 'Meteor' were released by the University of Minnesota Fruit Breeding Farm (Brooks and Olmo, 1997). The University of Minnesota introduced a number of plum hybrids throughout the 1900s. For example, 'La Crescent' was introduced in 1923 and 'Alderman' was released in 1985 (Brooks and Olmo, 1997).

Invasive potential

Invasive species are a substantial challenge facing the world today. According to Reichard and White (2001), an invasive species is defined as a species that has established or has the ability to spread into surrounding plant communities, build self-sustaining populations, and become a dominant and/or disruptive force in those systems. A species can become invasive when it gains an advantage over the native species through the removal of barriers that originally prevented the establishment of self-sustaining populations (Galatowitsch et al. 1999; Valéry et al. 2008). Through the alteration of the structure and processes of an ecosystem, invasive species threaten the

survival of the native flora (Deckers et al. 2005; Vanhellemont et al. 2009). Invasive species change nutrient cycling processes and fire regimes, hybridize with native species, reduce the survival of native fauna and flora, and result in many other negative ecological impacts (Mack et al. 2000). According to Gomez (2005), invasive species reduce the diversity of an ecosystem by choking out less-competitive native populations through the formation of dense, single species stands. Invasive species not only pose a severe threat to the biodiversity of ecological systems but also present a sizeable economic burden (Mack et al. 2000; Pimentel et al. 2000). The United States incurs about $137 billon total per year in losses due to invasive species (Pimentel et al. 2000) with around $13 billion due specifically to invasive plants (Peters et al. 2006). According to Mack et al. (2000), economic costs due to invasive species result first from financial loss associated with the reduction of economic output like reduced crop yields and second due to costs related to the control and eradication of these pests. The heavy toll that invasive species exact on ecosystems and economies underscores the importance of active prevention of potential escapes of non-indigenous species into surrounding environments (Pimentel et al. 2000).

The escape and the subsequent invasion of an ecosystem by a non-indigenous species occur in the three-stage process of introduction, colonization, and naturalization through asexual and sexual reproduction (Richardson et al. 2000). Human endeavors like agriculture, horticulture, and timber are often the vectors through which potentially invasive species are introduced into new environments (Kolar and Lodge, 2001; Mack et al. 2000; Reichard and White, 2001). According to Reichard and White (2001), in the United States alone, about 82% of woody invasive plants were introduced by humans to

meet needs in areas such as fruit production, landscape, or erosion control. Plant breeding is another human activity through which invasive plants may be introduced (Peters et al. 2006). Although many of the traits that are selected by plant breeders do not result in invasive genotypes, some characteristics can aid in the assimilation of the species into new, non-cultivated environments (Anderson et al. 2006). Plant breeders often select individuals that have high stress tolerance and are resistant to disease; although these traits are desirable for production, they can also result in high invasive potential (Anderson et al. 2006). Successful germination, high growth rates, hybrid vigor, high male and female fertility, stress tolerance, and disease resistance are characteristics that are often associated with high invasive potential (Anderson et al. 2006; Gomez, 2005). As a result, breeders and others should consider the invasive potential of a cultivar before increasing propagation or releasing the cultivar onto the market (Anderson et al. 2006).

After a species is introduced into an ecosystem, it must colonize through reproduction and then develop a self-sustaining population (Richardson et al. 2000). Subsequently, naturalization occurs after the species spreads, reproduces and establishes self-sustaining populations in new environments (Richardson et al. 2000). According to Anderson et al. (2006), high male fertility in addition to female fertility can increase the invasive potential of species. High fertility indicates the ability to successfully sexually reproduce which is vital for the colonization and establishment phases of the invasion process.

Due to the number of factors that impact invasive potential, predicting invasiveness can be challenging (Kolar and Lodge, 2001). One method of screening for

invasive potential is to examine important stages in the establishment and life cycles of potentially invasive plants. According to Guo et al. (2010), seed germination and seedling establishment are two of the most important stages in the establishment of a plant. As a result, these two stages are important for development of self-sustaining populations in new environments.

Some *Prunus* species, like *P. serotina* Ehrh., have escaped cultivation and become invasive in parts of Europe (Deckers et al. 2005). Originally, *P. serotina* was introduced for erosion control but it invaded agricultural land and fragments of woody habitat (Deckers et al. 2005). In order to control the spread of *P. serotina,* expensive eradication measures were put into place but these solutions were mostly ineffective and costly (Deckers et al. 2005). *P. americana* had also shown characteristics that are indicative of high invasive potential. According to Francis (2004), *P. americana* thrives in a variety of habitats across a vast geographical range. In addition to relatively high germination success, *P. americana* easily produces root suckers and thus, has the ability to form, thick stands vegetatively (Francis, 2004). The high invasive potential of *P. americana* is especially concerning due to the fact that it is in the background of many of the interspecific, winter-hardy plum cultivars. The history of invasive species within this genus provides a basis for concern in regards to whether or not winter-hardy *Prunus* have the potential to become invasive.

Pollen viability

Andersen and Weir (1967) stated that one of the largest challenges faced by the *Prunus* fruit-breeding program at the University of Minnesota was low pollen viability of

selections. Pollen viability plays a crucial role in successful fertilization and thus, seed set and fruit development (Nyéki and Soltész, 1996; Westwood, 1993). The main purpose of measuring pollen viability is to determine the potential of a certain cultivar's pollen grains to germinate upon arrival on the stigma and to fertilize the ovule (Firmage and Dafni, 2000). Low pollen viability can result in low fertilization and thus, potentially low fruit set (Gallotta et al. 2014). The question becomes whether or not winter-hardy cultivars, which were the result of selections from early breeding programs such as the University of Minnesota's program, have high pollen viability.

Within the context of plant breeding, the ability to store pollen for extended periods of time without a reduction in viability is important. Successful pollen storage allows for increased breeding efficiency and for the preservation and exchange of germplasm (Hanna and Towill, 1995). Although, long-term storage of pollen would be useful, very little is known about the longevity of many species' pollen, including *Prunus*, in storage (Hanna and Towill, 1995).

Objectives

The purpose of this thesis was to examine sexual reproduction and seedling establishment of winter-hardy *Prunus* cultivars through the lens of invasive potential as well as potential applications in breeding and production. This goal was achieved through two objectives, 1.) determine if winter-hardy *Prunus* species can germinate and establish in a Minnesota landscape, and 2.) estimate pollen viability of selected winter-hardy *Prunus* cultivars through the use of viability staining. The first chapter of this thesis examined seed germination and seedling establishment through two germination

experiments and one seedling establishment experiment. Specifically, the germination experiments assessed the effects of fruit type, germination environment, seed scarification, and seed storage on germination whereas the establishment experiment examined the effects of germination environment, parental fruit type and cultivar within a fruit type, and herbivore predation on seedling establishment. The second chapter assessed pollen viability and the longevity of pollen in storage of selected winter-hardy *Prunus* cultivars. By incorporating seed germination, seedling establishment, and pollen knowledge gained through this project, more informed decisions could be made in regards to future, potential breeding efforts.

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Chapter 1: Evaluation of seed germination and seedling establishment of winter-hardy *Prunus* species

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Summary

Invasive species threaten the survival of native flora through the alteration of the structure and processes of natural communities. After introduction, seed germination and seedling establishment are vital to form self-sustaining populations. In this study, we measured germination and seedling establishment for a selection of winter-hardy *Prunus* apricot, tart cherry, and plum cultivars. Germination experiments examined the effects of fruit type, cultivar within a fruit type, germination environment, seed scarification, and seed storage on germination whereas the establishment experiment examined the effects of germination environment, parental fruit type and cultivar, and herbivore predation on seedling establishment. We concluded that most of the cultivars we examined would not become invasive due to low seed germination and/or poor seedling establishment. Apricots had high overall seed % germination but poor seedling growth due to high herbivore predation indicating lower invasive potential. Tart cherries had lower seed germination, moderate seedling establishment coupled with low herbivore pressure and vegetative propagation via suckering indicating higher invasive potential. Progeny from the plum cultivars ‘Hazel’, ‘Whittaker’, ‘South Dakota’, and ‘Hennepin’ had high % germination, vigorous seedling growth, low to moderate herbivore pressure, and limited vegetative propagation via suckering, indicating invasive potential.

Introduction

An invasive species spreads after introduction and becomes a dominant, self-sustaining component of an ecosystem (Kolar and Lodge, 2001). Invasive species can significantly alter the biodiversity of natural landscapes; their control has significant economic costs (Kolar and Lodge, 2001; Vanhellemont et al. 2009). Through the alternation of the structure and processes of natural communities, invasive species gain a competitive advantage and threaten the survival of native flora (Vanhellemont et al. 2009).

The spread of invasive species is often the result of human activities including agriculture, horticulture, and forestry (Reichard and White, 2001; Vanhellemont et al. 2009). *Prunus,* a large and economically important genus, includes many crops that have a long and rich history of human cultivation (Das et al., 2011; Griffiths, 1994; Potter, 2012; Wen et al., 2008). Some *Prunus* species have escaped cultivation and become invasive. For example, *P. serotina* Ehrh., a species native to North America, has escaped cultivation in parts of Europe and become invasive (Deckers et al. 2005). *Prunus serotina* was introduced in Europe during the early 20th century for timber production but it escaped cultivation and became invasive (Deckers et al. 2005). In addition to *P. serotina, P. americana* Marsh. has high invasive potential because it is naturalized across a wide geographical range and is adapted to a variety of ecosystems (Francis, 2004). These examples from *Prunus* provide a basis for concern in regards to whether or not winter-hardy *Prunus* have the potential to become invasive. However, predicting invasive potential can be difficult due to the many characteristics and factors that affect it (Kolar

and Lodge, 2001). There are a substantial number of transitions and barriers that a species must overcome in order to become a dominant force in an ecosystem. Kolar and Lodge (2001) state that first a species must be transported/dispersed into an environment. Once in an environment, stand establishment must occur to ensure a viable population that can reproduce sexually and/or asexually (Kolar and Lodge, 2001). Seed germination and seedling establishment are important steps in establishing viable populations.

Longevity of seed prior to germination is important for establishment of seed banks and subsequently, self-sustaining populations. Some *Prunus* species, like *P. americana,* form seed banks and the seed can remain viable in the soil for years before germinating (Fryer, 2010). Prior to germination, seeds are often placed into cool, dry storage to prevent loss of viability. Huntzinger (1971) concluded that the seed of *P. serotina* can maintain viability for at least 3 years when stored at 0.6-5°C. How long a seed remains viable in storage depends on the species and storage conditions. On average, *P. cerasus* L. seed remains viable for 1-2 years whereas *P. domestica* L. seed maintains viability for 4-6 years (Westwood, 1993). Moisture content impacts the longevity and viability of seed in storage. If moisture content of the seed drops below 1 to 2%, germination will be reduced (Hartmann et al. 1997). However, moisture content that is too high (> 13%) will result in fungal activity and potential germination in storage (Hartmann et al. 1997).

Prunus seeds must overcome mechanical and deep physiological dormancy in order to germinate (Baskin and Baskin, 1998; Hartmann et al. 1997). Mechanical dormancy restricts radicle emergence by a covering (e.g. endocarp) around the seed

whereas physiological dormancy prevents radical emergence (Hartmann et al. 1997; Nikolaeva, 1969). In order to overcome the mechanical dormancy imposed by the endocarp, scarification of the endocarp might be required. Scarification of the endocarp has been shown to have variable effects on germination in *Prunus* species. For *P. americana*, *P. cerasus* L., and *P. persica* Batsch., scarification or the complete removal of the endocarp has increased germination percentages and resulted in faster germination rates (Chen et al. 2007; Grisez et al. 2008; Kristiansen and Jenson, 2009). In *P. domestica* L. and *P. angustifolia* Marsh., scarification or complete removal of the endocarp did not impact germination (Grisez et al. 2008; McMahon et al. 2015). Baskin and Baskin (1998) theorized that mechanical dormancy might not be a separate form of dormancy than physiological dormancy because some species still overcome dormancy through a period of cold stratification without scarification.

Deep physiological dormancy is overcome through a long period of moist, cold stratification at above freezing temperatures (Baskin and Baskin, 1998; Westwood, 1993). Prior to cold stratification, moist, warm stratification at 20-25°C, has been shown to increase seed germination percentages in the species *P. campanulata* Maxim., *P. angustifolia*, *P. cerasifera* Ehrh., *P. spinosa* L., and *P. virginiana* L. (Baskin and Baskin, 1998; Chen et al. 2007; Grisez et al. 2009; Westwood, 1993). The cold stratification period occurs at 0-10°C and is usually greater than 8 weeks but the specific length is species dependent (Hartmann et al. 1997; Jauron, 2000). For example, *P. armeniaca* L. cultivars require around 50 days of cold stratification whereas other species like *P. domestica* and *P. cerasus* require longer periods, 90 or 90-150 days respectively (Jauron,

2000; Grisez et al. 2008; Seeley and Damavandy, 1985). Longer periods of cold stratification often result in higher germination percentages. For example, germination in peach (*P. persica* Batsch.) begins after 8 weeks of cold stratification but continues to increase until about 12 weeks (Martınez-Gómez and Dicenta, 2001).

Two important stages in the life history and development of a plant are seed germination and seedling establishment (Guo et al. 2010). Both stages are affected by a variety of abiotic and biotic factors (Guo et al. 2010; Wilson and Jacobs, 2006). Characteristics such as high germination percentages, and successful stand establishment through vigorous growth, vegetative propagation via suckering, and lower herbivore pressure aid in the spread and establishment of potentially invasive species (Hock et al. 2015; Kolar and Lodge, 2001; Siemann and Rogers, 2001).

After seed germination, seedling establishment is an important step in determining invasive potential. Seedling establishment is dependent upon seedling quality. Wilson and Jacobs (2006) defined seedling quality as the ability of a seedling to survive and put on growth after planting. Since the term “seedling quality” is in itself not quantifiable, other performance related characteristics that are assessable must be measured in order to gauge the quality of seedlings (Ritchie, 1984). Morphological characteristics such as height and stem diameter have been shown to be effective metrics for predicting seedling survival, establishment, and quality in the field (Mattsson, 1996). Seedling stem diameter is one of the most useful morphological traits to estimate seedling quality (Grossnickle, 2012). According to Mexal and Landis (1990), seedling stem diameter captures a seedling’s response to the surrounding environment and thus, stem

diameter is correlated with many morphological characteristics including height, root and shoot weight, and root morphology.

It would be expected that high seed germination and successful seedling establishment would be characteristic of a cultivar with high invasive potential. According to Phartyal et al. (2009) around 44% of mature seed of the invasive *P. serotina* germinates. As a result, >50% germination would be considered high germination in the field and thus, be indicative of an invasive risk. Successful seedling establishment is characterized by high seedling survival percentages (>80%; Ritchie, 1984) coupled with vigorous seedling growth. Seedlings of cultivars that demonstrate these characteristics of high seed germination and seedling establishment would warrant further investigation.

The overall objective of this paper was to examine germination and seedling establishment success of open-pollinated, winter-hardy *Prunus* species. Two germination experiments and one seedling establishment experiment were carried out. The objective of Experiment I was to determine whether or not winter-hardy species could successfully germinate in different environments and whether scarification impacted germination. Experiment II additionally investigated whether or not cool, dry storage impacted germination of non-scarified and scarified seed in different environments. In a common field experiment, Experiment III examined seedling establishment of seedlings germinated from open-pollinated seed in Experiment II.

Materials and Methods

In Experiment I (2012/2013) we examined three fruit types of *Prunus* for germination of open pollinated seed including 28 *Prunus* cultivars while in Experiment II

(2014/2015), 22 *Prunus* cultivars were evaluated (Table 1). As a continuation of Experiment II, Experiment III (2015) examined seedling establishment for 21 *Prunus* cultivars. Fruit type was defined as apricot, tart cherry, or plum. These three fruit types represent multiple *Prunus* species (Table 1). Although there are two types of tart cherry varieties, amarelle and morello cultivars, all tart cherries were classified under one category for the purposes of this experiment (Brown et al. 1989). Cultivar refers to all breeding lines, accessions, and released cultivars used in this study. Apricot fruits were collected at maturity in 2012 and 2013 and not in 2014 due to lack of a crop. In 2012, all apricot, tart cherry, and plum fruits were collected from trees at the University of Minnesota research plots in Excelsior, MN (44°52’06.4” N lat., -93°38’00.5” W long.) during weeks 25-26, and 31-34. Week number is defined as the number of weeks from the first week of the year beginning January 1. Mature apricot fruits were collected during weeks 31-32 in 2013. In 2014, fruits of all tart cherry, except for *P. cerasus* ‘Northstar’, and all plum germplasm were collected at maturity from trees at University of Minnesota research plots in Excelsior, MN (44°52’06.4” N lat., -93°38’00.5” W long.) during weeks 29, 35-37. ‘Northstar’ fruits were collected during week 23 in 2013 and week 29 in 2014 from a tree in Saint Paul, MN (44°59’3.6744” N lat., -93°4’7.2546” W long.). In addition, mature ‘Mount Royal’ fruits were collected at University of Minnesota Saint Paul, MN (44°59’17.1” N lat., 93°10’53.8” W long.).

Experiment I (2012/2013)

A multistep series of seed treatments were used in Experiment I for each *Prunus* cultivar. For each cultivar, 48 seeds were randomly chosen and divided into two groups

of 24 each. The first group of 24 seeds were not mechanically scarified and served as the control. These were planted in 8 containers with 3 seeds per container. The second group of 24 seeds was mechanically scarified by cracking the endocarp. These scarified seeds were planted in 8 containers with 3 seeds per container. The containers, rather than individual seeds, were considered experimental units. Seeds were planted in 11.43 x 11.43 cm Jumbo Junior containers (Belden Plastics, St. Paul, MN) and were filled with either BM2 germination mix (Berger, Quebec Canada) or pasteurized field soil (Waukegin silt loam) that was collected from the University of Minnesota St. Paul campus (44°59’17.8” N lat., -93°10’51.6” W long.).

After planting, all containers were put through a common warm stratification treatment at room temperature (20-25°C, day/night) in darkness for two weeks, beginning week 41, 2012. Containers were monitored and watered as necessary for the duration of warm stratification.

After the warm stratification treatment, containers were divided into greenhouse and field environments. Containers, 4 non-scarified and 4 scarified per cultivar, for the greenhouse environment were placed in a cooler (5°C; complete darkness) for a 112-day period of cold stratification, week 43, 2012 – week 7, 2013. During the cold stratification period, containers were monitored for seed germination and hand-watered as necessary. Field containers, 4 scarified and 4 non-scarified per cultivar, were covered with fine netting to prevent rodents and other animals from removing seeds. These containers were placed in a field plot at the University of Minnesota Saint Paul, MN (44°59’18.4”N, -93°10’21.5”W) in week 43, 2012. Containers in the field were buried with the soil level

of the containers equal to the field soil level and placed in a randomized complete block design. As a result, about 2.5 cm of each container was visible above the soil. Containers in the field were overwintered. Average monthly soil temperature (10.2 cm depth) and the number of days with average temperatures above and below 0°C per month during Experiments I and II were calculated from average soil temperatures at the University of Minnesota St. Paul Climatological Observatory (44°59’25.1” N long., -93°10’35.2” W lat.; Minnesota DNR, 2016; Table 2).

When the cold stratification period in the cooler was completed in the greenhouse environment, containers were placed in a randomized complete block design in the greenhouse. The average day/night temperature for the greenhouse environment was 17.8°C. Germination was monitored for a seven-week period. A seed was considered germinated once the plumule was observed above the soil surface (Huntzinger, 1971). The week each seed germinated was denoted using a toothpick placed next the seedling. The average number of weeks for germination for each container was calculated by summing the number of weeks to germination for all germinated seedlings and then dividing by the number of seedlings that germinated in the container. If a seed did not germinate, it was not used to calculate average number of weeks for germination.

In the spring of 2013, the containers in the field were monitored for germination *in situ*. Starting when the first seedling’s pumule became visible, germination for all containers was monitored for seven weeks. Average number of weeks to germination for individual seedlings was recorded with the same methodology as in the greenhouse.

Experiment II (2014/2015)

Since environment (greenhouse and field) had a significant effect on germination in Experiment I, the experiment was repeated in both environments but each environment was treated as statistically independent. In addition to examining the effects of seed scarification and environment on seed germination, Experiment II also examined the effect of differing lengths of cold, dry seed storage on germination of *Prunus* cultivars. For apricot cultivars, seed storage for 26 months for 2012 seed and for 13 months for 2013 seed was examined. Due to the lack of an apricot crop in 2014, only stored seed was examined in Experiment II. For tart cherry cultivars, seed storage for 26 months for 2012 seed, 13 months for 2013 seed, and 2 months for 2014 seed were compared. The 2014 tart cherry seed was stored for 2 months as seed was collected during week 29 and the experiment began during week 39. For plum cultivars, the seed storage for 25 months for 2012 seed and 1 month for 2014 seed was examined. Since plum fruits were collected in weeks 35-37 and the experiment began in week 39, plum cultivars' 2014 seed was stored for 2 weeks to a month. Containers in the greenhouse went through a 120-day period of cold stratification in the cooler (5°C; complete darkness). After removal from the cooler, week 5 (2015), containers were placed in a greenhouse with an average temperature of 21°C and 16 hours of supplemental light (300 μmols m^{-2} s^{-1}). The containers in the field were randomized in a completely randomized design. In the spring, containers in the field were monitored for germination for six weeks after germination of first seed.

In the greenhouse environment, after monitoring germination for 7 weeks, seedlings within a container were transplanted in week 16 as a group into a larger container and placed in the greenhouse for two weeks. These groups of seedlings within a

container were considered a replication for the seedling establishment study and individual seedlings were considered measurement units. After week 18 in 2015, the containers were placed in a cold frame for five weeks to harden off until transplanting into the field. As germination occurred in the field environment of Experiment II, individual containers were removed and placed in the cold frame, similar to the greenhouse environment, until transplanting into the field.

Experiment III (2015)

Seedlings from both the greenhouse and the field environments of Experiment II were transplanted into a common field environment (Waukegin silt loam soil) at the University of Minnesota St. Paul campus (44°59'18.4"N, -93°10'21.5"W). The plot was rototilled prior to transplanting. Transplanting occurred during week 23 in 2015. Seedlings germinated in each container (n=1-3) were kept together when removed from containers to minimize disturbance to the root systems. Groups of seedlings (replications) were transplanted in the field in a generalized block design, blocking by germination environment. Replications were planted 15.24 cm apart. Initial height (cm) and initial stem diameter (mm) at 2.5 cm above the soil level was taken at transplanting for each seedling within a replication. Within a replication, initial height (cm) and initial stem diameter (mm) were averaged. Seedlings were monitored, regularly irrigated, and weeded as necessary.

Towards the end of the season, herbivore damage became apparent. This damage was caused by *Odocoileus virginianus* Zimmermann (white-tailed deer) and *Sylvilagus floridanus* Allen (eastern cottontail rabbit). Individual seedlings within a replication were

rated on a 1 to 5 scale for herbivore damage and the average rating per replication was recorded. This scale was adopted from a similar scale used by von Merév et al. (2012) with the ratings defined as 1 = No visible damage; 2 = less than 25% seedling damaged and/or removed; 3= 25% to 50% of seedling damaged and/or removed; 4 = 50% to 75% of seedling damaged and/or removed; 5 = greater than 75% of seedling damaged and/or removed. Final stem diameter (mm) at 2.5 cm above soil level, the number of root suckers, and damage rating was taken during week 36. Final plant height was not taken due to herbivore damage. Initial and final trunk cross-sectional area was calculated as: $TCA = \pi(\frac{Diameter}{2})^2$ (Robinson, 2008). Using the average initial and final stem diameters for a replication, the average change in stem diameter and trunk cross-sectional area (Δ TCA) was calculated. Change in stem diameter was calculated by subtracting the average initial stem diameter for each group of seedlings from the average final stem diameter. The Δ TCA was calculated by subtracting average initial TCA for each group of seedlings (replication) from the final TCA. Percent survival at the end of the experiment was calculated by dividing the total number of seedlings per cultivar divided by the total number of seedlings planted. Since all replications were pooled for percent survival calculations, no averages or average separations were calculated.

Statistical analysis

R version 3.2.3 (2015-12-10) was utilized for the majority of statistical analysis. For Experiments I, II, and III, quantitative data was first analyzed among fruit types (i.e. apricot, tart cherry, and plum) using univariate, linear model analysis of variance (ANOVA). Within a fruit type, quantitative data was analyzed among cultivars using

univariate, linear model ANOVA. For Experiments I, two tailed t-tests were utilized to compare non-scarified and scarified seed germination within a cultivar. In Experiment II, two-tailed t-tests were used to compare the effects of seed storage lengths on germination percentage and average weeks for germination within a cultivar. Scarification was not used as a factor when analyzing data in Experiment III because it was not significant across environments and fruit types in Experiment I. For both Experiments I and II, germination percentage data was transformed using an arcsine, square root transformation. Since variation within a fruit type was of interest, mean values for cultivars within a fruit type were compared using Tukey's Honestly Significant Difference test (HSD) at a significance level of $\alpha \leq 0.05$. Germination percentage data within each *Prunus* species or within the plum hybrids were correlated, using Pearson correlations ($\alpha \leq 0.05$), between field and greenhouse environments for Experiments I and II. For Experiment III, Pearson correlations were carried out between the factors, initial height, change in stem diameter, Δ TCA, final percent survival, and herbivore damage rating at a significance level of $\alpha \leq 0.05$.

Results

Experiment I

In Experiment I, environment ($p<0.001$) and fruit type ($p<0.001$) significantly impacted % germination across environments, fruit types and seed scarification treatments. Seed scarification ($p=0.998$) and the fruit type x scarification interaction ($p=0.292$) were not significant. Due to the significant effects of environment and fruit

type, % germination data was analyzed separately for each fruit type within an environment (Table 3).

Within the greenhouse environment of Experiment I there were significant effects of fruit type ($p<0.001$) and seed scarification ($p<0.01$) on % germination. The fruit type x scarification interaction was also significant ($p<0.05$). Due to the significant effect of fruit type, subsequent statistical analyses were carried out for each fruit type separately. For the field environment of Experiment I, there was a significant effect of fruit type on % germination ($p<0.001$). However, scarification and the fruit type x scarification interaction were not significant in this environment. Percent germination data was pooled for scarification treatment within the Experiment I field environment in future analyses (Table 3).

Fruit type ($p<0.01$), seed scarification treatment ($p<0.05$), and the interaction of fruit type x scarification ($p<0.01$) had significant effects on the average number of weeks for germination whereas environment did not have a significant effect ($p=0.09$). Average number of weeks for germination data was pooled over environments and analyzed separately for fruit type and scarification treatments in subsequent analyses (Table 3).

Experiment II

In the greenhouse environment of Experiment II, fruit type ($p<0.05$) and seed scarification ($p<0.001$) had significant effects on % germination across fruit types, seed storage, and scarification treatments whereas seed storage ($p=0.28$) did not have a significant effect. The fruit type x seed storage ($p=0.36$), fruit type x scarification ($p=0.81$), and fruit type x scarification x seed storage ($p=0.81$) interactions were all not

significant. Data within the greenhouse environment of Experiment II was analyzed separately for each fruit type and scarification treatment (Table 4).

Fruit type ($p<0.001$) and scarification treatment ($p<0.001$) had significant effects on average number of weeks for germination in the Experiment II greenhouse environment across fruit types, scarification, and seed storage lengths. Seed storage ($p=0.22$) and the interactions of fruit type x scarification ($p=0.14$), fruit type x seed storage ($p=0.89$), scarification x seed storage ($p=0.26$), and fruit type x scarification x seed storage ($p=0.96$) were all not significant. Since seed storage did not have a significant effect on % germination or average number of weeks for germination in the Experiment II greenhouse environment, data was pooled for storage in subsequent analysis (Table 4).

In the field environment of Experiment II, the main effects of fruit type ($p<0.05$) and seed storage ($p<0.05$) had significant effects on % germination across fruit types and seed storage lengths. Seed scarification did not have a significant effect ($p=0.16$). The interactions of fruit type x scarification ($p=0.99$), fruit type x seed storage ($p=0.78$), scarification x seed storage ($p=0.73$), and fruit type x scarification x seed storage ($p=0.73$) were not significant. In subsequent analyses in the field environment of Experiment II, % germination data was pooled for scarification treatment (Table 5).

Fruit type ($p=0.62$), scarification treatment ($p=0.38$), and seed storage ($p=0.98$) did not have significant effects on average number of weeks for germination in the Experiment II field environment. The fruit type x scarification ($p=0.35$), fruit type x seed storage ($p=0.16$), and scarification x seed storage ($p=0.14$) interactions were also not

significant. On average, all seeds in the field environment of Experiment II germinated by the end of week 2.

Experiment III

In Experiment III there was a significant effect of both germination environment ($p<0.001$) and fruit type ($p<0.001$) on initial seedling height across all environments and fruit types. Fruit type did not have a significant effect on initial stem diameter ($p=0.09$) whereas germination environment ($p<0.001$) and the germination environment x fruit type interaction ($p<0.01$) did have significant effects. Germination environment ($p<0.001$) and fruit type ($p<0.001$) had significant effects on change in stem diameter. The germination environment x fruit type interaction ($p<0.001$) also had a significant effect on change in stem diameter. The main effects of germination environment ($p<0.001$) and fruit type ($p<0.001$) as well as the germination environment x fruit type interaction ($p<0.001$) had significant effects on ΔTCA. Due to the significant impact of environment and fruit type on the majority of variables examined in Experiment III, data for these variables was analyzed separately within a fruit type and germination environment combination (Tables 6 and 7).

Fruit type ($p<0.001$) had a significant effect on average herbivore damage rating, but germination environment ($p=0.11$) and germination x fruit type interaction ($p=0.55$) were not significant. In subsequent analyses, average herbivore damage rating data was pooled over germination environments (Table 8).

Apricots

Experiment I

In the Experiment I greenhouse environment there was a significant effect of cultivar ($p<0.01$) on % germination within the apricot fruit type. Scarification ($p=0.77$) and the cultivar x scarification interaction ($p=0.12$) did not have significant effects. For non-scarified seed the range in average % germination was 33.3% for 'Westcot' to 100.0% for 'Moongold' with 'Westcot' differing significantly from both 'Moongold' and 'Sungold' (Table 3). There was no significant variation for average % germination among scarified apricot seed in the greenhouse environment with apricots having a pooled average of 69.4% germination (Table 3).

In the field environment of Experiment I there was a significant effect of cultivar ($p<0.01$) on % germination. The range in average % germination was 20.8% for 'Moongold' to 66.7% for 'Sungold' with 'Sungold' differing significantly from both 'Moongold' and 'Westcot' (Table 3).

Apricot cultivar ($p<0.001$) had a significant effect on average number of weeks for germination across environments. Scarification ($p=0.06$) and the cultivar x scarification interaction ($p=0.25$) did not have significant effects on average number of weeks for germination. For non-scarified apricot seed, averages ranged from 0.6 for 'Westcot' to 2.0 for 'Moongold' with both 'Westcot' and 'Sungold' differing significantly from 'Moongold' (Table 3). There was no significant variation among apricot cultivars for average number of weeks for germination of scarified seed with all germinating on average by the end of week 1.0 (Table 3).

Experiment II

In the greenhouse environment of Experiment II, apricot cultivar ($p<0.01$) had a significant effect on % germination whereas scarification ($p=0.18$) and the cultivar x scarification interaction ($p=0.59$) did not have significant effects. Within a seed scarification treatment, there was no significant variation in average % germination among apricot cultivars (Table 4). Non-scarified seed had a pooled % germination average of 54.2% and scarified seed had a pooled average of 67.4% (Table 4). Within an apricot cultivar there was no significant differences between non-scarified and scarified seed for % germination (Table 4).

Scarification ($p<0.01$) had a significant effect in the greenhouse environment on the average number of weeks for germination whereas apricot cultivar ($p=0.27$) and the cultivar x scarification interaction ($p=0.17$) did not. For non-scarified seed, the average number of weeks for germination ranged from 0.8 for 'Debbie's Gold' to 2.0 for MN203, with MN203 being significantly different than all other apricot cultivars (Table 4). There was no significant variation among scarified seed of apricot cultivars with all germinating on average by the end of week 1.0 (Table 4). For 'Debbie's Gold' there was a significant difference in average number of weeks for germination between non-scarified and scarified seed (Table 4).

Apricot cultivar ($p<0.001$) significantly affected % germination in the Experiment II field environment whereas seed storage ($p=0.14$) and the cultivar x seed storage interaction ($p=0.53$) did not. For seed stored for 26 months, averages ranged from 25.0% for 'Westcot' to 70.8% for 'Moongold' with 'Westcot' being significantly different than 'Moongold' (Table 5). Average % germination of seed stored for 13 months ranged from

4.2% for MN203 to 64.6% for ‘Sungold’ with ‘Westcot’ and ‘Sungold’ differing significantly from MN203 (Table 5). Within an apricot cultivar there was no significant difference in average % germination between 26 and 13 months in storage (Table 5).

Experiment III

There was a significant effect of cultivar on initial height for apricots germinated in the greenhouse ($p<0.05$) but not in the field ($p=0.51$). Within a germination environment (i.e. greenhouse or field), there was no significant variation among apricot cultivars for initial height with pooled averages of 67.9 cm for the greenhouse seedlings and 24.4 cm for the field seedlings (Tables 6 and 7).

For apricot seedlings started in the greenhouse or the field environments, cultivar did not have a significant effect on initial stem diameter ($p=0.48$ and $p=0.16$, respectively), change in stem diameter ($p=0.52$ and $p=0.19$, respectively), or ΔTCA ($p=0.84$ and $p=0.22$, respectively). As a result, there was no significant variation within a germination environment among apricots for initial stem diameter, change in diameter, and ΔTCA (Tables 6 and 7). Pooled averages for apricot seedlings started in the greenhouse were 5.25 mm for initial diameter, 1.14 mm for change in diameter, and 8.34 mm^2 for ΔTCA (Table 6). For apricot seedlings started in the field, pooled averages for initial diameter, change in diameter, ΔTCA were 2.33 mm, 1.56 mm, and 8.16 mm^2, respectively (Table 7). Average herbivore damage ratings for apricot seedlings were ≥ 4.7 with no significant variation among apricot cultivars (Table 8).

No apricot seedlings germinated in the greenhouse environment produced root suckers (Table 9). Two replications of seedlings from ‘Moongold’ and one replication of

seedlings from ‘Westcot’ started in the field produced one root sucker (Table 9). At the end of the experiment, % survival of apricot seedlings germinated in the greenhouse ranged from 83% for ‘Westcot’ to 95% for ‘Debbie’s Gold’ while % survival for seedlings germinated in the field ranged from 81% for ‘Westcot’ to 100% for ‘Moongold’ (Table 10).

Tart Cherries

Experiment I

Within the tart cherry fruit type, cultivar ($p=0.65$) and scarification ($p=0.20$) did not significantly affect % germination in the greenhouse environment of Experiment I. In the Experiment I field environment, cultivar ($p=0.15$) did not have a significant effect on % germination. In either germination environment, % germination of tart cherries was ≤ 33.3% with no significant variation among cultivars (Table 3).

Cultivar ($p=0.60$), scarification ($p=0.24$) and cultivar x scarification interaction ($p=0.07$) did not have significant effects on average number of weeks for germination. On average, all tart cherry seeds germinated by week 1.6 with no significant variation among cultivars (Table 3).

Experiment II

Within the tart cherry fruit type there was a significant effect of scarification ($p<0.001$) on % germination in the Experiment II greenhouse environment whereas the main effect of cultivar ($p=0.45$) and the cultivar x scarification interaction ($p=0.74$) were not significant. There was no significant variation among cultivars within a scarification treatment (Table 4). Pooled average % germination for non-scarified seed was 35.0%

whereas for scarified seed the pooled average was 52.5% (Table 4) ‘Meteor’ had a significant difference in average % germination between non-scarified and scarified seed (Table 4).

In the Experiment II greenhouse environment, the main effects of tart cherry cultivar ($p<0.05$) and scarification ($p<0.001$), and the cultivar x scarification interaction ($p<0.01$) had significant effects on average number of weeks for germination. For non-scarified seed, average number of weeks for germination ranged from 1.0 for ‘Bali’ to 3.6 for ‘Northstar’ with ‘Northstar’ differing significantly from ‘Suda’ and ‘N87155’ (Table 4). Average % germination of ‘Meteor’ non-scarified seed was significantly different than ‘N81755’ (Table 4). For scarified seed there was no significant variation in average number of weeks for germination among tart cherry cultivars with all germinating by the end week 2.0 (Table 4). The tart cherry ‘Northstar’ had a significant difference in average number of weeks for germination between non-scarified and scarified seed (Table 4).

In the Experiment II field environment there was a significant effect of both cultivar ($p<0.05$) and seed storage ($p<0.05$) on % germination. The cultivar x seed storage interaction ($p=0.06$) was not significant. There was no significant variation in % germination among cultivars with seed stored for 26 months with tart cherries having a pooled average of 19.8% (Table 5). Average % germination among tart cherry seed stored for 2 months ranged from 8.3% for ‘Northstar’ to 58.3% for ‘Bali’ with ‘Northstar’ differing significantly from ‘Bali’ (Table 5). For ‘Bali’ there was a significant difference in average % germination between seed stored for 26 months and for 2 months (Table 5).

Experiment III

For tart cherry seedlings started in either the greenhouse or field environments, there was no significant effect of cultivar on initial height ($p=0.36$ and $p=0.12$, respectively), initial stem diameter ($p=0.21$ and $p=0.19$, respectively), change in stem diameter ($p=0.32$ and $p=0.09$, respectively), and ΔTCA ($p=0.26$ and $p=0.21$; Tables 6 and 7). Within a germination environment there was no significant variation among tart cherry seedlings for initial height, initial stem diameter, change in diameter, and ΔTCA (Tables 6 and 7). Pooled averages for tart cherry seedlings started in the greenhouse were 31.1 cm for initial height, 5.93 mm for initial diameter, 1.95 mm for change in diameter, and 25.35 mm^2 for ΔTCA (Table 6). For seedlings started in the field, pooled averages were 7.4 cm for initial height, 1.57 mm for initial diameter, 3.31 mm for change in diameter, and 18.60 mm^2 for ΔTCA (Table 7). Average damage ratings for all tart cherry seedlings were ≤3.3 with no significant variation among cultivars (Table 8).

Replications of seedlings started in the greenhouse from ‘Bali’, ‘Northstar’, and ‘Suda’ produced one or more root suckers with seedlings from ‘Northstar’ and ‘Suda’ producing the largest number (Table 9). No tart cherry replications started in the field produced root suckers (Table 9). At the end of the experiment, % survival of tart cherry seedlings germinated in greenhouse ranged from 67% for ‘Suda’ to 92% for ‘Meteor’ (Table 10). For seedlings started in the field, % survival ranged from 89% for ‘N81755’ to 100% for ‘Northstar’ (Table 10).

Plums

Experiment I

Within the plum fruit type, cultivar ($p<0.001$) and scarification ($p<0.001$), and the cultivar x scarification interaction ($p<0.01$) had significant effects on % germination in the greenhouse environment of Experiment I. Averages for non-scarified seed of plum cultivars ranged from 0.0% for ‘Winona’ to 100.0% for ‘Opal’ (Table 3). For non-scarified seed there was significant variation among plum cultivars with multiple cultivars differing from each other (Table 3). The range in average % germination for plum cultivars’ scarified seed in the greenhouse environment was 16.7% for ‘Tecomseh’ to 91.7% for ‘La Crescent’ and ‘Whittaker’ (Table 3). There was significant variation among plum cultivars for average % germination of scarified seed (Table 3). Within the plums ‘Hennepin’, ‘Superior’, and ‘Winona’ there were significant differences for % germination between non-scarified and scarified seed ($p<0.05$; Table 3).

In the field environment of Experiment I there was a significant effect of plum cultivar ($p<0.001$) on average % germination. Average germination percentages ranged from 0.0% for ‘Mount Royal’, ‘Stanley’, and ‘Tecomseh’ to 62.5% for ‘Hennepin with there being significant variation among plum cultivar means (Table 3).

Plum cultivar ($p<0.01$) and scarification ($p<0.001$) had significant effects on average number of weeks for germination. The cultivar x scarification interaction was not significant ($p=0.06$). There were no significant differences among cultivars for average number of weeks for germination of non-scarified or scarified seed (Table 3). Non-scarified seed had a pooled average of 1.9 weeks for germination whereas scarified seed had an average of 1.6 weeks (Table 3). Within the plums ‘Gracious’, ‘Superior’, and

‘Winona’ there were significant differences between non-scarified and scarified seed for average number of weeks for germination (Table 3).

Experiment II

In the greenhouse environment of Experiment II there was a significant effect of both cultivar ($p<0.001$) and scarification ($p<0.001$) on % germination whereas the cultivar x scarification interaction ($p=0.45$) did not have a significant effect. For non-scarified seed, averages ranged from 4.2% for ‘Alderman’ to 75.0% for ‘Todd’ (Table 4). There was significant variation among cultivars for % germination of non-scarified seed (Table 4). No significant variation was observed among plum cultivars for % germination of scarified seed with plum cultivars having a pooled average of 56.9% (Table 4). For ‘Alderman’, ‘Gracious’, and ‘Winona’ there were significant differences between non-scarified and scarified seed (Table 4).

In the greenhouse environment of Experiment II, cultivar ($p=0.08$), scarification ($p=0.10$) and the cultivar x scarification interaction ($p=0.38$) did not have significant effects on average number of weeks for germination. On average, all plum cultivars germinated by the end of week 2 (Table 4).

In the field environment of Experiment II, cultivar ($p<0.001$) had a significant effect on % germination whereas seed storage ($p=0.14$) and the cultivar x seed storage interaction ($p=0.54$) did not. For seed stored for 25 months, averages ranged from 4.2% for ‘Monitor’, ‘Mount Royal’ and ‘Pipestone’ to 95.8% for ‘Hennepin’ (Table 5). There was significant variation among cultivars for % germination of seed stored for 25 months (Table 5). Average % germination for seed stored for 1 month ranged from 0.0% for

‘Monitor’ to 75.0% for ‘Hennepin’ with significant variation being observed among cultivars (Table 5).

Experiment III

Plum cultivar had a significant effect on initial height of seedlings started in the greenhouse ($p<0.05$) and in the field ($p<0.001$). On average, plum seedlings started in the greenhouse had a pooled average of 61.1 cm for initial height with no significant variation among cultivars (Table 6). Average initial heights of plum seedlings started in the field ranged from 8.5 cm for seedlings from ‘Mount Royal’ to 25.7 cm for seedlings from ‘Whittaker’ (Table 7). There was significant variation for initial height among plum seedlings started in the field (Table 7).

Cultivar had a significant effect on initial stem diameter of plum seedlings started in the greenhouse ($p<0.001$) and the field ($p<0.001$). For plum seedlings started in the greenhouse, average initial diameters ranged from 4.21 mm for seedlings from ‘South Dakota’ to 6.88 mm for seedlings from ‘Mount Royal’ with there being significant variation among cultivars (Table 6). Averages for seedlings started in the field ranged from 1.16 mm for seedlings from ‘Monitor’ to 2.39 mm for seedlings from ‘Todd’ with ‘Todd’ differing significantly from a number of plum cultivars (Table 7).

For plum seedlings started in the greenhouse, cultivar ($p=0.65$) did not have a significant effect on change in stem diameter and there was no significant variation among cultivars (Table 6). Seedlings started in the greenhouse had a pooled average change in diameter of 0.67 mm (Table 6). For plum seedlings started in the field, there was a significant effect of cultivar ($p<0.001$) on change in stem diameter with a range of

1.69 mm for seedlings from multiple cultivars to 4.64 mm for ‘Whittaker’ (Table 7). Among plum seedlings started in the field there was significant variation for average change in stem diameter with ‘Whittaker’ being significantly different than four different plums (Table 7)

Cultivar ($p=0.71$) did not have a significant effect on ΔTCA for plum seedlings started in the greenhouse and there was no significant variation among plum seedlings (Table 6). Seedlings started in the greenhouse had a pooled average ΔTCA of 6.90 mm^2 (Table 6). For plum seedlings started in the field, cultivar had a significant effect on ΔTCA ($p<0.001$) with average ΔTCA ranging from 3.18 mm^2 for seedlings from ‘Mount Royal’ to 36.89 mm^2 for seedlings from ‘Whittaker’ (Table 7). Seedlings from ‘Whittaker’ differed significantly from half of the other plum cultivars (Table 7).

Average herbivore damage ratings for plums ranged from 2.7 for seedlings from ‘Hazel’ to 4.8 for seedlings from ‘Todd’ (Table 8). There was significant variation among plums for average herbivore damage rating with seedlings from ‘Hazel’ differing significantly from seedlings of three cultivars (Table 8).

Of the plums germinated in the greenhouse, replications of seedlings from ‘Todd’, ‘Gracious’, ‘Monitor’, ‘South Dakota’, and ‘Whittaker’ produced one or more root suckers during the experiment (Table 9). Five replications from ‘South Dakota’ produced the most root suckers with a pooled total of 8 (Table 9). There were ≤ 4 replications from ‘Todd’, ‘Gracious’, ‘Monitor’, and ‘Whittaker’ that produced suckers (Table 9). In contrast to seedlings started in the greenhouse, replications of plum seedlings started in field produced fewer total root suckers (Table 9). One sucker was produced by two

replications from ‘Hazel’ and ‘South Dakota’, and single replications from ‘Compass’, ‘Gracious’, ‘Monitor’, and ‘Whittaker’ (Table 9).

At the end of Experiment III, % survival among plum seedlings started in the greenhouse ranged from 33% for seedlings from ‘Pipestone’ to 89% for seedlings from ‘Mount Royal’ (Table 10). Percent survival among plum seedlings started in the field ranged from 43% for seedlings from ‘Winona’ to 100% for seedlings from multiple cultivars (Table 10).

Correlations

Experiments I and II

In Experiment I, the only significant correlation between % germination in the greenhouse and field was for the plum hybrids, *Prunus spp.* ($r=0.19$, $p<0.05$; Table 11). The remaining correlation coefficients for Experiment I were not significant (Table 11). For Experiment II, there were significant positive correlations between % germination in the greenhouse and field for *P. cerasus* ($r=0.28$, $p<0.05$), *P. domestica* ($r=0.62$, $p<0.001$), and the plum hybrids, *Prunus spp.* ($r=0.30$, $p<0.01$; Table 11).

Experiment III

Pearson correlations were carried out between the factors, initial height, initial stem diameter, change in stem diameter, ΔTCA, average herbivore damage rating, and % survival to the end of experiment. Initial height was negatively correlated with change in stem diameter ($r=-0.36$, $p<0.001$) and ΔTCA ($r=-0.15$, $p<0.01$; Table 12). Change in stem diameter was also negatively correlated with average herbivore damage rating ($r=-0.41$, $p<0.001$) and positively correlated with ΔTCA ($r=0.88$, $p<0.001$; Table 12). ΔTCA was

also negatively correlated with average herbivore damage rating ($r=-0.41$, $p<0.001$; Table 12).

Discussion

The introduction of invasive species often begins with human activities like horticulture or forestry (Reichard and White, 2001; Vanhellemont et al. 2009). Many winter-hardy *Prunus* cultivars have been cultivated since the early 1900s to 1980s (Anderson and Weir, 1967; Brooks and Olmo, 1997), but many of these cultivars have not been extensively propagated. Some *Prunus* species have escaped cultivation and have become invasive in certain parts of the world. For example, *P. serotina* has become invasive in parts of Europe (Deckers et al. 2005). *P. americana* has also demonstrated high invasive potential as it is adapted to a variety of habitats and spread across a wide geographic range (Francis, 2004). Whether other *Prunus* species and cultivars will become invasive is not known. According to Kolar and Lodge (2001) as well as Siemann and Rogers (2001), invasive species are first introduced and then, through germination and stand establishment out compete native species.

Successful germination is the first step towards establishing a self-sustaining population and as a result, species with higher % germination compared to native species may be more likely to become invasive (Hock et al. 2015). In our experiments, seed germination across environments for apricots was high. Tart cherries were characterized by low germination. The plum cultivars we studied had variable % germination, which is perhaps due to the diverse genetic background (Table 1). Some plum cultivars like *P. americana* 'Hazel', *P. munsoniana* 'Whittaker', and Japanese-American hybrids 'South

Dakota', and 'Hennepin' had high seed germination across both environments, scarification treatments and seed storage. In contrast, *P. domestica* 'Mount Royal' and *Prunus spp.* 'Monitor' had variable germination percentages across environments, scarification treatments and seed storage. In comparison to native species, cultivars with higher % germination across environments could potentially become invasive compared to cultivars with low germination (Hock et al. 2015).

Inbreeding depression could potentially provide an explanation for why we observed low % germination among tart cherry cultivars. Most tart cherry cultivars are self-compatible but naturally outcrossing and thus, inbreeding depression is possible in tart cherry progeny (Lansari and Iezzoni, 1990; Krahl et al. 1991). According to Baskin and Baskin (2015), inbreeding has a variable effect on germination; in some cases inbreeding depression has a negative relationship with germination. Lansari et al. (1994) states that inbreeding depression in almond (*P. dulcis* Miller) can result in reduced seed germination. Inbreeding depression in the tart cherry cultivars tested could have played a role in the lower germination observed. Even though most tart cherry cultivars had low % germination, germination still did occur, indicating invasive potential. Other factors that may affect a cultivar's invasive potential include crop load, seed dispersal mechanism, and seedling establishment (Bullock et al. 2002; Deckers et al. 2008). According to Deckers et al. (2008) the invasive *P. serotina* has inconsistent crop loads but its avian dispersal system makes it highly effective at spreading throughout the landscape. Tart cherries are often consumed completely or damaged by birds (Lindell et al. 2012). The

potential for seed dispersal via birds coupled with good stand establishment may result in higher invasive potential.

One of the questions of this study was whether or not cold, dry storage of seed would result in reduced germination. According to Cochran et al. (1961) plum seeds once dried can be stored for up to four years without a significant loss of viability. Grisez et al. (2008) reported that after 18 months in storage at 7-10°C, *P. americana* seed had 70% germination. Similarly, Kristiansen and Jenson (2009) concluded that germination of *P. cerasus* 'Stevnsbaer Brigitte' seed did not differ significantly from recently harvested seed. The majority of seeds of cultivars in our study successfully stored for over 2 years without a significant decrease in germination, which led to the conclusion that seed storage does not decrease % germination.

Germination can be impeded at many steps in the processes. The uptake of water initiates germination (Chong et al. 1994). Hard seed coats or at times, stony endocarps can prevent or reduce water uptake (Chong et al. 1994; Hartmann et al. 1997). The endocarp of stone fruits prevents the expansion of the embryo so no radical emergence can occur (Hartmann et al. 1997). These seed types often need to be cracked or softened through scarification to initiate water uptake and thus, germination (Chong et al. 1994; Hartmann et al. 1997). In our experiments, endocarps of seeds were mechanically scarified prior to planting. Scarification had a significant effect on % germination in the greenhouse environments but not in the field environments. A potential reason for this is the freeze-thaw cycle. According to Chong et al. (1994), scarification of the seed can result through the freeze-thaw action of the soil. During the overwintering period in our

field experiments, the soil at a 10.2 cm depth oscillated above and below 0°C (Table 2). Scarification via freezing and thawing of the soil in the field could have been sufficient enough to crack the endocarp of non-scarified seeds and resulted in similar germination between non-scarified and scarified seed.

Kristiansen and Jenson (2009) observed greater % seed germination for *P. cerasus* seeds with the endocarp removed whereas Grisez et al. (2008) reported that after 90 days of cold stratification, *P. armeniaca* seeds achieved 95% germination with an intact endocarp. McMahon et al. (2015) observed no significant difference for % germination between non-scarified and scarified *P. angustifolia* seed and reasoned that the lower percentages of seeds germinating could have been caused by not enough of the endocarp being removed. For example, when Kristiansen and Jenson (2009) removed the entire endocarp of *P. cerasus* seed, there was a significant positive effect on % germination. In the greenhouse environments of our study, scarification had a significant effect on germination and average number of weeks to germination. However, within most cultivars there was not a significant difference for % germination between non-scarified and scarified seed in the greenhouse environments. In the majority cultivars tested in our study, the combination of warm and cold stratification may have sufficiently overcome dormancy and eliminated the need for scarification. Higher % germination was observed for scarified seed in most cultivars where there was a significant difference for % germination between non-scarified and scarified seed in the greenhouse environments. For most cultivars, there was not a significant difference for average number of weeks for germination between non-scarified and scarified seed. Germination percentages that were

similar or lower for scarified seed compared to non-scarified seed may indicate that some cultivars do not require scarification for successful germination.

In this study, we determined there was very little variation among cultivars within a fruit type for the average number of weeks for germination. Most cultivars germinated within three weeks after removal from the cooler in the greenhouse or after the first seed germinated in the field. Regardless of the environment, once germination began, the majority of seeds germinated quickly.

Chong et al. (1994) states that moisture is the most important factor for initiation of seed germination and lack of consistent moisture during germination can result in drying of the seed leading to failed germination and potentially seed death. Across fruit types, we observed higher percent seed germination in the greenhouse than the field. In the greenhouse environments, containers were consistently monitored and watered whereas in the field watering ceased once the field soil froze and did not begin again until the soil thawed. Inconsistent moisture in the field soil could have resulted in lower germination across fruit types.

Lockley (1980) recorded a significant positive correlation between greenhouse and field environments for germination and seedling emergence of *P. virginiana* L, leading to the conclusion that germination in the greenhouse was indicative of germination in the field. If the environments in our germination experiments were correlated, germinated seed in the greenhouse could be predictive of germination under field conditions. This would be a useful tool for quickly screening multiple genotypes. However, we found that within most species there was no significant correlation for %

germination between the two environments. In both germination experiments there was a significant positive correlation between environments for the hybrid plums (*Prunus spp.* L.). In tart cherries (*P. cerasus*) and European plums (*P. domestica*) evaluated, we found positive significant correlations between germination in the two environments for one experiment. The significant positive correlation between greenhouse and field environments within some *Prunus* species indicates that % germination in the greenhouse could be used to predict field germination.

After successful germination, vigorous growth, vegetative propagation via suckering, and lower herbivore pressure of the introduced genotype is imperative to outcompete native species (Kolar and Lodge, 2001; Siemann and Rogers, 2001). In many important timber species, stem diameter and seedling height are used as predictors of field survival and growth (Mattson, 1996). According to Mexal and Landis (1990), seedling diameter provides a snapshot into a seedling's response to the surrounding environment and as a result is correlated with many morphological characteristics including seedling height, root and shoot weight, and root morphology. Stem diameter can provide an estimate of seedling vigor and the size of the root system (Grossnickle, 2012). A significant positive correlation between height and stem diameter was reported by Khadivi-Khub et al. (2012) among species of the *Cerasus* subgenus of *Prunus.* We also found that initial height was significantly correlated with initial diameter, changes in stem diameter and ΔTCA. As a result, we concluded that stem diameter can be used as a measurement of vigor and thus, as a metric for stand establishment.

We found that across fruit types, initial height and diameter was larger for seedlings germinated in the greenhouse than in the field. However, change in diameter, ΔTCA, and survival of seedlings germinated in the greenhouse was lower than in the field at the end of the experiment. Wilson and Jacobs (2006) observed that taller sawtooth oak (*Quercus acutissima* Carruth) seedlings had lower survival upon planting. Esen et al. (2012) observed that taller *P. avium* L. seedlings, up to 70 cm, had higher survival because these seedlings were better able to compete with surrounding vegetation. According to Grossnickle (2012), taller seedlings are better able to compete for light. However, Esen et al. (2012) found that this height and survival relationship became negative once seedlings were taller than 70 cm. Although not significant, initial height and stem diameter had negative correlation coefficients with % survival across all cultivars' seedlings in our experiment. Initial height was negatively correlated with change in diameter and ΔTCA. A potential reason for the lower growth and survival for greenhouse compared to field seedlings may be transplant shock. According to Close et al. (2005), transplant shock is defined as reduction of growth and/or mortality of seedlings after planting. Jacobs et al. (2005) states that transplant shock in newly planted hardwood seedlings is common and often a symptom of stress related to moisture and/or nutrient availability. After transplanting, smaller trees are able to return to vigorous growth more quickly in comparison to larger trees because larger trees require a longer period of time to reestablish their original root to shoot ratio (Watson, 2005). In our study, seedlings from the greenhouse were on average taller with more foliage, which

could have resulted in higher moisture stress when transplanted leading to reduced vigor in the field.

Change in stem diameter and ΔTCA was negatively impacted by herbivory in our experiment. Seedlings with higher herbivory ratings tended to have lower changes in stem diameter and ΔTCA. Although apricot seedling survival and initial height was relatively high, apricots sustained severe herbivore damage and had low changes in stem diameter and ΔTCA, which translated to relatively low vigor and low to moderate establishment. Tart cherries had moderate seedling survival, growth in stem diameter and ΔTCA, low to moderate herbivore damage and root suckers. Large changes in diameter and ΔTCA are indicative of vigorous growth and successful establishment, which may lead to invasiveness. Seedling establishment within the plum fruit type was variable. Seedlings from cultivars *P. domestica* 'Mount Royal', and 'Todd', and *Prunus spp.* 'Winona' had low to moderate seedling survival, high herbivore damage, low changes in diameter and ΔTCA, and low numbers of root suckers. In contrast, *P. americana* 'Hazel', *P. munsoniana* 'Whittaker', and hybrids *Prunus spp.* 'South Dakota' and 'Hennepin' had moderate changes in diameter and ΔTCA, low to moderate herbivore damage, and some seedlings produced suckers. We concluded that the progeny of *P. americana, P. munsoniana,* and some Japanese-American hybrids have a higher likelihood of becoming invasive than progeny from *P. domestica* cultivars.

Deckers (2005) states that invasive species like *P. serotina* has primarily invaded areas of agricultural landscapes characterized as forest fragments, patches of woody habitat, or old agricultural fields. Brown and Antos (2012) examined seed germination

and early survival of bitter cherry (*P. emarginata* Douglas ex Hook) and found that the highest seedling emergence and survival occurred in an open environment and the second highest at the edge of a stand. However, in a more open environment, seedlings are more exposed and accessible to herbivores. As observed in this study, *Prunus* seedlings are not immune to herbivore predation. The main sources of herbivore damage in our field study were White-tailed deer and Eastern cottontail rabbit.

Kullberg and Bergström (2010) observed preferential browsing by large herbivores on different deciduous species. In Kullberg and Bergström's (2010) study, oak (*Quercus robar* L.) alder (*Alnus glutinosa* L.), and beech (*Fagus silvatica* L.) were heavily browsed while species like cherry (*P. avium*) was moderately browsed and species like ash (*Fraxinus excelsior* L.) were only lightly browsed. We observed preferential browsing with significant differences in damage ratings among fruit types and cultivars within a fruit type.

McNaughton (1983) argues that although high levels of herbivory do not result in maximum plant fitness, low to moderate levels of herbivory may result in long-term increases in fitness. Herbivore predation can induce more vegetative growth and thus, result in increased fitness (McNaughton, 1983). According to Kolar and Lodge (2001), the invasive potential of a species increases if that species has historically propagated vegetatively. If a species that has a tendency to sucker is often subjected to low or moderate herbivory, the invasive potential of that species may be very high because of the induction of vegetative propagation. Some *Prunus* species produce root suckers. Franken-Bembenek and Gruppe (1984) concluded that 14% of *P. cerasus* self-pollination

progeny and anywhere from 0% to 90% of interspecific seedlings with *P. cerasus* as a parent produced suckers. Reigard et al. (2013) assessed *P. americana* as a potential rootstock for *P. persica* and found that *P. americana* has a tendency to sucker. We observed that herbivore damage was negatively correlated with change in stem diameter and ΔTCA. Seedlings from apricots cultivars that had less vigorous growth also tended to have high herbivore ratings. Although most seedlings were browsed, in most cases herbivory did not result in seedling mortality by the end of the experiment. Most seedlings responded to herbivore predation with new growth. Seedlings from some tart cherry cultivars, *P. munsoniana,* and some hybrid plums produced root suckers. For example, one replication of seedlings from 'Northstar' produced five root suckers after being damaged by herbivores (Table 9). The majority of seedlings that produced root suckers had low to moderate damage ratings, which indicates that herbivory may stimulate vegetative growth as was argued by McNaughton (1983). From an invasive standpoint, removal of the apical bud of a tart cherry or plum seedling through herbivore predation could result in vegetative propagation via suckering. In turn, vegetative propagation could increase the size of a stand and result in further spread of the species.

Conclusions

Many factors contribute to the invasive potential of a species including seed germination, vigor of seedlings, tendency to vegetatively propagate, herbivore pressure, crop load, and seed dispersal mechanisms (Deckers et al. 2008; Kolar and Lodge, 2001; Siemann and Rogers, 2001). Many of the *Prunus* cultivars examined in this study will probably not become invasive due to poor germination and/or poor seedling

establishment. However, some of the tart cherries and plums exhibited traits that would indicate the potential to become invasive. Although, tart cherries had relatively low % germination, moderate seedling establishment coupled with low herbivore pressure and the ability to produce suckers is indicative of potential invasiveness. Suckering of tart cherry seedlings could allow for successful stand establishment without requiring a large number of seeds to germinate. According to Brooks and Olmo (1997) tart cherry cultivars like 'Northstar' and 'Meteor' tend to be productive and bear regularly. On average, a 10 to 20 year old tart cherry tree ('Montmorency') produces 36 kg to 45 kg (Me-Nsope, 2009). Even with relatively low germination, high fruit yields could result in large numbers of propagule units and thus, could potentially result in a moderate number of seedlings. Progeny from the plum cultivars *P. americana* 'Hazel', *P. munsoniana* 'Whittaker', and the hybrids 'South Dakota' and 'Hennepin' exhibited high germination, vigorous growth, moderate to low herbivore pressure, and produced some root suckers, indicating the potential to become invasive.

Even though some cultivars examined in these experiments exhibit characteristics indicative of the potential to become invasive, escapes from cultivation by these cultivars have not yet been documented. Horticultural practices like mowing, tilling, hand pulling, and the application of herbicides can control the spread invasive species (Beasley and Pijut, 2010; Culley and Hardiman, 2007). As a result of these practices, horticulturalists may inadvertently be preventing the escape of *Prunus* cultivars into surrounding environments. However, winter-hardy *Prunus* cultivars may become invasive if present in an abandoned field or in a circumstance where horticultural control practices are not

applied, as has occurred with the invasive, ornamental Pyrus *calleryana* Dcne in parts of the United States (Culley and Hardiman, 2007; Taylor et al. 1996).

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Table 1: Fruit type, species, and cultivar for germplasm examined in Experiment I, II, and III. Collection location (HRC=University of Minnesota research plots in Excelsior, MN; UMN St. Paul=University of Minnesota St. Paul campus) and years open pollinated *Prunus* seed was collected is indicated along with the groups of seedlings (# replications) started in either the greenhouse or field in Experiment III.

				Experiments I and II Years Seed Collected			Experiment III # Replications	
Fruit Type	Species	Cultivar	Collection Location	2012	2013	2014	Greenhouse	Field
Apricot	*P. armeniaca* L.	'Debbie's Gold'	HRC		x		8	7
		MN203	HRC		x		-	-
		'Moongold'	HRC	x			8	8
		'Sungold'	HRC	x	x		8	13
		'Westcot'	HRC	x	x		15	10
Tart Cherry	*P. cerasus* L.	'Bali'	HRC	x		x	12	8

Table 1: Continued

				Experiments I and II Years Seed Collected			Experiment III # Replications	
Fruit Type	Species	Cultivar	Collection Location	2012	2013	2014	Greenhouse	Field
Tart Cherry	*P. cerasus*	'Mesabi'	HRC	x			-	-
		'Meteor'	HRC	x		x	10	7
		N81755	HRC	x		x	13	10
		'Northstar'	Saint Paul, MN		x	x	11	3
		‘Suda'	HRC	x		x	10	10
Plum	*P. americana* L.	'Hazel'	HRC	x		x	12	14
	P. besseyi x P. hortulana L.	'Compass'	HRC	x		x	11	9
	P. domestica L.	'Mount Royal'	HRC & UMN	x		x	7	5

Table 1: Continued

				Experiments I and II Years Seed Collected			Experiment III # Replications	
Fruit Type	Species	Cultivar	Collection Location	2012	2013	2014	Greenhouse	Field
Plum	*P. domestica*	'Opal'	HRC	x			-	-
		‘Stanley'	HRC	x			-	-
		'Todd'	HRC	x		x	15	14
	P. munsoniana Wright and Hedrick	'Whittaker'	HRC	x		x	16	17
	P. nigra Aiton	'Bounty'	HRC	x			7	5
	Prunus spp. L.	'Alderman'	HRC	x		x	14	12
		'Gracious'	HRC	x		x	16	16

Table 1: Continued

				Experiments I and II Years Seed Collected			Experiment III # Replications	
Fruit Type	Species	Cultivar	Collection Location	2012	2013	2014	Greenhouse	Field
Plum	*Prunus spp.*	'Hennepin'	HRC	x		x	13	2
		'La Crescent'	HRC	x			-	-
		MN598	HRC	x			-	-
		'Monitor'	HRC	x		x	13	2
		'Pipestone'	HRC	x		x	6	4
		'Redcoat'	HRC	x		x	-	-
		'South Dakota'	HRC	x		x	16	15
		'Superior'	HRC	x			-	-
		'Toka'	HRC	x			-	-

Table 1: Continued

				Experiments I and II Years Seed Collected			Experiment III # Replications	
Fruit Type	Species	Cultivar	Collection Location	2012	2013	2014	Greenhouse	Field
Plum	*Prunus spp.*	'Underwood'	HRC	x			-	-
		'Winona'	HRC	x		x	6	7

Table 2: Average monthly soil temperature (°C) from October to May at 10.2 cm depth and number of days with average soil temperatures below and above 0°C. Temperature data was collected at the University of Minnesota Saint Paul campus (Minnesota DNR, 2016).

Experiment I				
Month	Year	Avg. Temp. (C)	Days below 0°C	Days above 0°C
October	2012	10.5	0	31
November	2012	3.3	6	24
December	2012	0.4	3	28
January	2013	-1.9	27	4
February	2013	-1.9	28	0
March	2013	-0.3	29	1
April	2013	3.6	5	15*
May	2013	13.8	0	31
Experiment II				
Month	Year	Average Temp	Days below 0°C	Days above 0°C
October	2014	8.7	0	31
November	2014	2.0	0	30
December	2014	0.2	12	19
January	2015	-14.4	26	5
February	2015	-4.5	28	0
March	2015	-0.2	10	21
April	2015	6.4	0	30

Table 2: Continued

Month	Year	Average Temp	Days below 0°C	Days above 0°C
May	2015	13.7	0	31

*Temperature probe failed for 10 days during April 2013

Table 3: Average % germination (Avg. % Germ.) and average number of weeks for germination (Avg. # of Weeks for Germ.) after cold stratification for non-scarified and scarified *Prunus* seeds in the greenhouse and field environments of Experiment I. In the field environment, % germination was pooled for seed scarification treatments. Average number of weeks for germination was pooled for the two environments (i.e. greenhouse and field). For the greenhouse environment, mean separations (a-c) for % germination were among cultivars within a fruit type and scarification treatment. For the field environment, mean separations (a-e) for % germination were among cultivars within a fruit type for pooled scarification treatment (field). Mean separations (a-b) for average number of weeks for germination were among cultivars within a fruit type and scarification treatment. An asterisk refers to a significant difference ($p<0.05$) within a cultivar across scarification treatments.

		Avg. % Germ.			Avg. # of Weeks for Germ.	
		Greenhouse		Field		
Fruit Type	Cultivar	Non-Scarified	Scarified	Germination	Non-Scarified	Scarified
Apricot	'Moongold'	100.0 a	83.3	20.8 b	2.0 a	1.0
	'Sungold'	83.3 a	66.7	66.7 a	0.7 b	0.5

Table 3: Continued

		Avg. % Germ.			Avg. # of Weeks for Germ.	
		Greenhouse		Field		
Fruit Type	Cultivar	Non-Scarified	Scarified	Germination	Non-Scarified	Scarified
Apricot	'Westcot'	33.3 b	58.3	25.0 b	0.6 b	0.4
	Pooled	72.2	69.4	37.5	1.1	0.6
Tart Cherry	'Bali'	16.7	33.3	8.3	0.6	1.2
	'Mesabi'	16.7	33.3	0.0	0.4	1.0
	'Meteor'	25.0	0.0	0.0	0.9	0.0
	‘N81755’	33.3	8.3	16.7	1.6	0.5
	'Suda'	16.7	0.0	16.7	0.6	0.4
	Pooled	21.7	15.0	8.3	0.8	0.6
Plum	'Hazel'	25.0 abc	50.0 ab	25.0 abc	0.6	1.2

Table 3: Continued.

		Avg. % Germ.						Avg. # Weeks for Germ.	
		Greenhouse				Field			
Fruit Type	Cultivar	Non-Scarified		Scarified		Germination		Non-Scarified	Scarified
Plum	'Compass'	33.3	abc	83.3	ab	29.2	abc	1.0	1.5
	'Mount Royal'	41.7	abc	25.0	ab	0.0	e	1.2	0.4
	'Opal'	100.0	a	75.0	ab	4.2	de	1.5	1.1
	'Stanley'	33.3	abc	25.0	ab	0.0	e	0.7	0.8
	'Todd'	41.7	abc	58.3	ab	12.5	bcde	0.6	1.1
	'Whittaker'	58.3	ab	91.7	a	41.7	abcd	1.0	1.1
	'Bounty'	41.7	abc	75.0	ab	50.0	abc	1.4	2.0
	'Alderman'	16.7	abc	58.3	ab	8.3	cde	0.5	1.0
	'Gracious'	16.7	abc	58.3	ab	25.0	abc	0.3 *	1.4 *

Table 3: Continued.

		Avg. % Germ.			Avg. # Weeks for Germ.	
		Greenhouse		Field		
Fruit Type	Cultivar	Non-scarified	Scarified	Germination	Non-Scarified	Scarified
Plum	‘La Crescent’	91.7 a	91.7 a	16.7 bcde	2.1	2.1
	MN598	25.0 abc	50.0 ab	0.0 e	1.3	1.2
	‘Monitor’	25.0 abc	33.3 ab	16.7 bcde	0.8	1.9
	‘Pipestone’	41.7 abc	50.0 ab	8.3 cde	2.0	1.7
	‘Redcoat’	8.3 bc	41.7 ab	16.7 bcde	0.5	1.5
	'South Dakota'	75.0 ab	75.0 ab	54.2 ab	1.5	1.2
	'Superior'	8.3 bc*	75.0 ab*	8.3 cde	0.3 *	3.0 *
	'Toka'	66.7 ab	66.7 ab	41.7 abcde	1.3	1.0
	'Underwood'	25.0 abc	50.0 ab	4.2 de	1.0	1.4

Table 3: Continued

		Avg. % Germ.			Avg. # Weeks for Germ.	
		Greenhouse		Field		
Fruit Type	Cultivar	Non-Scarified	Scarified	Germination	Non-Scarified	Scarified
Plum	'Winona'	0.0 c*	75.0 ab*	8.3 cde	0.4 *	1.6 *
	Pooled	39.0	58.0	20.6	1.0	1.3

Table 4: In the greenhouse environment of Experiment II, average % germination and average week for germination of scarified and non-scarified *Prunus* seeds. Percent germination and average number of weeks for germination were pooled for seed storage. Mean separations (a-e) among cultivars were within a fruit type and seed scarification treatment (a-e). An asterisk (*) refers to a significant difference ($p<0.05$) within a cultivar between seed scarification treatments.

		Greenhouse Environment			
		Avg. % Germ.		Avg. # of Weeks for Germ.	
Fruit Type	Cultivar	Non-Scarified	Scarified	Non-Scarified	Scarified
Apricot	'Debbie's Gold'	66.7	83.3	1.0 b*	0.5 *
	MN203	8.3	33.3	2.0 a	0.7
	'Moongold'	50.0	66.7	1.0 b	1.0
	'Sungold'	66.7	83.3	0.8 b	0.6
	'Westcot'	66.7	62.5	1.0 b	0.6
	Pooled	54.2	67.4	1.0	0.7

Table 4: Continued

		Avg. % Germ.			Avg. # of Weeks for Germ.			
Fruit Type	Cultivar	Non-Scarified		Scarified	Non-Scarified		Scarified	
Tart Cherry	‘Bali’	45.8		58.3	1.0	abc	1.6	
	‘Meteor’	25.0*		50.0*	2.9	abc	1.7	
	N81755	41.7		62.5	1.2	c	1.2	
	‘Northstar’	25.0		54.2	3.6	a*	1.4	*
	‘Suda’	37.5		37.5	1.7	bc	1.7	
	Pooled	35.0		52.5	2.1		1.5	
Plum	‘Hazel’	54.2	abcd	50	1.6		2.0	
	‘Compass’	25	abcde	45.8	2.5		1.9	
	‘Mount Royal’	16.7	cde	29.2	1.8		1.2	
	‘Todd’	75	a	75	1.1		1.6	
	‘Whittaker’	62.5	abc	70.8	1.4		1.8	

Table 4: Continued

Fruit Type	Cultivar	Avg. % Germ.				Avg. # of Weeks for Germ.	
		Non-scarified		Scarified		Non-Scarified	Scarified
	'Alderman'	4.2	e*	37.5	*	_z	1.3
	'Gracious'	45.8	abcde*	75	*	2.4	1.9
	'Hennepin'	58.3	abcd	58.3		2.1	1.6
	'Monitor'	54.2	abcd	66.7		2.1	2.0
	'Pipestone'	12.5	de	29.2		2.0	1.5
	'South Dakota'	66.7	ab	79.2		1.6	1.1
	'Winona'	20.9	bcde*	66.7	*	2.3	1.4
	Pooled	41.3		56.9		1.9	1.6

Table 5: Average % germination in the field environment of Experiment II for *Prunus* cultivars. Data was pooled for seed scarification treatment. Mean separations among cultivars were within fruit type and seed storage (a-d). An asterisk (*) refers to a significant difference ($p<0.05$) within a cultivar across months in storage.

		Field Environment		
		Months in Storage		
Fruit Type	Cultivar	26 months		13 months	
Apricot	'Debbie's Gold'	-		37.5	ab
	MN203	-		4.2	b
	'Moongold'	70.8	a	-	
	'Sungold'	54.2	ab	64.6	a
	'Westcot'	25.0	b	45.8	a
	Pooled	50.0		38.0	

		Months in Storage			
		26 months	13 months	2 months	
Tart Cherry	'Bali'	12.5*	-	58.3	a*
	'Meteor'	8.3	-	41.7	ab
	N81755	29.2	-	37.5	ab
	'Northstar'	-	4.2	8.3	b
	'Suda'	29.2	-	29.2	ab
	Pooled	19.8	4.2	35.0	

Table 5: Continued

		Months in Storage			
		25 months		1 month	
Plum	'Hazel'	50.0	bc	70.8	a
	'Compass'	20.8	cd	29.2	abc
	'Mount Royal'	4.2	d	16.7	ab
	'Todd'	50.0	bc	58.3	ab
	'Whittaker'	62.4	abc	75.0	a
	'Alderman'	16.7	cd	16.7	bc
	'Gracious'	25.0	bcd	54.2	ab
	'Hennepin'	95.8	a*	75.0	a*
	'Monitor'	4.2	d	0.0	c
	'Pipestone'	4.2	d	12.5	ab
	'South Dakota'	70.8	ab	70.8	ab
	'Winona'	20.8	cd	16.7	ab
	Pooled	35.8		41.3	

Table 6: Parameters measured for *Prunus* seedlings started in the greenhouse included initial height (cm), initial stem diameter (mm), change in stem diameter (mm), and change in trunk cross-sectional area (ΔTCA). Mean separations (5% Tukey's HSD) were among cultivars within fruit type within each parameter.

Female Parent			Greenhouse Replication			
Fruit Type	Species	Cultivar	Initial Height (cm)	Initial Diameter (mm)	Change in Diameter (mm)	ΔTCA (mm^2)
Apricot	*P. armeniaca*	'Debbie's Gold'	64.1	5.00	0.78	7.72
		'Moongold'	58.7	5.00	1.90	10.00
		'Sungold'	69.1	5.29	1.34	10.86
		'Westcot;	79.5	5.69	0.52	4.77
		Pooled	67.9	5.25	1.14	8.34
Tart cherry'	*P. cerasus*	'Bali'	31.7	5.81	2.30	27.52
		'Meteor'	26.7	5.08	2.66	32.03
		N87155	36.8	6.47	2.59	40.74
		'Northstar'	27.4	5.39	0.84	9.40

Table 6: Continued

Female Parent			Greenhouse Replication			
Fruit Type	Species	Cultivar	Initial Height (cm)	Initial Diameter (mm)	Change in Diameter (mm)	ΔTCA (mm^2)
Tart Cherry	*P. cerasus*	'Suda'	33.0	6.89	1.35	17.07
		Pooled	31.1	5.93	1.95	25.35
Plum	*P. americana*	'Hazel'	57.7 ab	4.94 abc	0.71	4.16
	P. bessyi x P. hortulana	'Compass'	77.2 a	5.93 abc	0.62	7.39
	P. domestica	'Mount Royal'	60.7 ab	6.88 a	1.33	9.69
		'Todd'	42.0 ab	6.43 ab	0.58	6.03
	P. munsoniana	'Whittaker'	49.7 ab	4.35 bc	1.14	7.49
	Prunus spp.	'Alderman'	67.7 ab	6.60 ab	1.31	15.02
		'Gracious'	58.9 ab	4.90 abc	0.84	13.35
		'Hennepin'	61.1 ab	4.64 abc	0.92	6.43
		'Monitor'	51.0 ab	4.29 bc	0.19	4.53

Table 6: Continued

Female Parent			Greenhouse Replication			
Fruit Type	Species	Cultivar	Initial Height (cm)	Initial Diameter (mm)	Change in Diameter (mm)	ΔTCA (mm^2)
Plum	*Prunus spp.*	‘Pipestone’	74.6 ab	5.98 abc	0.02	0.09
		‘South Dakota’	54.1 ab	4.21 c	-0.25	4.87
		‘Winona’	77.9 a	6.09 abc	0.34	3.76
		Pooled	61.1	5.44	0.65	6.90

Table 7: Parameters measured for *Prunus* seedlings started in the field included average initial height (cm), initial stem diameter (mm), change in stem diameter (mm), and ΔTCA (mm^2). Mean separations (5% Tukey's HSD) were among cultivars within fruit type and within a parameter.

Female Parent			Field Replication			
Type	Species	Cultivar	Initial Height (cm)	Initial Diameter (mm)	Change in Diameter (mm)	Change in TCA (mm^2)
Apricot	*P. armeniaca*	'Debbie's Gold'	24.8	2.40	1.52	7.95
		'Moongold'	24.0	2.28	1.56	7.96
		'Sungold'	24.3	2.44	1.12	5.34
		'Westcot;	24.5	2.20	2.02	11.39
		Pooled	24.4	2.33	1.56	8.16
Tart Cherry	*P. cerasus*	'Bali'	8.6	1.54	4.16	24.56
		'Meteor'	8.2	1.67	3.45	20.24
		N87155	7.8	1.65	3.97	24.43
		'Northstar'	6.1	1.26	2.63	10.80

Table 7: Continued

Female Parent			Field Replication			
Fruit Type	Species	Cultivar	Initial Height (cm)	Initial Diameter (mm)	Change in Diameter (mm)	ΔTCA (mm^2)
Tart Cherry	*P. cerasus*	Suda	6.4	1.74	2.32	12.96
		Pooled	7.4	1.57	3.31	18.60
Plum	*P. americana*	‘Hazel’	22.5 ab	2.11 ab	3.67 ab	24.58 ab
	P. bessyi x P. hortulana	‘Compass’	15.8 bcd	1.68 b	1.73 b	7.42 b
	P. domestica	‘Mount Royal’	8.5 d	1.90 ab	0.83 b	3.18 b
		‘Todd’	13.3 d	2.39 a	1.47 b	8.02 b
	P. munsoniana	‘Whittaker’	25.7 a	2.15 ab	4.64 a	36.89 a
	Prunus spp.	‘Alderman’	19.1 abcd	2.00 ab	2.74 ab	16.96 ab
		‘Gracious’	17.5 bcd	1.78 b	1.69 b	10.43 b
		‘Hennepin’	25.5 a	2.11 ab	3.98 ab	27.08 ab

Table 7: Continued

Female Parent			Field Replication			
Fruit Type	Species	Cultivar	Initial Height (cm)	Initial Diameter (mm)	Change in Diameter (mm)	ΔTCA (mm^2)
Plum	*Prunus spp.*	‘Monitor’	12.2 d	1.16 b	2.41 ab	8.88 b
		‘Pipestone’	12.5 d	1.40 b	2.73 ab	12.23 ab
		‘South Dakota’	21.8 abc	1.85 b	4.24 ab	27.38 ab
		‘Winona’	13.9 cd	1.52 b	1.83 ab	9.97 b
		Pooled	17.4	1.84	2.66	16.08

Table 8: Average herbivore damage rating of *Prunus* seedlings in Experiment III, pooled over germination environments. Mean separations (5% Tukey's HSD) were among cultivars within a fruit type within a parameter.

Fruit type	Species	Cultivar	Herbivore rating	
Apricot	*P. armeniaca*	'Debbie's Gold'	4.7	
		'Moongold'	4.8	
		'Sungold'	4.8	
		'Westcot'	4.8	
		Pooled	4.8	
Tart Cherry	*P. cerasus*	'Bali'	2.0	
		'Meteor'	2.6	
		N81755	2.3	
		'Northstar'	2.8	
		'Suda'	3.3	
		Pooled	2.6	
Plum	*P. americana*	'Hazel'	2.7	c
	P. besseyi x Prunus spp.	'Compass'	3.4	bc
	P. domestica	'Mount Royal'	4.5	ab
	Prunus spp.	'Todd'	4.8	ab
		'Whittaker'	3.5	bc
		'Alderman'	4.5	ab
		'Gracious'	4.1	abc

Table 8: Continued

Fruit type	Species	Cultivar	Herbivore rating	
Plum	*Prunus spp.*	'Hennepin'	3.1	bc
		'Monitor'	4.2	abc
		'Pipestone'	4.0	abc
		'South Dakota'	2.9	bc
		'Winona'	3.8	abc
		Pooled	3.8	

Table 9: The fraction of the replications (Fraction Reps.) of *Prunus* seedlings started in the greenhouse or the field that produced suckers and the total number of suckers (# suckers) produced by seedlings across replications. Only *Prunus* cultivars that had replications with suckers are displayed.

Female Parent		Greenhouse		Field	
Fruit Type	Cultivar	Fraction Reps.	# Suckers	Fraction of Reps.	# Suckers
Apricot	'Moongold'	0	0	1/8	2
	'Westcot'	0	0	1/10	1
Tart Cherry	'Bali'	1/12	2	0	0
	'Northstar'	2/11	7	0	0
	'Suda'	4/10	7	0	0
Plum	'Hazel'	0	0	2/14	2
	'Compass'	0	0	1/9	1
	'Todd'	1/15	1	0	0
	'Gracious'	4/14	6	1/12	1

Table 9: Continued

Female Parent		Greenhouse		Field	
Fruit Type	Cultivar	Fraction Reps.	# Suckers	Fraction Reps.	# Suckers
Plum	'Monitor'	2/13	4	1/2	1
	'South Dakota'	5/16	8	2/15	2
	'Whittaker'	2/6	4	1/7	1

Table 10: Percent survival of *Prunus* seedlings at the end of Experiment III, based on fruit type and female parent cultivar, for seedlings started in the greenhouse (Grhs.) and the field.

Fruit Type	Cultivar	% Survival	
		Grhs.	Field
Apricot	'Debbie's Gold'	95	91
	'Moongold'	80	100
	'Sungold'	86	94
	'Westcot'	83	81
Tart Cherry	'Bali'	87	92
	'Meteor'	92	91
	N81755	77	89
	'Northstar'	87	100
	'Suda'	67	92
Plum	'Hazel'	69	100
	'Compass'	80	100
	'Mount Royal'	89	100
	'Todd'	85	97
	'Whittaker'	88	92
	'Alderman'	70	100
	‘Gracious’	73	80
	'Hennepin'	86	90

Table 10: Continued

Fruit Type	Cultivar	% Survival	
		Grhs.	Field
Plum	'Hennepin'	86	90
	'Monitor'	67	73
	'Pipestone'	33	100
	'South Dakota'	78	96
	'Winona'	67	43

Table 11: Pearson correlations between % germination in the greenhouse environment and % germination in the field environments of Experiments I or II. An asterisk (*) refers to a significant correlation ($p<0.05$) between the two environments (i.e. greenhouse and field) within a species and an experiment.

Fruit Type	Species	Experiment I	Experiment II
Apricot	*P. armeniaca*	0.04 ns	0.13 ns
Tart Cherry	*P. cerasus*	-0.03 ns	0.28 *
Plum	*P. americana*	-0.38 ns	-0.31 ns
	P. besseyi x Prunus spp.	-0.28 ns	-0.25 ns
	P. domestica	0.10 ns	0.62 *
	P. munsoniana	-0.34 ns	0.00 ns
	P. nigra	-0.67 ns	_z
	Prunus spp.	0.19 *	0.30 *

[z] *P. nigra* was not examined in Experiment II due to lack of seed set

Table 12: Correlations between initial height (cm), initial stem diameter (mm), change in diameter (mm), change in trunk cross-sectional area (TCA; mm^2), herbivory rating, and percent survival of *Prunus* seedlings to end of the Experiment III. Asterisks indicate significant correlations[z].

	Initial Height	Initial Diameter	Change Diameter	Change TCA	Herbivory Rating	Percent Survival
Initial Height	1.00					
Initial Diameter	0.75***	1.00				
Change Diameter	-0.36***	-0.36***	1.00			
Change TCA	-0.15**	-0.05	0.88***	1.00		
Herbivory Rating	0.08 ns	0.01 ns	-0.41***	-0.41***	1.00	
Percent Survival	-0.25 ns	-0.25 ns	0.15 ns	0.10 ns	-0.01 ns	1.00

[z] ** indicates $p<0.01$ and *** indicates $p<0.001$

Chapter 2: The effects of cultivar and storage on pollen viability in winter-hardy *Prunus* species

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Summary

Pollen viability plays an important role in seed set and, thus, sexual reproduction in plants. The measurement of pollen viability has applications in plant breeding. For many species, binucleate pollen collected at dehiscence can be stored frozen for several months or years without a significant reduction in viability. The objective of this study was to examine pollen viability and the stability in storage of 21 hardy *Prunus* cultivars. Aniline blue was used to assess pollen viability. Pollen samples were collected at dehiscence and stored at -20°C, then were sampled once a month for nine months. Initial % stainability for all cultivars was > 50%, which is indicative of male-fertile individuals. Initially, apricot cultivars had an average of 97% stainability with no significant variation among cultivars. There was significant variation for initial samples % stainability among cultivars within the tart cherry and plum fruit types. Initial % stainability for tart cherries ranged from 69% to 90% whereas the initial range for plums was 81% to 98%. High initial pollen stainability is particularly critical for tart cherries, which are self-compatible. Across all fruit types, many cultivars did not have significant, linear decreases in % stainability after storage, indicating that pollen may still be used for crosses after nine months in storage. Average % stainability for plums 'La Crescent', 'Monitor', and 'Toka' decreased significantly after seven months in storage whereas 'Superior' had a significant decrease in as few as three months in storage. Most *Prunus*

had >50% stainability after storage for 9 months and could be used for pollination in the following year after pollen collection.

Introduction

Prunus is an economically important genus in Rosaceae that includes fruit crops like apricots, almonds, plums, sweet and tart cherries, and peaches (Potter, 2012). Early spring frosts, lack of winter hardiness, low temperatures, and short life spans limit the *Prunus* species that can be successfully cultivated in northern latitudes (Andersen and Weir, 1967; Ramming and Cociu, 1991; Taylor, 1965). As a result, early breeding programs focused on improving winter hardiness through the hybridization of native species, which often had poor fruit quality, with non-hardy but high quality fruiting species like *P. domestica* L. or *P. salicina* Lindl. (Andersen and Weir, 1967; Ramming and Cociu, 1991).

One of the many challenges that confronted early breeding programs working with winter-hardy *Prunus* selections was low pollen viability (Andersen and Weir, 1967). The term pollen viability has been utilized to describe fertilization ability, germinability, stainability, pollen maturity, and pollen longevity (Dafni and Firmage, 1999). Ultimately, the purpose of measuring pollen viability is to determine the potential of a cultivar's pollen grains to germinate on the stigma and to fertilize the ovule (Firmage and Dafni, 2000). As a result, pollen viability describes male fertility.

Seed set and thus, the formation of fruit is dependent upon successful pollination and fertilization in fruit crops like *Prunus* species (Westwood, 1993). Pollen viability, in addition to other factors such as compatibility and environmental conditions, significantly impacts fertilization and seed set. Low pollen viability also can result in poor fruit set

(Davarynejad et al. 2008; Gallotta et al. 2014; Westwood, 1993). The success of different crosses made by breeders is dependent initially upon the viability of pollen. Thus, the assessment of pollen viability in cultivars of interest has applications in horticulture (Firmage and Dafni, 2000) and plant breeding.

According to Dafni and Firmage (1999), the most accurate method of estimating pollen viability is seed set. However, assessment of seed set is time-consuming. Other procedures such as *in vivo* and/or *in vitro* pollen germination can provide an accurate evaluation of viability but are laborious to develop the proper media and may not be indicative of male fertility (Dafni and Firmage, 1999; Hanna and Towill, 1995). Pollen stainability is a fast and easy measurement of pollen viability (Bolat and Pirlak, 1999; Hanna and Towill, 1995). Staining procedures provide an estimate of pollen viability (Liedl and Anderson, 1993) and thus are an appropriate method to inform breeding decisions. Bolat and Pirlak (1999) found that in their studies of *Prunus* pollen viability that specific stains were correlated with pollen germination.

Within the context of plant breeding, the ability to store pollen without a reduction in viability is important. Using stored pollen allows for crosses to be made among diverse individuals with differing flowering periods and across years (Hanna and Towill, 1995). Pollen storage not only increases breeding efficiency but also allows for the preservation and exchange of germplasm (Hanna and Towill, 1995). Temperature and moisture are two of the most important factors that affect the longevity of pollen in storage with low temperature and reduced moisture being conducive to pollen preservation (Akihama and Omura, 1986; Hanna and Towill, 1995). The number of nuclei in pollen grains at dehiscence also affects the longevity of pollen in storage.

Binucleate pollen can successfully be stored for extended periods of time under cold, dry conditions whereas the viability of trinucleate pollen decreases rapidly within days or even hours (Liedl and Anderson, 1993). Species in the Rosaceae are characterized by binucleate pollen (Brewbaker and Emery, 1962), which indicates that pollen of *Prunus* cultivars could be potentially stored without a significant reduction in viability. Akihama and Omura (1986) reported that *P. persica* Maxim. pollen has been successfully stored for up to nine years at -20 C. Although, extended storage of pollen would be useful, little is known about the longevity of pollen for many species (e.g. *Prunus*) and cultivars (Hanna and Towill, 1995).

This study examined pollen viability and the longevity of pollen in storage in a selection of winter-hardy *Prunus* cultivars. Staining of each cultivar's pollen at anthesis, anther dehiscence, was used to determine male fertility. Longevity of pollen in cold dry storage at - 20°C over a nine-month period was examined in order to determine if pollen could be potentially used in future crosses without a significant decrease in viability.

Materials and Methods

Twenty-one winter-hardy *Prunus* cultivars, classified into three fruit types, were included in the experiment (Table 1). Fruit type is defined as apricot, tart cherry, or plum. Although there are two types of tart cherry varieties, amarelle or clear juice cultivars such as 'Meteor', and morello or dark red juiced cultivars such as all the remaining tart cherry cultivars in this experiment, all tart cherries were classified under one category for the purposes of this experiment (Brown et al. 1989). The three fruit types in this experiment represent multiple *Prunus* species (Table 1). Cultivar refers to all breeding lines,

accessions, and released cultivars used in this study. The cultivars included in this study were introduced between the years 1896 and 1982 (Table 1).

From March through May 2015, selected trees at the University of Minnesota research plots in Excelsior, MN (44°52’06.1”N lat., -93°37’59.9”W long) were monitored for bud break. When flowers were in the balloon stage (Burgos et al. 1993; Hartmann and Neumüller, 2008), flowers were collected, placed in a collection bag and transported in a cooler back to a laboratory (University of Minnesota, St. Paul, MN). Flowers for the apricots were collected during week 16; tart cherry flowers were collected during week 18; and plum flowers were collected between weeks 17 and 18. Week number refers to the number of weeks since January 1st, 2015.

Prior to anther removal, the laboratory bench was sanitized with a 70% ethanol solution. Anthers were removed by scraping the flowers across a colander and the anthers were then collected in a petri dish. The petri dish containing the anthers was placed in a desiccator at room temperature (25°C) overnight to allow pollen dehiscence. After dehiscence, for initial pollen stainability, a sample of the cultivar’s pollen was placed on a glass microscope slide and stained with 1% aniline blue in 80% propionic acid for 5+ minutes (Anderson and Ascher, 1993) and then examined under a light microscope (Tungsten lamp). The total number of stained and unstained pollen grains was counted in four, random microscope fields on each microscope slide with n>20 grains/field (Anderson and Ascher, 1993). A viable pollen grain stains a dark blue color due to an intact cytoplasm taking up the stain (Anderson and Ascher, 1993). An aborted or non-viable pollen grain will not take up the stain and thus, will be translucent or only light blue in color (Anderson and Ascher, 1993). Each of the microscope fields served as a

replication with 4 replications per cultivar per date. From these counts, percent stainable pollen was calculated.

After a sample of pollen was stained for initial pollen stainability estimates, the remaining pollen of each cultivar was placed in a gel cap in a sealed bag containing a desiccant, and stored in a freezer (-20°C). Every month for nine months, the staining process was repeated for each cultivar.

Statistical analysis

R version 3.2.3 (2015-12-10) was used for statistical analyses. Percent stainability data was transformed using the arcsine square root transformation. All replicated, quantitative data was analyzed across fruit types, cultivars, and months in storage using univariate, linear model repeated measures analysis of variance (ANOVA). Within a certain of month of storage, replicated quantitative data was analyzed using univariate, linear model analysis of variance (ANOVA). Within a cultivar, replicated data was analyzed using linear model ANOVA. Means were compared using Tukey's Honestly Significant Difference test (HSD) at $\alpha = 0.05$.

Results

The main effect, cultivar, had a significant effect on initial % stainability ($p<0.001$) across all fruit types. There was no significant variation among apricot cultivars for average initial % stainability, having a pooled average of 97% (Table 2). Variation for initial % stainability among tart cherry cultivars was observed with averages ranging from 69% for 'Northstar' to 90% for 'Surecrop' (Table 2). The tart cherry 'Northstar' differed significantly from 'Surecrop' (Table 2). Initial % stainability for plums ranged from 81% for 'Toka' to 98% for 'Mount Royal' with significant

variation among cultivars (Table 2). ‘Toka’ differed significantly from ‘South Dakota’ followed by ‘Mount Royal’ with Superior’ and ‘La Crescent’ was also significantly different than ‘Mount Royal’ (Table 2).

As pollen was evaluated over the course of nine months, both the main effects of cultivar ($p<0.001$) and months in storage ($p<0.001$), as well as the interaction between cultivar x months in storage ($p<0.001$) had significant effects on % stainability. Across all months in storage there was no significant variation among apricot cultivars, with all maintaining averages of ≥ 92% pollen stainability (Table 2). Within an apricot cultivar, average % stainability did not significantly vary throughout the experiment (Table 2).

Except for at five and eight months in storage, significant variation among tart cherry cultivars was observed with all tart cherries having averages of ≥ 55% throughout the experiment (Table 2). When there was variation among tart cherry cultivars, ‘Surecrop’ often had the highest average % stainability and differed significantly from one or more of the other tart cherry cultivars with the exception of ‘Suda’ (Table 2). Within ‘Bali’, ‘Mesabi’, and ‘N87155’ there was no significant variation for average % stainability among different months in storage (Table 2). Significant variation was observed, however, within ‘Meteor’, ‘Northstar’, ‘Suda’, and ‘Surecrop’ (Table 2). However, in ‘Meteor’, ‘Northstar’, ‘Suda’, and ‘Surecrop’ there was not a consistent or linear decrease in % stainability as the experiment progressed (Table 2).

Throughout the experiment there was significant variation in average % stainability among plum cultivars (Table 2). All plums maintained averages of ≥48% with six cultivars having averages ≥70% throughout the duration of the experiment (Table 2). Average % stainability across months in storage within ‘Compass’,

‘Alderman’, ‘South Dakota’, and ‘Underwood’ did not vary significantly (Table 2). There was some significant but inconsistent variation within ‘Mount Royal’ and ‘Gracious’ (Table 2). Average % stainability for ‘La Crescent’, ‘Monitor’, and ‘Toka’ decreased significantly after seven or more months in storage whereas ‘Superior’ had significant decreases after just three months in storage (Table 2).

Discussion

According Anderson and Ascher (1993), >50% pollen stainability is indicative of a potentially male-fertile individual, providing a benchmark for determining pollen viability for cultivars. However, Anderson and Ascher (1993) point out that one intact male gamete has the potential to fertilize an ovule, which indicates that pollen with % stainability of >0% could possibly result in seed set. In *Prunus*, where there is one ovule/ovary and only one seed is produced/fruit, a single pollen grain would be sufficient for seed and fruit set. Pollen viability among and within *Prunus* species is often variable; Burgos et al. (2004) reported that the majority of apricot (*P. armeniaca* L.) cultivars are not male-sterile and have high percentages of viable pollen. Gallota et al. (2014) examined pollen stainability at anthesis in five apricot cultivars and found consistently high percentages (>95%). Asma (2008) found that depending upon the cultivar, average % stainability after a short time in storage (4°C) for apricots ranged from 61.1% to 91.9%. Bolat and Pirlak (1999) reported that depending on the stain used, average % stainability at anthesis for *P. cerasus* L. ‘Kutahya’ ranged from 66.8% to 85.2%. According to Ćalić et al. (2013) average % stainability for *P. domestica* cultivars range from 66% to 99% whereas Andersen and Weir (1967) found many hybrid plums (e.g. Japanese-American) had lower pollen viability when compared to *P. domestica* cultivars.

In this study, all *Prunus* cultivars examined were potentially male-fertile at anthesis as all cultivars had initial % stainability averages of >50% (Table 2). Similar to what was observed by Gallotta et al. (2014), Asma et al. (2008), and Burgos et al. (2004), the apricots in this study had high initial % stainability with a pooled average of 97%. Tart cherries had lower and more variable initial % stainability. However, initial % stainability for all tart cherry cultivars was also greater than 50%, meaning that their self-compatible nature would most likely result in seed set. Similar to what Ćalić et al. (2013) reported for *P. domestica*, we found that *P. domestica* 'Mount Royal' had high % stainability. Initial % stainability for the hybrid plums (*Prunus spp.*) was variable but still ≥ 80%; the variability could be attributed to varying species in the plum germplasm. Pollen stainability of >50% for all cultivars in this study indicates that these cultivars could potentially be used successfully in breeding crosses. However, although % stainability was >50%, this does not necessarily indicate the potential to germinate (Bolat and Pirlak, 1999; Hanna and Towill, 1995). Also, other factors like self and cross-compatibility and environmental conditions also impact pollination, fertilization, and fruit set (Westwood, 1993).

Even though, 'Compass', 'Mount Royal', and 'Suda' have been around for more than a century, % stainability was >50% throughout this experiment (Tables 1 and 2). Without purging action of sexual reproduction via meiosis, multiple cycles of asexual propagation allow the accumulation of deleterious alleles and, thus the operation of Muller's ratchet (Anderson et al. 2010; Bull and Charnov, 1985). Muller's ratchet proposes that populations undergoing periods of asexual reproduction accumulate deleterious alleles due to random genetic drift (Stephan et al. 1993). According to

Anderson and Ascher (1994), a potential consequence of the operation of Muller's ratchet is reduced male and/or female fertility. Since *Prunus* species are asexually propagated, based on Muller's ratchet, we would have expected that older cultivars would have reduced fertility due to more cycles of asexual propagation. For example, Anderson et al. (2010) concluded that many cycles of asexual propagation resulted in reduced fertility in Easter lily (*Lilium longiflorum* Thunb.). However, in the present study low % stainability was not observed in many of the old cultivars. One reason for this could be that these woody perennial cultivars may have not been as extensively propagated, less propagule units, as lily, which is grown as an annual for Easter flowering. Also, in self-compatible crops such as tart cherries, pollen viability would be selected for regardless of the number of propagation cycles; such selection would not have occurred in lily. Further investigations are necessary to determine the extent of clonal decline in *Prunus* cultivars.

The majority of tart cherry cultivars are self-compatible (Lansari and Iezzoni, 1990), indicating the capability of producing seed after self-pollination occurs (Liedl and Anderson, 1993). In this study, all tart cherries had initial mean % stainability of >50% (Table 2). Self-compatibility, coupled with % stainability of >50%, has interesting horticultural. From a horticultural production viewpoint, self-compatibility and viable pollen reduces the need for separate pollen sources and, thus, is conducive to monocultures. Self-compatibility necessitates self-fertilization, which results in consistent reproduction of inbreds and an increase in the number of propagule units (Liedl and Anderson, 1993; Van Kleunen and Johnson, 2007). As a result, a single, isolated tart cherry tree could potentially produce a self-sustaining population, which could, in turn,

result in the further spread of the species based on the germination and establishment of inbred cherry seedlings (Kostick, 2016).

Successful storage of pollen allows for outcrossing of diverse individuals as well as the exchange and preservation of germplasm (Hanna and Towill, 1995). However, if a cultivar's % pollen stainability drops below 50% while in storage, it may no longer be useful for potential crosses (Anderson and Ascher, 1993). Since *Prunus* pollen is binucleate (Brewbaker and Emery, 1962), we expected that the pollen of these winter-hardy cultivars could successfully be stored at low temperatures. In this study, most cultivar % stainability did not decrease significantly throughout the experiment. Each of the apricot cultivars' % stainability remained ≥92% and there were no significant decreases in % stainability during the experiment (Table 2). For most tart cherries, % stainability did not significantly decrease during the experiment despite minor variation (Table 2). The apparent decrease and subsequent increase in % stainability of some tart cherries was most likely due to random, sampling error. After seven months in storage, % stainability decreased significantly for the plums 'La Crescent', 'Monitor', and 'Toka', which indicates that relatively short-term (≤6 months) storage of pollen may not result in a significant reduction of viability. In contrast, 'Superior' had a significant decrease in % stainability after only three months in storage (Table 2). Even though some cultivars in this study had significant decreases in % stainability, most still had final percentages at ~50% or higher (Table 2). Since potentially only one viable pollen grain is required for seed set, cultivars with reduced % stainability could still fertilize an ovule and produce a seed or fruit (Anderson and Ascher, 1993).

Conclusions

High male fertility as measured with pollen viability is one important factor that impacts the success of a potential cross. In this study, all of the cultivars examined are potentially male-fertile as all had initial % stainability averages >50%. This indicates that pollen of these cultivars could possibly be used in crosses.

Longevity of pollen in storage varied by fruit type and among cultivars within a fruit type. Except for the plums 'Monitor' and 'Superior', all cultivars had >50% stainability at the end of the experiment, which indicates that the majority of cultivars' pollen could potentially remain viable in storage for at least nine months. Since all cultivars had >0% stainability, the pollen of these cultivars could potentially fertilize an ovule.

Although all cultivars may be male-fertile, further experimentation could be done to characterize pollen viability of these cultivars. Future work could focus on pollen germination and/or seed set in specific crosses between winter-hardy cultivars. One potential question to examine in future research is whether or not the pollen of winter-hardy *Prunus* cultivars germinates and effects seed set (fruits) after an extended period in storage. It would also be of value to study the potential intra- and interspecific compatibility among winter-hardy *Prunus* cultivars.

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Table 1: Fruit type (i.e. apricot, tart cherry and plum), species, cultivar name, year of introduction, and pollen collection week (2015) of flowers and pollen for *Prunus* cultivars evaluated in this experiment.

Fruit Type	Species	Cultivar	Introduction Year	Collection Week	Citation
Apricot	*P. armeniaca* L.	'Moongold'	1961	Week 16	Brooks and Olmo, 1997
		'Pioneer'	Unknown	Week 16	NA
		'Sungold'	1961	Week 16	Brooks and Olmo, 1997
		'Westcot'	1982	Week 16	Brooks and Olmo, 1997
Tart Cherry	*P. cerasus* L.	'Bali'	Unknown	Week 18	NA
		'Mesabi'	1964	Week 18	Brooks and Olmo, 1997
		'Meteor'	1952	Week 18	Brooks and Olmo, 1997
		'N81755'	Unknown	Week 18	NA
		'Northstar'	1950	Week 18	Brooks and Olmo, 1997

Table 1: Continued

Fruit Type	Species	Cultivar	Introduction Year	Collection Week	Citation
		'Suda'	~1900	Week 18	Hedrick et al. 1915
		'Surecrop'	Unknown	Week 18	NA
	P. besseyi x *P. hortulana*				
Plum	L.	'Compass'	1896	Week 18	Waugh, 1899
	P. domestica L.	'Mount Royal'	~1900	Week 18	Andersen and Weir, 1967
	Prunus spp. L.	'Alderman'	1985	Week 18	Brooks and Olmo, 1997
		'Gracious'	1957	Week 18	Brooks and Olmo, 1997
		'La Crescent'	1923	Week 18	Brooks and Olmo, 1997
		'Monitor'	1920	Week 18	Brooks and Olmo, 1997
		'South Dakota'	1949	Week 18	Brooks and Olmo, 1997
		'Superior'	1933	Week 17	Brooks and Olmo, 1997

Table 1: Continued

Fruit Type	Species	Cultivar	Introduction Year	Collection Week	Citation
Plum	*Prunus. spp.* L.	'Toka'	1911	Week 17	Hansen, 1940
		'Underwood'	1921	Week 18	Brooks and Olmo, 1997

Table 2: Average percent stainability for pollen of winter-hardy *Prunus* cultivars over nine months in storage. Mean separations across all fruit types were analyzed within the same month of storage (columns) based on Tukey's Honestly Significant Difference test (HSD, $\alpha \leq 0.05$) and are designated by upper-case (A-J) letters. Whereas, mean separation within a cultivar (row) across months in storage are indicated by lower-case letters (a-f).

	Months in storage																	
Cultivar	Initial (0 Mo.)		1 Mo.		2 Mo.		3 Mo.		4 Mo.		5 Mo.		6 Mo.		7 Mo.		8 Mo.		9 Mo.	
Apricots																				
'Moongold'	98%	**A**	96%	**AB**	96%	**A**	95%	**A-D**	96%	**A-D**	93%	**AB**	93%	**A-C**	87%	**A-D**	91%	**A-D**	92%	**A-C**
	a		a		a		a		a		a		a		a		a		a	
'Pioneer'	96%	**AB**	100%	**A**	98%	**A**	100%	**A**	95%	**A-C**	96%	**A**	96%	**AB**	98%	**A**	97%	**A**	96%	**A**
	a		a		a		a		a		a		a		a		a		a	
'Sungold'	97%	**A**	98%	**AB**	98%	**A**	96%	**A-D**	98%	**A**	97%	**A**	95%	**A-C**	97%	**AB**	96%	**AB**	95%	**AB**
	a		a		a		a		a		a		a		a		a		a	
'Westcot'	96%	**A-C**	98%	**AB**	97%	**A**	97%	**A-C**	96%	**A-C**	95%	**A**	96%	**A**	96%	**AB**	93%	**A-C**	95%	**AB**
	a		a		a		a		a		a		a		a		a		a	

Table 2: Continued.

	Months in Storage																	
Cultivar	Initial (0 Mo.)		1 Mo.		2 Mo.		3 Mo.		4 Mo.		5 Mo.		6 Mo.		7 Mo.		8 Mo.		9 Mo.	
Tart Cherry																				
'Bali'	77% a	**D-F**	73% a	**C**	75% a	**BC**	71% a	**H**	70% a	**FG**	66% a	**CD**	62% a	**F-H**	60% a	**E-G**	62% a	**F-H**	69% a	**E-J**
'Mesabi'	73% a	**EF**	75% a	**C**	74% a	**BC**	75% a	**F-H**	68% a	**FG**	68% a	**CD**	57% a	**GH**	61% a	**E-G**	63% a	**F-H**	57% a	**G-J**
'Meteor'	74% a-c	**EF**	76% a-c	**C**	85% a	**A-C**	82% ab	**D-H**	76% a-c	**E-G**	73% a-c	**B-D**	65% bc	**E-H**	76% a-c	**C-G**	69% a-c	**E-H**	61% c	**F-J**
'N81755'	76% a	**EF**	73% a	**C**	72% a	**BC**	70% a	**H**	72% a	**FG**	68% a	**CD**	66% a	**E-J**	65% d-g	**D-G**	68% a	**E-H**	62% a	**F-J**
'Northstar'	69% ab	**F**	75% a	**C**	70% ab	**C**	71% ab	**H**	68% ab	**FG**	55% b	**D**	58% ab	**G-H**	57% ab	**FG**	56% ab	**H**	53% ab	**IJ**
'Suda'	88% ab	**A-F**	84% ab	**BC**	91% a	**AB**	86% ab	**B-H**	79% ab	**C-G**	77% b	**B-D**	74% b	**D-H**	74% b	**C-G**	77% b	**D-G**	74% b	**D-I**

Table 2: Continued

	Months in Storage																			
Cultivar	Initial (0 Mo.)		1 Mo.		2 Mo.		3 Mo.		4 Mo.		5 Mo.		6 Mo.		7 Mo.		8 Mo.		9 Mo.	
'Surecrop'	90% ab	**A-E**	95% a	**AB**	91% ab	**A-C**	92% ab	**A-E**	92% ab	**A-E**	78% b	**B-D**	85% ab	**A-F**	76% b	**C-G**	79% ab	**D-G**	85% ab	**A-F**
Plums																				
'Alderman'	90% a	**A-F**	92% a	**A-C**	86% a	**A-C**	83% a	**D-H**	86% a	**B-F**	83% a	**A-C**	80% a	**B-G**	82% a	**B-F**	85% a	**B-E**	80% a	**B-G**
'Compass'	91% a	**A-D**	92% a	**A-C**	94% a	**A**	96% a	**AB**	94% a	**A-E**	90% a	**AB**	86% a	**A-E**	84% a	**A-F**	91% a	**A-D**	91% a	**A-D**
'Gracious'	91% ab	**A-E**	94% a	**AB**	92% ab	**A-C**	90% ab	**B-F**	87% ab	**B-F**	84% ab	**A-C**	88% ab	**A-D**	86% ab	**A-E**	94% ab	**A-D**	81% b	**B-G**
'La Crescent'	82% ab	**C-F**	77% ab	**C**	75% a-c	**BC**	80% ab	**E-H**	84% a	**B-F**	77% ab	**B-D**	79% ab	**C-G**	68% b-d	**D-G**	53% d	**H**	54% cd	**H-J**
'Monitor'	87% a	**A-F**	85% a	**BC**	86% a	**A-C**	88% a	**B-G**	81% a	**B-G**	76% ab	**B-D**	75% ab	**D-H**	70% a-c	**D-G**	58% bc	**GH**	48% c	**J**

Table 2: Continued

Cultivar	Months in Storage																	
	Initial (0 Mo.)		1 Mo.		2 Mo.		3 Mo.		4 Mo.		5 Mo.		6 Mo.		7 Mo.		8 Mo.		9 Mo.	
'Mount Royal'	98% a	**A**	94% ab	**A-C**	98% a	**A**	93% ab	**B-F**	88% b	**A-F**	90% ab	**AB**	92% ab	**A-D**	93% ab	**A-C**	93% ab	**A-D**	91% ab	**A-D**
'South Dakota'	95% a	**A-C**	97% a	**AB**	93% a	**AB**	95% a	**A-D**	97% a	**AB**	92% a	**AB**	94% a	**AB**	91% a	**A-C**	82% a	**C-F**	97% a	**A-E**
'Superior'	86% a	**B-F**	78% ab	**C**	78% a-c	**BC**	72% b-d	**H**	62% d-f	**G**	66% c-e	**CD**	56% ef	**H**	53% ef	**G**	56% d-f	**H**	49% f	**J**
'Toka'	81% a	**D-F**	75% ab	**C**	70% a-c	**C**	74% ab	**GH**	69% a-c	**FG**	72% ab	**CD**	68% a-c	**E-H**	64% bc	**E-G**	65% bc	**F-H**	53% c	**IJ**
'Underwood'	91% a	**A-E**	87% a	**C**	88% a	**A-C**	84% a	**CH**	78% a	**D-G**	77% a	**B-D**	78% a	**C-G**	85% a	**A-D**	78% a	**D-G**	77% A	**C-H**

Conclusions

This thesis characterized the invasive potential of winter-hardy *Prunus* cultivars by analyzing male fertility, seed germination and establishment. Variability in seed germination and seedling establishment as well as relatively the need for scarification and high male fertility characterizes the winter-hardy germplasm examined herein. Germination and seedling establishment were impacted by germination environment, fruit type, and cultivar (Chapter 1). Herbivory significantly affected the establishment success of *Prunus* seedlings and, thus, may play an important role in preventing some *Prunus* species from becoming established (Chapter 1).

The majority of *Prunus* cultivars studied in Chapter 1 will probably not become invasive because of low seed germination (<50%) in the field and/or poor seedling establishment due to herbivore pressure and other environmental factors. However, a few cultivars were identified that exhibited traits indicative of high invasive potential. *Prunus americana* Marsh. 'Hazel', *P. munsoniana* Wight and Hedrick 'Whittaker', *Prunus spp.* 'Hennepin', and 'South Dakota' demonstrated high germination percentages (>50%) in the field environments of Experiments I and II and high seedling establishment in Experiment III, indicating a higher invasive risk (Chapter 1). Tart cherries had low germination percentages in the field but had relatively high seedling establishment, which was characterized by vigorous seedling growth, low herbivore pressure, and the ability to vegetatively propagate via suckering (Chapter 1). Although tart cherries had relatively low germination percentages, self-compatibility due to high male fertility levels (Chapter 2), coupled with the ability to vegetatively propagate via suckering, could result in high

invasive potential because a single tree could potentially develop a self-sustaining population (Chapter 1). Since many factors contribute to the invasive potential of a species including seed germination, vigor of seedlings, tendency to vegetatively propagate, herbivore pressure, crop load, and seed dispersal mechanisms, future work should focus on other aspects of invasive risk (e.g. propagule pressure and dispersal mechanism) for the plum cultivars ‘Hazel’, ‘Whittaker’, ‘Hennepin’, and ‘South Dakota’ as well as all the tart cherry cultivars.

All cultivars were characterized as male-fertile, with all having >50% initial pollen stainability (Chapter 2). Pollen stainability of >50% indicates that if all other factors, environmental and compatibility, are conducive to fruit set, the cultivars examined could potentially be used as male parents in future crosses. High male fertility of self-compatible species like the tart cherries indicates that a single tree could potentially develop a self-sustaining population without a separate pollen source, which could result in higher invasive potential. Future work with pollen viability of winter-hardy cultivars should focus on inter and intra-specific cross compatibility to examine the potential uses of these cultivars in future breeding efforts as well as characterize invasive potential.

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