A Perennial Flax (*Linum spp.*) Breeding Program Using Ideotype Models to Select for Oilseed, Garden, and Cut Flower Cultivars

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Dedication

This thesis is dedicated to my late grandfather Dr. James R. King, a Professor of History, and a lasting inspiration for my academic work. Your lessons continue to guide my path, and your “incurable optimism” is an ideal that I aspire to every day.

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Literature Review

Introduction

Annual flaxseed (*L. usitatissimum* L.) contains several compounds beneficial to human and animal health including soluble and insoluble fiber, lignans, and most importantly, high levels of ω-3 fatty acids. These functional aspects have generated demand for flax as a health food and high-value oilseed crop. The *Linum* genus also includes important ornamental species with potential for added value as cut flowers. The Forever Green Initiative (FGI; University of Minnesota) is pursuing domestication of wild perennial flax species for high-value oilseed and ornamental uses. Most species in the genus share the unique profile of common flax (*L. usitatissimum*), making them well suited to satisfy growing demand for flaxseed. Perennial species have the added benefit of providing ecosystem services like soil retention, water quality improvement, and pollinator services. The long-term goal of this project is to provide perennial flax cultivars with suitable agronomic and horticultural performance to Minnesota producers. Oilseed, cut flower, and garden bedding plant ideotypes have been used to identify top candidate species for domestication to advance the continued development of perennial flax as a new specialty crop in Minnesota.

History of flax cultivation and usage to the present day

Common flax (*Linum usitatissimum*), also known as linseed, is one of the oldest and most widely utilized crops in the world. The multitude of uses for the aboveground portions of the plant are reflected in the specific epithet, *usitatissimum*, which is Latin for "most useful". Flax was one of the first crop species domesticated by humans. Archaeological evidence indicates that humans began using flaxes for oil and fiber ~10,000 yr. ago, with cultivation beginning ~8,000 yr. ago in the fertile crescent (Vaisey-Genser and Morris, 2003). More recent archaeological work has reported remnants of woven and dyed flax fibers dating to ~30,000 yr. ago from a cave in modern day Georgia (Kvavadze et al., 2009). Early indicators of domestication include increases in seed size, seed and oil yield, and indehiscent capsules which prevent seed shattering. Flax was historically cultivated as a dual use crop for both fiber and seed, although distinct varietal differences appeared in early civilizations like Egypt and Abyssinia. Tall unbranched types were favored for fiber production, while short, large-seeded, and highly branched forms were used as a cereal and oil source (Vaisey-Genser and Morris, 2003).

Fiber

Historically, fiber has been the most important use of flax. After retting or removal of the woody portion of the stem, the long, hollow fibers were used to make sails, rugs, clothing, and other textiles. Other important products include baskets, nets, rope, and paper (Pengilly, 2003; Vaisey-Genser and Morris, 2003). The widespread use of linen cloth throughout history is due, in part, to its strength and wicking ability, the

latter of which gives linen clothing a natural cooling effect. In ancient Egypt, products derived from the seed and fiber of the plant were central to daily life. The ancient Egyptians used planting density and timing of harvest to influence fiber quality, with denser planting resulting in reduced branching and longer, higher quality fibers. Harvesting early while the stem was still green yielded fine, light fabric reserved for royalty, while late harvests gave coarse, strong fabric, and seed used to re-plant the crop (Vaisey-Genser and Morris, 2003).

Linen was the preferred material for sailcloth throughout much of history, as its wicking ability provided added strength when wet (Vaisey-Genser and Morris, 2003). Flax cultivation and processing were central to the history of maritime trade and conquest, from the early Phoenician traders in the Mediterranean, all the way up to the British colonial expansion (Eastman, 1968; Vaisey-Genser and Morris, 2003). Decline in the use of flax fiber was precipitated by two inventions which revolutionized the textile industry. The first was the spinning frame, patented in 1769 by Richard Arkwright, which could spin cotton thread as strong as linen (Hammond and Hammond, 1919). Then, a few decades later in 1794, Eli Whitney patented the Cotton Gin, which enabled quick processing of raw cotton. These revolutionary inventions spurred the development of an industrialized textile industry in the United States which led to widespread decline in the use of linen fabric (Vaisey-Genser and Morris, 2003).

Whole flax & flaxseed oil

Whole flaxseed and flaxseed oil have been used throughout history for a wide array of uses including cosmetics, religious ceremonies, art, tanning and leather making, quarrying stone (to transport blocks by dragging), lamp fuel, and the preservation of wooden structures (Pengilly, 2003; Vaisey-Genser and Morris, 2003). Flaxseed oil is a drying oil, meaning that the fatty acids polymerize to form a thin, solid film in the presence of oxygen (Eastman, 1968; Vaisey-Genser and Morris, 2003). This property made flaxseed oil a key component in the manufacture of paints, inks, varnishes, and other protective coatings. These products were used to preserve tools, furniture, buildings, machinery, paintings, and countless other items throughout history (Eastman, 1968; Vaisey-Genser and Morris, 2003). Flax based paints were used as early as Ancient Egypt, and were the medium for countless renaissance-era paintings; flaxseed oil was even a key ingredient of the ink used by Johannes Gutenberg's mechanical printing press (Eastlake, 1960; Eastman, 1968; Vaisey-Genser and Morris, 2003). Flaxseed oil was the predominant drying oil used throughout the western world until the mid-20th century, when it gradually became replaced by new synthetic resins and binders (Eastman, 1968).

Throughout history, whole flaxseed has also been added to livestock rations, and consumed by humans in polentas, breads, and porridges, or used medicinally in the form of poultices, balms, salves, ointments, and teas (Pengilly, 2003; Vaisey-Genser and Morris, 2003). Other modern uses for flaxseed oil include the manufacture of linoleum flooring, patent leather, adhesives, and, more recently, use as a concrete preservative (Eastman, 1968; Vaisey-Genser and Morris, 2003).

Future sources of demand for flaxseed

Flax as a functional food

While medicinal and food uses have been secondary to industrial applications historically, there is growing demand and research interest around the use of flax as a functional food. A functional food is defined as "one which is similar in appearance to a conventional food, consumed as a part of the usual diet, with demonstrated physiological benefits, and/or to reduce the risk of chronic disease beyond basic nutritional functions" (Goyal et al., 2014). The high concentration of ω-3 α-linolenic acid (ALA) that gives flaxseed oil its drying properties is also the primary molecule of interest for the functional food aspects of flax. The consumption of ω-3 fatty acids has been widely studied and is associated with decreased cardiovascular disease, hypertension, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders (Gogus and Smith, 2010; Goyal et al., 2014; Vaisey-Genser and Morris, 2003). In addition to high levels of ALA, flax is also an excellent source of soluble and insoluble fiber, which has been shown to reduce blood glucose, cholesterol levels, and treat digestive ailments. Finally, flax contains very high levels of phenolic molecules called lignans. These are being studied for anticarcinogenic properties, especially towards hormone-dependent cancers like those of the breast, endometrium and prostate (Goyal et al., 2014; Vaisey-Genser and Morris, 2003).

The functional foods market is growing rapidly, with an estimated value of US $309 billion by 2027 (Precedence Research, 2020; Vergari et al., 2010). One of the main

drivers of this growth is increased awareness of the need for ω-3 fatty acids in the human diet (Dean, 2003; Goyal et al., 2014). Fish has received much of the attention when it comes to incorporating ω-3 fatty acids into the diet, but issues with the sustainability of global fisheries, and concerns about bioaccumulation of heavy metals challenge the viability of fish as a sustainable ALA source (Bosch et al., 2016; Kojadinovic et al., 2006; Lenihan-Geels et al., 2013; Okpala et al., 2018). Plant sources of ω-3 fatty acids avoid these issues and can also be easily incorporated into grain-based foods like bread, baked goods, cereals, and granola (Dean, 2003; Goyal et al., 2014; Vaisey-Genser and Morris, 2003). Given that flaxseed oil has the highest concentration of ω-3 among vegetable oils, it is well-positioned to meet this growing demand (Dean, 2003).

Recent decades have also seen renewed interest in using whole or whole-ground flax in pet and livestock feeds to increase animal health and livestock production (Dean, 2003; Duguid, 2009; FCOC, 2019a). Premium pet foods containing flaxseed are promoted as a solution to digestive and skin problems (FCOC, 2019a). One challenge limiting the direct consumption of flax by certain animals is the presence of anti-nutritional compounds such as cyanogenic glycosides and cadmium. Plant breeders are working to reduce these levels in order to remove limits on the amount of flaxseed that can be safely included in livestock rations (Dean, 2003; Duguid, 2009). It is now well established that including flaxseed in animal rations can increase the ω-3 fatty acid content of animal products such as eggs, meat, milk, which can then be marketed as premium functional foods (Cherian and Quezada, 2016; Kouba and Mourot, 2011; Palmquist, 2009; Pérez-Juan et al., 2014; Scheideler, 2003; Vaisey-Genser and Morris,

2003). This recent surge of interest in the functional properties and health benefits of flaxseed is driving growth in the flax market which is expected to continue.

Other flax-derived products

There are several minor products associated with flax which have potential for future growth. Linoleum is a type of flooring made by combining flaxseed oil, resin, and cork onto a backing made of burlap or canvas. It is then heated to polymerize the flaxseed oil, resulting in an inexpensive, yet durable material (Vaisey-Genser and Morris, 2003). Linoleum flooring was very popular prior to the invention of cheaper synthetic flooring (Eastman, 1968). Recent improvements to the manufacturing process and growing interest in natural products are generating renewed demand for linoleum, which is both non-allergenic and biodegradable (Duguid, 2009; Vaisey-Genser and Morris, 2003).

Oilseed flax production also provides fiber as a by-product. These fibers are shorter and lower quality than what is achieved when growing true fiber-flax cultivars. Traditionally, this seed-flax straw was collected and processed into fine papers like bond or cigarette paper (Eastman, 1968; Vaisey-Genser and Morris, 2003). New and emerging uses for by-product flax fibers include pulp sweeteners to strengthen recycled paper, geotextiles for erosion control, insulation, and plastic composites (Duguid, 2009; FCOC, 2019a). Flax straw is also being investigated for use as a carbon-neutral alternative fuel source (Duguid, 2009).

History of flax in Minnesota

Flax was one of the first crops brought by European settlers of the American Colonies (Eastman, 1968; Meyers, 2003). At that time, it was primarily used for fiber production, which was labor intensive and often required the participation of an entire community (National Park Service, 2015; Peterson, 1947). As settlers moved west, flax was planted after "breaking prairie," eventually causing most of the production to be centered in Midwest states such as Iowa, Minnesota, Nebraska, North Dakota, and South Dakota (Coffin, 1902; Eastman, 1968). After the invention of the cotton gin, demand for flax fiber waned; by the mid-19th century most of the remaining demand was for industrial applications of flax (linseed) oil, with a secondary market for livestock feed (Meyers, 2003; Peterson, 1947; Vaisey-Genser and Morris, 2003).

The processing of one 56 lb. bushel of flaxseed produces an average of 20 lb. of oil and 36 lb. of meal, also called press cake. Throughout the 20th century, the protein-rich meal was primarily exported to Europe, where it was valued as a premium animal feed for its high nutritional content (Eastman, 1968). The extracted flaxseed oil was then used to make paints, varnishes, and linoleum flooring (Eastman, 1968; National Park Service, 2013).

By the turn of the 20th century, Minneapolis, MN had become the center of linseed processing in North America (Eastman, 1968). Notable flax processors in Minneapolis included the Archer-Daniels-Midland Linseed Company, the Bisbee Linseed Company, and the Minnesota Linseed Oil Company (now a National Historic Site)

(Eastman, 1968; National Park Service, 2013). In the United States, demand for flaxseed oil spiked during both World Wars due to the large quantity of wartime machinery being manufactured, all of which required protective coating (Figure 1-1) (Eastman, 1968; Minnesota Agricultural Experiment Station, n.d.). Thus, by the 1920s, linseed oil had become Minneapolis's fourth largest industry. For 18/29 years between 1921-1950, Minnesota was the nation's leading producer of flax; at its peak in 1948, Minnesota accounted for > 1/3 of all American flax production (Eastman, 1968; National Park Service, 2013). The decline of the flax production and processing industry began with price fluctuations driven by the war, plus periods of domestic crop shortages and high foreign imports. These factors prompted a gradual shift to synthetic resins and binders among flax consuming industries like paint manufacturing, and the industry never recovered (Figure 1-1) (Eastman, 1968; National Park Service, 2013; Oplinger et al., 1997).

Prior flax breeding at the University of Minnesota

From 1894-1984 the University of Minnesota (UMN) had an active flax breeding and disease research program which had close collaboration with North Dakota State University (NDSU) (Eastman, 1968). Flax was extremely susceptible to wilt (*Fusarium lini*) and rust (*Melampsora lini*), so in 1890, Minnesota Governor W.R. Merriam appointed university botanist Dr. Otto Lugger to "make all necessary experiments to find, if possible, a remedy against this disease [wilt]", thus initiating the first flax experiment

in Minnesota (Department of Agronomy and Plant Genetics, 2000; Eastman, 1968). Formal breeding for disease resistance soon began under Dr. Willet Hays in 1894 (Table 1-1) (Department of Agronomy and Plant Genetics, 2000). Over the next 90 yr., breeders at the UMN and NDSU released numerous high yielding flax varieties with resistance to flax rust and wilt (Table 1-1 andTable 1-2) (Department of Agronomy and Plant Genetics, 2000). Notably, these efforts led to the development of the famed gene for gene hypothesis by plant pathologist Dr. Harold H. Flor, who received his Ph.D. from the UMN in 1929, and went on to study flax rust at NDSU (Department of Agronomy and Plant Genetics, 2000; Loegering and Ellingboe, 1987). By the mid-20th century, interest in flax breeding declined as production waned (Figure 1-1), and the UMN breeding program was discontinued after the last flax breeder, Dr. Verne Comstock, retired in 1984 (Table 1-1) (Department of Agronomy and Plant Genetics, 2000). Until recently, NDSU housed the only active (common) flax breeding program in the United States. The majority of flax breeding programs are now located at public institutions throughout Canada, primarily in Alberta, Manitoba, and Saskatchewan (Duguid, 2009).

Justification for a new perennial flax breeding program

Recent data published by the Food and Agriculture Organization of the United Nations (FAO) records increases in global flaxseed (linseed) production over the last 10 yr., from 1.66 million tonnes in 2007 to 2.97 million tonnes in 2017 (Figure 1-2a) (FAO, 2019). Trends toward “green” products and functional foods have generated

increased awareness of flax-derived products, which may explain this recent production increase (Duguid, 2009). Between 2007-2017, Canada was the largest producer of flax, followed by China and the Russian Federation. The United States ranks 5th among top producing countries, just ahead of India (FAO, 2019). Despite increases in global production, the United States has seen a decrease in total production from 2007-2017 (Figure 1-2b) (FAO, 2019). However, production has been increasing gradually in Minnesota in recent years (Figure 1-3) (USDA, n.d.).

The expanding global market for flaxseed indicates that there is still opportunity for US public institutions to become active in flax improvement. To meet this growing demand for flaxseed, the UMN is investigating the feasibility of perennial flax improvement for agronomic and horticultural applications. These efforts are being conducted as part of the Forever Green Initiative (FGI), a research group at the UMN with the goal of encouraging year-round cover on the Minnesota agricultural landscape as a means of counteracting the environmental impacts of modern agricultural practices. The FGI focuses primarily on winter cover crops and perennial or biennial grain species that are capable of overwintering in Minnesota (FGI, 2019).

Qualities of wild flax species

Most wild species of flax have high levels of ALA, similar to *L. usitatissimum*, making them a suitable alternative for the health foods market (Poliakova and Lyakh, 2017; Westcott and Muir, 2003). Perennial *Linum* species are also ideal candidates for the

ornamental market, which is relatively untapped apart from limited breeding efforts on *L. grandiflorum* Desf., *L. perenne* L., and *L. lewisii* Pursh (Cullis, 2011; Fu, 2019; Lyakh et al., 2018; Ogle et al., 2006). Marketing perennial flax as a new cut flower filler crop is a potential source of added horticultural value, although nothing is known about the potential vase life of any *Linum* species (Dole and Wilkins, 2005).

Certain flax species possess ornamental qualities which have resulted in their cultivation in home gardens, landscaping, and botanic gardens. Several species from the *Linum* section of the genus (See Chapter 2 for a detailed taxonomic review) are reported to possess impressive flowers, notably *L. austriacum* L., *L. perenne*, and *L. narbonense* L., which are typically shades of blue, white, or violet (Diederichsen and Richards, 2003; McDill et al., 2009). Also within sect. *Linum* is the annual flax species *L. grandiflorum*, which is characterized by large crimson-red flowers, although breeding efforts have produced shades of pink, white, apricot, copper, and lilac, as well as a variety of flower shapes resulting from varying degrees of longitudinal or marginal petal folding (Lyakh et al., 2018). Another section containing commonly cultivated ornamental flax is *Dasylinum*, which includes species such as *L. hypericifolium* Salisb., *L. hirsutum* L., and *L. viscosum* L. The section *Syllinum* contains one widely cultivated ornamental, *L. flavum* L., which possesses bright yellow flowers (Diederichsen and Richards, 2003).

Ecosystem services to address Minnesota environmental issues

Perennial flax is uniquely positioned to meet the growing demand for flax products, while also providing added ecosystem services. Cold hardy perennial flax retains green vegetation late into the fall and begins regrowth early in the spring, resulting in decreased soil erosion and improvements in water quality (Colson et al., 2005). Mitigating these environmental effects of agriculture is of urgent importance in the State of Minnesota. It has been estimated that up to 45% of Minnesota cropland is eroding at a rate greater than the NRCS defined tolerable level of five tons per acre per year (Minnesota Board of Water & Soil Resources, 2002). In some areas up to 19 in of topsoil has already been lost (DeJong-Hughes et al., 2018). Intensive tillage practices and lack of winter cover are cited as major factors contributing to the high rate of erosion in Minnesota (DeJong-Hughes et al., 2018). These practices also increase soil compaction, damage soil structure, and reduce soil organic matter—all factors which increase rates of erosion (DeJong-Hughes, 2018; DeJong-Hughes and Daigh, 2018; Overstreet and DeJong-Hughes, 2018). Prior research has suggested that it takes at least 100 y for an inch of topsoil to accumulate naturally, further illustrating the severity and urgency of these issues (Hipple, 2006).

Minnesota is also experiencing widespread water quality issues driven by erosion and agricultural runoff (The Water Resources Center, 2017). Eroded soil particles which contain nitrogen and phosphorus are capable of causing algal blooms when deposited in surface waters (DeJong-Hughes et al., 2018). Agricultural runoff is of even greater concern, as it is estimated that 78% of nitrates and 48% of phosphorus in Minnesota water comes from agricultural sources (The Water Resources Center, 2017). The

Minnesota Department of Health (MDH) reported in 2017 that 537 public supply wells in Minnesota contained elevated levels of nitrates, which are costly to remove and dangerous to infants and young children (MDH, 2017). Best management practices outlined by the Minnesota Pollution Control Agency (MPCA) for reducing nutrient losses from agricultural fields recommend integrating “perennials planted in riparian areas or marginal cropland; extended rotations with perennials" into current agricultural systems as part of the solution to this problem (MPCA, 2013). Furthermore, studies of perennial grain crops, such as intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey subsp. *intermedium*), have demonstrated increased rates of nutrient capture over a 2 y period, proving that this is an effective method of reducing levels of agricultural pollutants to Minnesota waterways (Crews et al., 2016; Culman et al., 2013; Jungers et al., 2019). Development of perennial flax cultivars with suitable economic value to the farmer would, therefore, be an effective means of addressing many of the soil and water quality issues experienced in Minnesota as a by-product of agriculture.

An additional ecological concern is the widespread decline in pollinator populations worldwide due to loss of habitat, pesticide use, and disease (Boecking and Veromann, 2020; Maccagnani et al., 2020; MDA, 2014; Potts et al., 2010; Vaughan and Black, 2006). It is estimated that one-third of global food production relies on pollinators. Important insect pollinated crops include apples, almonds, blueberries, and many forage species, in addition to countless wild plant species (Bauer and Wing, 2010; MDA, 2014). A major issue on the agricultural landscape is a lack of forage sources for pollinators, especially late in the season (Vaughan and Black, 2006). Perennial flax can provide

forage for pollinators at times when annual flax and other bee pollinated crops are not blooming. Many of the perennial flax genotypes in the FGI breeding program have a long flowering period that begins early in the spring, followed by a second period of flowering which begins later in the season, continuing until the first frost (D. Tork, unpublished data, 2018-2021). Integrating perennial flax into the existing agricultural landscape would therefore be an effective means of filling gaps in the availability of forage for native and managed bee populations.

Resources for perennial flax breeding

Conservation status

As with most crop species, the preservation of flax genetic resources is a concern for the long-term adaptability of modern breeding programs to new biotic and abiotic stresses. While *ex situ* conservation has been successful in preserving infraspecific diversity in *L. usitatissimum,* it is now rare to find *in situ*, or "on farm" conservation, and most landraces of fiber and seed flax have now been replaced by modern breeding lines (Diederichsen and Richards, 2003). Fiber flax, in particular, has lost much of its overall genetic diversity due to lack of cultivation (Diederichsen, 2019).

Certain wild *Linum* species have had an increased geographical distribution through their cultivation as ornamentals, although most still exist almost exclusively in their natural habitats. Their conservation is, therefore, dependent on the preservation of these natural ecosystems. For example, studies on the distribution of the *L. perenne* group

in Europe have found that "discontinuities in the distribution are not an artifact of uneven collecting but are real, and that considerable extinction has occurred in the last hundred years" (Ockendon, 1971). Wild species are typically underrepresented in germplasm collections because they are mostly outcrossing and, therefore, difficult to regenerate due to the need to control for cross pollination. Failure to properly isolate wild species may result in interspecific hybrids which have the potential to confuse taxonomic studies of the genus (Diederichsen, 2019; Diederichsen and Richards, 2003; Fu, 2019). Improvement of perennial flax species is, therefore, also an important means of selecting and maintaining genetic diversity within the genus.

Genetic resources

Most flax species are diploid, including common flax (*L. usitatissimum*), which has a relatively small genome size of ~370 Mb. The genome sizes of other species in sect. *Linum* are likely even smaller, as it is thought that common flax and its progenitor, *L. bienne* Mill., likely arose out of a whole genome duplication event followed by loss of certain chromosomes (Wang et al., 2012). The genome of common flax (*L. usitatissimum*) was completed in 2012 by whole-genome shotgun sequencing (Wang et al., 2012). Already, researchers have begun to conduct genome-wide association studies and association mapping of key agronomic traits (Soto-Cerda et al., 2014; Xie et al., 2018). There has also been work done to characterize the range of genetic variability for 27 traits in the Canadian core collection of common flax held in the Plant Gene

Resources of Canada (PGRC) (You et al., 2017). These investigations into the genetics of common flax will enable faster progress when genetic tools for perennial flax are developed. Once the perennial flax genome is sequenced, gene ontology analysis and comparison of paralogous genes will allow for more rapid discovery of agronomically relevant traits.

Domestication of perennial grain crops

Pipeline strategy for domestication

While ornamental herbaceous as well as fruit and vegetable perennial crops have been bred for millennia, the concept of domesticating perennial grain crops is relatively new. There are now several existing programs involved in the domestication of wild perennial grains. Prominent examples include intermediate wheatgrass (*Thinopyrum intermedium*), perennial sunflower (*Helianthus spp.*), and silphium (*Silphium integrifolium* Michx.) (DeHaan et al., 2018, 2016; Van Tassel et al., 2014; Vilela et al., 2018). The lessons gained from initial perennial grain domestication efforts are summarized in a review by DeHaan et al., (2016), which outlines a framework for perennial grain crop domestication using a pipeline approach. The initial phase of this pipeline involves defining the agricultural target, followed by a broad screening of large numbers of wild plants. The goal of this initial phase is to characterize the strengths and weaknesses of each species evaluated. No single species is expected to possess all of the

desired traits, but the goal is to find “species with traits that will make the process of domestication rapid and less difficult” (DeHaan et al., 2016).

After initial screening, it is recommended that several top candidate species be fed into the pipeline to undergo initial rounds of selection. The process of selection is important for ranking the top candidates, as the ease of breeding and response to selection are important factors in determining the long-term feasibility of a domestication program (DeHaan et al., 2016). Over time, the number of species present in the pipeline decreases, and the focus shifts from a wide screening to a more intensive evaluation of the most promising candidates. This is primarily when long-term research, breeding, and marketing strategies are developed. The final phase of domestication involves continued improvement of yield and critical limiting traits, while also laying the groundwork for seed production and marketing of the crop (DeHaan et al., 2016).

Critiques of perennial grain domestication programs.

One of the most charged debates regarding the feasibility of perennial grain crop domestication has centered around the idea of resource tradeoffs. Critics often argue that there are fundamental tradeoffs between perenniality/winter survival and seed production (Denison, 2016; Smaje, 2015). These arguments are based on the notion that there is a finite amount of photosynthate available in a single growing season. In annual crops, all available photosynthate can be directed into seed production at the end of the season during senescence, whereas perennials must shunt at least some of this photosynthate into

storage organs for winter survival (Denison, 2016; Smaje, 2015). This is the basis for the argument put forward by Denison (2016), who claims that the rapid increases in yield component traits achieved by intermediate wheatgrass breeders (Van Tassel and DeHaan, 2013) will eventually plateau if winter survival is held constant. Again, this argument is based on the notion that photosynthate is a limited resource. In other words, the total amount of photosynthate that can be shifted from vegetative to reproductive tissue without compromising winter hardiness will, in theory, eventually run out, resulting in a plateau in yield increases over time (Denison, 2016).

The critiques of perennial grain domestication argued by adherents to the tradeoff hypothesis were addressed directly in a study by DeHaan et al. (2018), which looked at phenotypic correlations between selected and non-selected traits over six intermediate wheatgrass (*T. intermedium*) breeding cycles. The results contradicted the idea that yield gains would come at the expense of other growth components, as seed yield per head was found to be positively correlated with competing allocations such as plant height, rhizome production and regrowth after harvest (DeHaan et al., 2018). These results suggest that the tradeoffs between yield and other physiological components may be less strict than imagined for a population which is still undergoing adaptation to the agricultural environment (DeHaan et al., 2018). However, further testing is needed to prove that this trend will continue once the plants are locally adapted and able to maximize the use of available resources. Fortunately for perennial grain breeders, pursuing answers to these questions of resource tradeoffs is one of the best justifications for the continued improvement of these crops.

Ideotype breeding

One of the greatest challenges of breeding undomesticated perennial crops is the sheer number of traits that require improvement. Most domesticated crops have several traits in common, including increased grain/fruit size, reduced branching, gigantism, non-shattering, loss of seed dormancy, synchronous maturation, and loss of toxic compounds. These traits are commonly referred to as the "domestication syndrome," as they are changes that took place during domestication that make crops more agronomically productive and useful to humans (Ross-Ibarra et al., 2007). For a perennial grain to successfully integrate into our current agricultural system, it will need to possess most, if not all of these traits.

In the FGI perennial flax program, these challenges of domestication are being addressed using an ideotype framework. The concept of a "crop ideotype" was first proposed by Donald (1968) who defined it as "a biological model which is expected to perform or behave in a predictable manner within a defined environment" (Donald, 1968). To begin building an ideotype model, one must first define traits of interest based on prior knowledge. For each of these traits, an ideal phenotype is then defined. For example, Donald's original ideotype integrated multiple traits expected to maximize wheat yield by reducing competition between plants in a crop community. These traits were chosen based on contemporary knowledge of crop physiology, as well as ease of measurement (Donald, 1968). It is important to note that ideotype models are a

complement to, not a replacement for, traditional breeding methods. The usefulness of the ideotype approach comes through the process of goal setting, prioritization of traits, testing, and finally, adjusting the model based on the results. Using this approach, an ideotype model should never be static, but should change as more knowledge is gained about the particular trait or system. Challenges of using the ideotype approach include correctly identifying target traits, and adjusting to deal with unforeseen genetic mechanisms like negative correlations between traits, pleiotropy, and trait compensation (Rasmusson, 1991). However, if used correctly, the ideotype approach helps to prioritize multiple unrelated traits, and generate testable hypotheses about how changes in one trait might affect the overall performance of the plant or crop community.

Perennial flax ideotypes have recently been defined for oilseed, cut flower, and garden breeding objectives, and are being used to drive selection in the FGI perennial flax breeding program. The traits of interest, goals for selection, and rationale behind these ideotypes are expanded upon in our recent review paper (Tork et al., 2019). The results obtained from screening perennial flax accessions and breeding populations using this ideotype framework are presented in Chapter 2 of this thesis. Vase life studies were also performed to investigate traits relevant to the cut flower ideotype; the background and results of these studies are presented in Chapter 3. Finally, improving winter hardiness is a universal breeding goal for all perennial flax ideotypes, as any plant grown in Minnesota must be capable of surviving winter conditions characteristic of USDA Plant Hardiness Zones 4 and 3. Controlled freezing studies were used to screen perennial flax for freezing tolerance, which may be useful as a corollary selection method for

winter hardiness. The background and results of this controlled freezing study are presented in Chapter 4.

Summary

Domestication of wild perennial flax will require an intensive breeding effort, yet this undertaking is justified by the many benefits that could be gained from this program in the long-term. As long as the environmental issues of soil erosion, water pollution, and pollinator decline continue to exist, there will be a demand for management-based solutions to these problems. The MPCA has stated that "research to develop the appropriate perennials and marketable uses needs to be a priority" (MPCA, 2014). Breeding of perennial species is time-intensive, so continued research and development must be pursued if perennial species are to become a standard management tool for addressing ecological and environmental concerns. The perennial flax breeding program can benefit from the growing demand for flax as a functional food, and the simultaneous development of ornamental varieties will encourage the use of these pollinator-friendly plants in the home garden, creating an added source of value for the breeding program. In summary, the diverse uses and potential benefits of perennial flax suggest that this crop is well-positioned to become an important addition to the FGI and the future Minnesota agricultural landscape.

Tables

Table 1-1. University of Minnesota faculty involved with flax breeding listed by retirement date.

Faculty member	From	To	Specialty and achievements
O. Lugger	-	-	First experiments on flax wilt in 1890. Preceded discovery of the causal fungus, *Fusarium lini*, by NDSU scientist H.L. Bolley in 1901, leading to first wilt-resistant var. North Dakota Resistant 52 in 1908 (Department of Agronomy and Plant Genetics, 2000; Eastman, 1968; Walster, 1950).
W.M. Hays	1888	1905	General agronomy, plant breeding; developed flax 'Primost' (MN No. 25) in 1894, the first pure line flax variety produced in US (Department of Agronomy and Plant Genetics, 2000).
J.J. Christensen	-	-	Plant pathology; established flax wilt nursery in 1913 where Norman Borlaug gathered thesis data (Department of Agronomy and Plant Genetics, 2000).
A.W. Henry	1923	1927	Plant pathology, flax rust (Ausemus, 1943; Hiruki, 1988).
A.H. Moseman	1944	1945	USDA, flax improvement (Department of Agronomy and Plant Genetics, 2000).
A.C. Arny	1909	1946	Agronomist; Released purified 'Redwing' reselection in 1930. Led flax development at MN ~1925-1945. Released 'Biwing' and 'Redson' in 1943; 'Minerva', 'Dakota' in 1949 (Arny, 1945, 1943; Department of Agronomy and Plant Genetics, 2000).
J.O. Culbertson	1937	1957	USDA, flax improvement. Released 'Redwood' in 1952. Developed varieties Arny, Marine 62, Windom, Nored, and Norstar (Department of Agronomy and Plant Genetics, 2000).
R.S. Dunham	1945	1958	Weed science; developed effective flax herbicide treatments (Department of Agronomy and Plant Genetics, 2000).
W.M. Myers	1932	1963	Plant Breeding 1932-37; department head 1962-63. Studied inheritance of flax rust prior, influencing H.H. Flor's work (Loegering and Ellingboe, 1987).
A.C. Dillman	-	-	USDA; introduced 'Dakota' in 1949; published paper 'Improvement of flax, which appeared in USDA's Yearbook of Agriculture in 1936 (Department of Agronomy and Plant Genetics, 2000; Walster, 1950).
C.R. Burnham	1938	1972	Genetics, cytogenetics, flax wilt (Department of Agronomy and Plant Genetics, 2000; Sherbakoff, 1949).
J.H. Ford	1960	1973	USDA, flax improvement (Department of Agronomy and Plant Genetics, 2000).
V.E. Comstock	1954	1984	USDA, flax improvement. Led MN flax project to 1972 when it was moved to ND. He continued flax development until retirement: 'Culbert', 'Verne' his namesake, 1985) (Department of Agronomy and Plant Genetics, 2000).
D. Wyse	2018	present	Perennial flax breeding for oilseed & ornamental use
N.O. Anderson	2018	present	Perennial flax breeding for oilseed & ornamental use

Table 1-2. University of Minnesota flax releases (*L. usitatissimum* L.) (Minnesota Agricultural Experiment Station, n.d.).

Variety	Date
Primost	1900
Redwing	1916
Winona	1922
Chippewa	1923
Redson	1943
Biwing	1943
Crystal	1944
Minerva	1949
Dakota	1949
Redwood	1952
Arny	1961
Marine 62	1962
Windom	1962
Nored	1968
Norstar	1969
Culbert	1975
Verne	1985

Figures

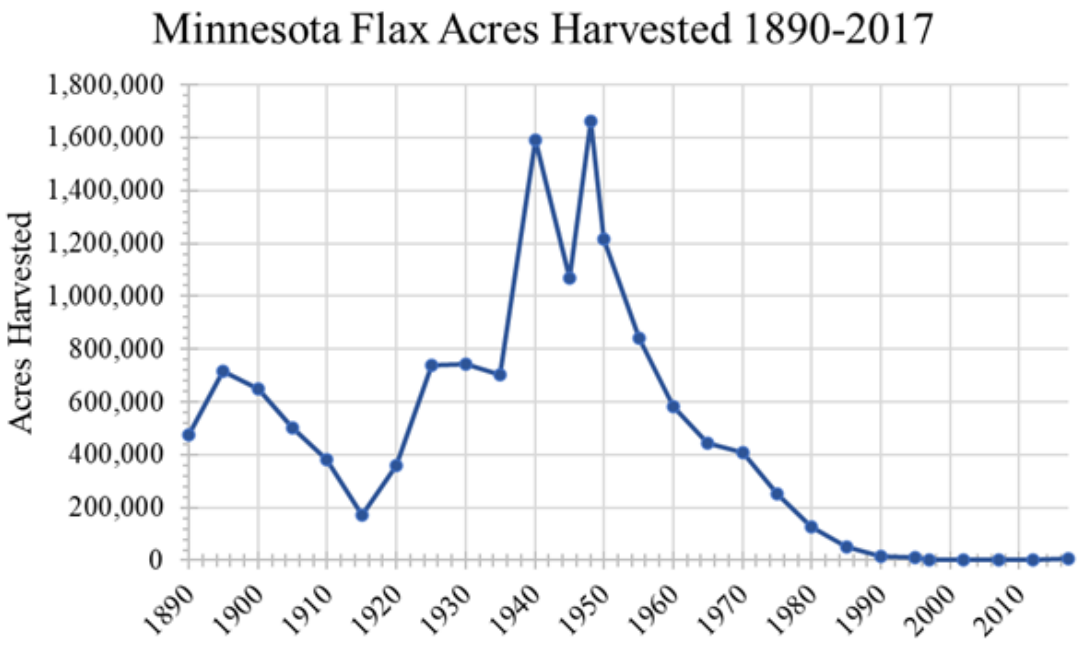

Figure 1-1. Acres of flax harvested in Minnesota between the years of 1890 and 2017 (Department of Agronomy and Plant Genetics, 2000; Walster, 1950)

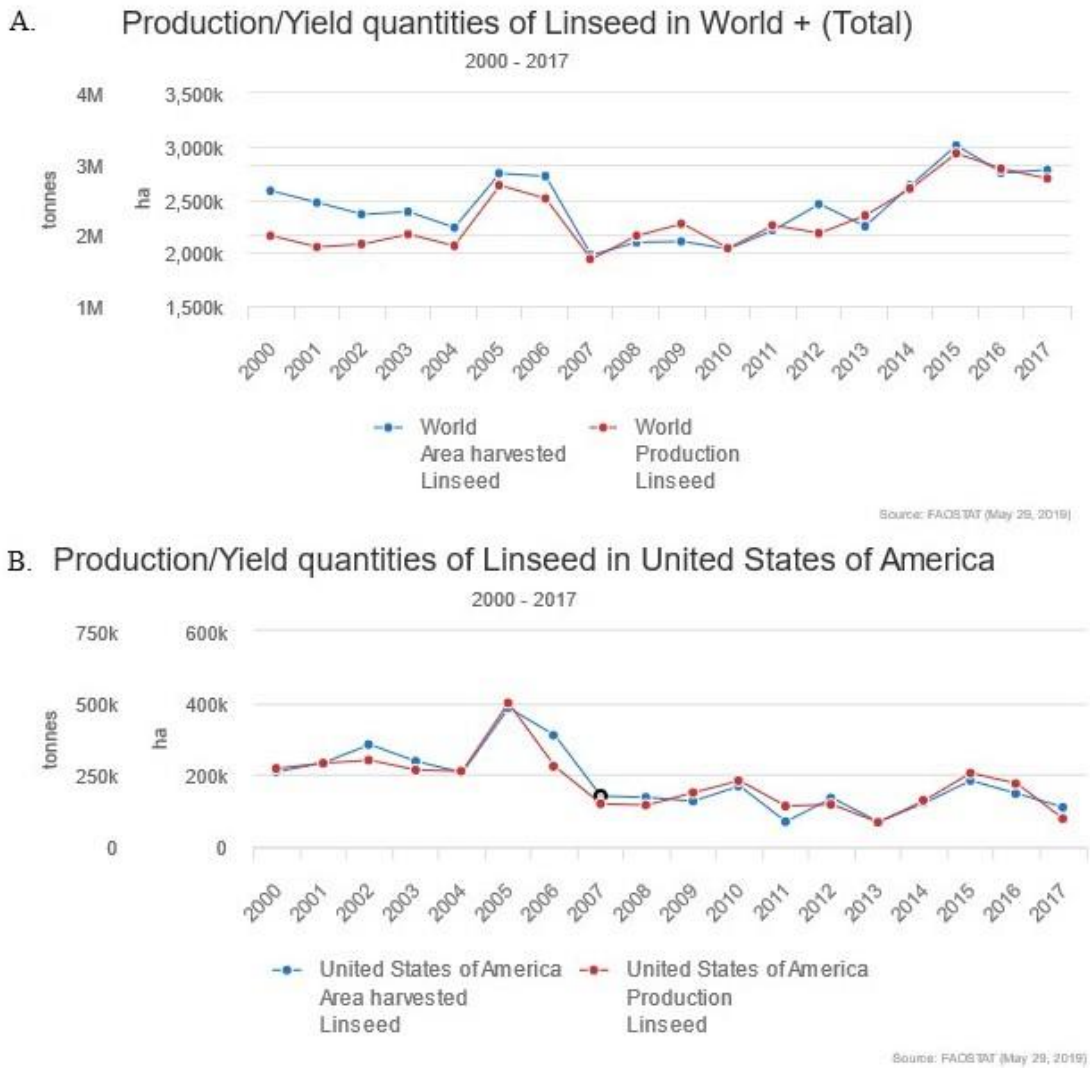

Figure 1-2. (A) Production (t) and area harvested (ha) of flaxseed/linseed from 2007-2017 globally and (B) in the United States of America (FAO, 2019).

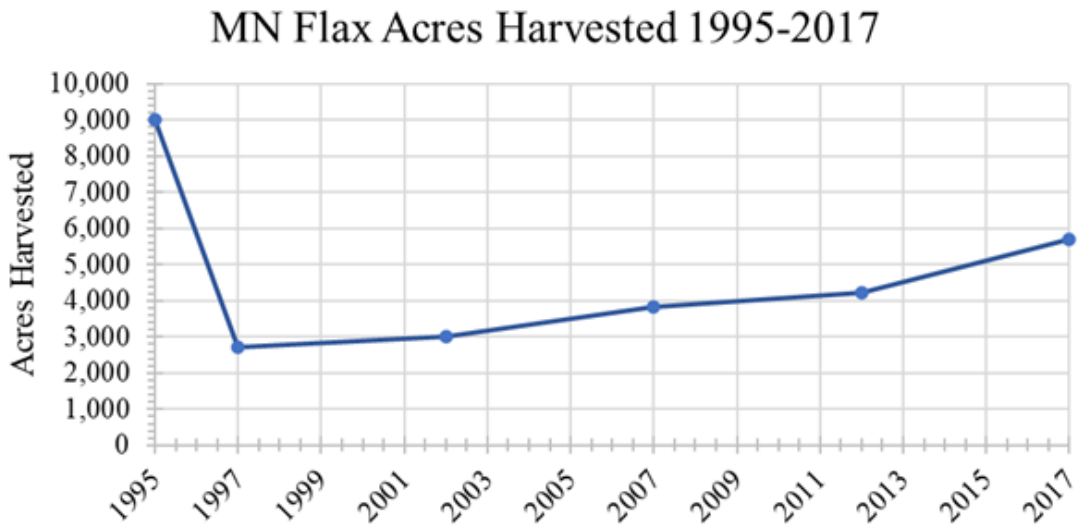

Figure 1-3. Gradual increase in acres of flax harvested in Minnesota between 1997 and 2017 (USDA, n.d.).

Chapter 2

Selection of Perennial Flax (*Linum* spp.) for Yield, Reproductive, and Plant Habit Traits to Achieve Oilseed, Cut Flower, and Herbaceous Perennial Ideotypes

Manuscript to be submitted to *Crop Science.*

Flaxseed has gained popularity as a health food for its high ω-3 fatty acid composition. Wild relatives of common flax (*Linum usitatissimum* L.) possess similar oil composition, showy flowers, and long flowering periods. Thus, perennial flax domestication was initiated at the University of Minnesota in 2008 to develop oilseed, cut flower, and garden cultivars that also provide ecosystem services. The objective of this study was to phenotype oilseed and cut flower breeding populations alongside accessions (annual, perennial) in a common garden to quantify the impact of selection and identify the top candidate species for breeding. Traits measured were based on perennial flax ideotypes and included: yield, seed weight, shattering, seed capsule diameter, flower diameter, flowering period, stem length and diameter, plant width and height, germination by week, summer and winter survival, style morph, flower shape, and petal overlap. In year one of growth, the oilseed population had the highest average seed yield. The oilseed and cut flower populations also had smaller seeds, higher levels of shattering, smaller capsule and stem diameters, longer flowering periods, larger plant size, more uniform growth, lower percent germination, and improved winter survival compared to wild species. *Linum austriacum* L. was the top candidate among wild species for oilseed and cut flower breeding, and it had a comparable performance with *L. perenne* L. for

garden ornamental breeding. These data illustrate the effect that 1-5 yr of selection can have on target and non-target traits in wild species, and implications for future breeding are discussed.

Introduction

The future of modern agriculture requires that several significant and mounting environmental challenges be addressed, namely soil erosion, nutrient runoff, and pollinator decline (Bauer and Wing, 2010; Boecking and Veromann, 2020; Hegde et al., 2011; Potts et al., 2010; Wu et al., 2018). Current production systems use intensive tillage practices and leave topsoil exposed throughout the winter months, facilitating increased rates of erosion and nutrient runoff (Hegde et al., 2011; Liu et al., 2008; Sprague and Gronberg, 2012; Streeter et al., 2018). These problems have gained widespread attention in Minnesota, where it is estimated that up to 45% of cropland is experiencing unsustainable levels of soil erosion and a majority of water nutrient pollution is linked to agricultural sources (Minnesota Board of Water & Soil Resources, 2002; The Water Resources Center, 2017). These problems contribute to algal blooms in surface waters, and contamination of public drinking water supplies as nutrients leach into the groundwater aquifers (Conley et al., 2009; Liu et al., 2008; MDH, 2017; Sutton et al., 2013; Wu et al., 2018). Pollinator populations have also declined significantly due to habitat loss and pesticide use associated with annual monoculture cropping systems, which are generally not capable of sustaining pollinator populations due to their limited

flowering period (Boecking and Veromann, 2020; Maccagnani et al., 2020; MDA, 2014; Potts et al., 2010; Vaughan and Black, 2006).

One proposed solution to these problems is increasing the amount of year-round cover on agricultural land through cover crops or perennial cropping systems (Cox et al., 2006; Crews et al., 2018, 2016; FAO, 2014). This challenge has been the focus of the University of Minnesota Forever Green Initiative (FGI) for over a decade (FGI, 2019). The FGI brings together academic, industry, and legislative partners to advance the development of winter cover crops, as well as perennial or biennial species capable of overwintering in Minnesota (FGI, 2019). The core philosophy of FGI is that all crops should provide economic benefits to the farmer, as well as ecosystem services that relieve the environmental impacts of agriculture. Perennial grain crops bred by FGI researchers, such as intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey subsp. *intermedium*), have demonstrated increased rates of nutrient capture over a two-year period, proving that this is an effective method of reducing levels of agricultural pollutants to Minnesota waterways (Crews et al., 2016; Culman et al., 2013; Jungers et al., 2019).

Domesticated annual flax (*L. usitatissimum* L.), also known as common flax or linseed, is one of the oldest cultivated plants and was domesticated in the fertile crescent ~8,000 B.C.E (McDill et al., 2009; Vaisey-Genser and Morris, 2003). Throughout history, flax has been highly valued as a multi-use crop for fiber, feed, and industrial applications (Vaisey-Genser and Morris, 2003). Wild relatives of annual flax are being evaluated for domestication as a new perennial crop. Although wild species of *Linum* L.

have less total oil content, most share the unique oil profile of annual flax that is high in ω-3 fatty acids (Poliakova and Lyakh, 2017; Westcott and Muir, 2003). The health benefits associated with consuming ω-3 fatty acids are expected to drive increased demand for flaxseed (Dean, 2003; Goyal et al., 2014; Vaisey-Genser and Morris, 2003). Therefore, one of the primary objectives of FGI perennial flax breeding is to develop perennial oilseed flax with added ecosystem services, e.g. reduced soil erosion and water quality improvement. Perennial flax can also fill gaps in the food supply for pollinators because of its long flowering period and potential to flower out-of-season. The prolific flowers of some perennial flax species make them ideal candidates for ornamental uses, such as a garden bedding plant or cut flower. In a unique collaboration between agronomy and horticulture breeders, the FGI perennial flax breeding program is simultaneously pursuing both oilseed and ornamental breeding objectives. Current breeding efforts are being facilitated using an ideotype approach (Tork et al., 2019).

The genus *Linum* contains ~180-200 species distributed throughout temperate and subtropical regions of the world (Bolsheva et al., 2017; McDill et al., 2009). Over the years, many taxonomic classifications and revisions of *Linum* have been proposed, based on morphological features (Ockendon, 1971; Ockendon and Walters, 1968; Planchon, 1848, 1847; Rogers, 1969; Small, 1907; Winkler, 1931). More recent molecular phylogenetic analysis supports Winkler's (1931) division of the genus into five "sections" which form two major lineages: a blue-flowered clade containing sects. *Linum* and *Dasylinum* (Planchon) Juz. and a yellow-flowered clade containing sects. *Linopsis* (Reichenb) Engelmann, *Syllinum* (Griseb.), and *Cathartolinum* (Reichenb.) Griseb.

(McDill et al., 2009). This study also supports a proposed re-classification of the morphologically and karyologically distinct species *L. stelleroides* Planch. from sect. *Linum* to a new monotypic section of the blue flowered clade, sect. *Stellerolinon* (McDill et al., 2009; Yuzepchuk, 1974).

Within the blue-flowered clade, sect. *Linum* contains ~50 species, the majority of which have bright blue petals. The section can be further subdivided into the *Linum* and *Adenolinum* (Rchb.) Engelm. groups based on morphology and chromosome number (Bolsheva et al., 2017; McDill et al., 2009). The *Adenolinum* group is commonly called the *L. perenne* group, and it is the source of most of the perennial flax germplasm being evaluated by the FGI breeding program. It includes Eurasian species such as *L. perenne* L. and *L. austriacum* L., and the North American species *L. lewisii* Pursh (McDill et al., 2009; Rogers, 1969). Several species within sect. *Linum*, such as *L. perenne*, *L. narbonense* L., and *L. grandiflorum* Desf. have ornamental value, although there are few reports on the variation available for ornamental breeding (Cullis, 2011; Fu, 2019). Section *Linum* also encompasses notable species such as common flax (*L. usitatissimum*) and its progenitor *L. bienne* Mill. (formerly *L. angustifolium* Huds.) (McDill et al., 2009). Most of the species in sect. *Dasylinum* occur in the Mediterranean and southwest Asia, and several have been cultivated as ornamentals, such as *L. hypericifolium* Salisb., *L. hirsutum* L. and *L. viscosum* L. (Diederichsen, 2007; Diederichsen and Richards, 2003; McDill et al., 2009).

Within the yellow-flowered clade, sect. *Linopsis* (syn. *Linastrum*) is widely distributed, with species occurring in the Mediterranean, Africa, and South America

(Diederichsen, 2007; Diederichsen and Richards, 2003; McDill et al., 2009). The other sections in the yellow flowered clade, *Syllinum* and *Cathartolinum*, are nested within the sect. *Linopsis* based on molecular data (McDill et al., 2009). Most species in sect. *Syllinum* occur throughout the Mediterranean and southwest Asia and are characterized by yellow or white flowers and relatively large leaves. One example is the yellow-flowered perennial *L. flavum* L., which is widely cultivated as an ornamental (Diederichsen and Richards, 2003; McDill et al., 2009). Section *Cathartolinum* was once thought to contain many of the yellow-flowered North American species such as *L. sulcatum* Riddell, *L. alatum* (Small) H.J.P. Winlk, and *L. rigidum* Pursh (Diederichsen, 2007; Small, 1907), but molecular data suggest that these species are instead members of sect. *Linopsis*, and that sect. *Cathartolinum* may instead be a monophyletic clade containing only *L. catharticum* L. (McDill et al., 2009). A recent review by Diederichsen (2019) highlights the need for extensive taxonomic revision in *Linum* to resolve inconsistent terminology, define species boundaries based on morphological and genetic features, and reach a consensus on the number of species in the genus, all of which will facilitate improved conservation and breeding efforts.

In general, flax grows a dominant primary shoot (prostrate to erect), with lateral branching occurring primarily from the base to form a distinct crown. The primary stem connects to an underground taproot. The green, sometimes glabrous leaves are alternate linear to linear-lanceolate in shape and 15-55 mm in length (Diederichsen and Richards, 2003; Poliakova and Lyakh, 2017). New flowers open each morning with petal drop starting midday (Diederichsen and Richards, 2003; Eastman, 1968). The reproductive

structures are all quinquepartite: five sepals, five petals, five pistils and stamens, and a five chambered seed pod, or capsule. Each chamber of the seed pod can have up to two seeds, for a maximum of ten, although the average for cultivated flax is ~6 seeds per capsule (Oplinger et al., 1997; You et al., 2017). Unlike most crops, shattering or capsular dehiscence is not completely fixed in domesticated flax. More northern (Canadian) types typically dehisce slightly at the apex, although not enough for seed shatter, which helps the seeds to resist weathering and disease by allowing excess moisture to escape the capsule (FCOC, 2019b). Petals can be shades of blue, white, pink, violet, red, or yellow, depending on the species. Petal veins, stamens, and anthers can all exhibit the same range of color as the petals, with coloration in different flower parts showing independent inheritance (Diederichsen and Richards, 2003). There is variation within the genus for all reproductive traits, including sepal and petal shape, flower size and color, seed size and color, and degree of capsule opening (Poliakova and Lyakh, 2017). Many species also exhibit a form of sexual dimorphism (heterostyly) and sporophytic self incompatibility, both of which promote outcrossing (Ruiz-Martín et al., 2018). In the classic case of distyly, there exist two flower morphs: approach herkogamous (pin), where the styles are longer than the stamens, and reverse herkogamous (thrum) where the styles are shorter than the stamens. In this type of self incompatibility, only pollen from the reciprocal flower morph will result in successful pollination. There are also species in *Linum*, such as domesticated *L. usitatissimum*, which are self-compatible and monomorphic homostylous, meaning that styles and stamens are at equal height in all flowers (Ruiz-Martín et al., 2018). Finally, there is the

unique case of *L. lewisii*, which is monomorphic approach herkogamous, yet self-compatible (Pendleton et al., 2008). This floral trait is one of the few morphological distinctions between North American *L. lewisii* and Eurasian *L. perenne*, which has caused misidentifications (Pendleton et al., 2008).

Initial FGI evaluations of perennial flax from 2005-2008 occurred in a randomized common garden containing *Linum altaicum* Ledeb. ex Juz., *L. austriacum, L. baicalense* Juz., *L. bienne, L. campanulatum* L., *L. flavum, L. hirsutum, L. lewisii*, *L. perenne*, *L. sulcatum*, *L. tauricum* Willd., *L. tenuifolium* L., and *L. thracicum* Degen (N. Anderson and K. Betts, unpublished data). These early generations of seed were open pollinated, so the current species composition of these populations is unknown, and may include interspecific hybrids (Jhala et al., 2008; Seetharam, 1972). Plants which survived the 2005 winter were selected for yield in 2006 and 2007 and replanted in 2008. Remnant seed from 2008 was planted in spring 2017, out of which the most vigorous plants were visually selected and used to start an "elite restart" nursery in fall 2017 (K. Betts, personal communication, 2020-21). In fall 2018, selections for cut flower (CF) and oilseed (OS) traits were made from the 2018 elite restart nursery, as well as from an additional small nursery containing open pollinated *L. austriacum*, *L. lewisii*, and *L. perenne*. These selections were based on the ideotypes outlined by Tork et al. (2019). For the OS population, the primary consideration was first year yield, with secondary priority given to seed weight. The CF selections were selected primarily for flower diameter, stem length, and overall vigor.

The primary objective of this research was to compare ideotype trait values among wild accessions and breeding populations which had undergone 1-5 yr of selection. A second objective was to look for significant phenotypes which might be introgressed into existing breeding populations. Trait evaluation tests the assumptions and hypotheses about the perennial flax crop ideotypes (Tork et al., 2019). We hypothesize that the CF and OS selection populations will possess mean trait values exceeding the species accessions for the traits under selection.

Materials and Methods

Experiment 1

Plant material

Accessions of wild perennial flax were obtained from the Germplasm Resources Information Network (GRIN) of the USDA-ARS, Plant Gene Resources of Canada (GRIN-CA), Kew Millennium Seed Bank, and several commercial sources. In total, 137 accessions were studied of *L. alatum, L. altaicum, L. aristatum, L. austriacum, L. baicalense, L. bienne, L. decumbens, L. flavum, L. grandiflorum, L. hirsutum, L. hudsonoides, L. leonii, L. lewisii, L. narbonense, L. pallescens, L. perenne, L. stelleroides, L. strictum, L. sulcatum, L. virgultorum,* and *L. viscosum*; eight accessions of domesticated *L. usitatissimum* were included as check lines (Table 2-1). The study also included two selection populations of unknown species makeup derived from previously established breeding populations. Population ‘Selections - CF’ is 17 selections made in

2018 for the cut flower (CF) ideotype (Tork et al., 2019), based on growth habit, stem length, and flower diameter. The 'Selections - OS' population consists of 25 genotypes selected in 2018 for oilseed (OS) traits, primarily yield and seed size (1000 seed wt.) (Table 2-1). Within both populations, the top 9-10 parent genotypes were propagated as vegetative cuttings from field plants in fall 2018. Ten stem tip cuttings per genotype (>5 cm length) were harvested from the crown, labeled, sealed in bags [1.2 ml Get Reddi® Sandwich Bags, United States Plastic Corporation], and put into a cooler on ice for transport to MN Ag. Exp. Station Plant Growth Facility, University of Minnesota (44°59'17.8" N, -93°10'51.6" W) before rooting. Cuttings were trimmed to 5-7 cm length using a sterile razor [GEM Carbon Steel Extra Sharp Single Edge Blade, The Razor Blade Co., CA], the lower leaves removed, and the cut stem base dipped into 1000 ppm Indole-3-butyric Acid (IBA), after which cuttings were inserted into pre-moistened foam propagation strips [ROOTCUBES® PLUS WEDGE®, Oasis Grower Solutions, Kent, OH]. Cuttings were rooted for 5 wk in a glass mist house (21/21 °C, day/night, 16 h; 0600–2200 h lighting with high pressure sodium high intensity discharge lamps or HIDs at a minimum set point of 150 μmol m^{-2} s^{-1} at plant level). An intermittent mist system, at a mist frequency of 10 min intervals (mist nozzles, reverse osmosis water) during 0600-2200 h with a 7 s duration was used. After rooting, cuttings were transplanted into 10.12 cm square deep pots [SVD-355-DEEP-BK-40, T.O. Plastics, Clearwater, MN] filled with a soilless medium [Promix Mycorrhizae, Premier Horticulture Inc., Quakertown, PA] and grown in a glass greenhouse at 16.7/15.5 °C day/night daily integral and a 16 h photoperiod (0600–2200 h; long days). Supplemental

lighting was supplied during winter months and cloudy days by 400 w high pressure sodium high intensity discharge (HPS-HID) lamps, at a minimum of 150 μmol m^{-2} s^{-1} at plant level, with an 16 h photoperiod. These were grown as stock plants and then for cuttings in spring 2019. The open-pollinated (OP) seed from these clonal genotypes was also planted for evaluation in 2019 if ≥ 59 seeds were available, in addition to the other selections.

Establishment of common garden nursery

In spring 2019, all CF selections (17), OS selections (25), and species accessions (137) were grown in a common garden nursery in Rosemount, MN to compare phenotypic traits of interest within the same environment. Accessions were sown in 288 plug trays [Landmark Plastic, Akron, OH] with soilless germination media [Berger BM2 Germination Mix, Berger, Saint-Modeste, Quebec, Canada] and covered with fine vermiculite [Palmetto Vermiculite Medium A-2, Palmetto Vermiculite, Woodruff, SC] in wk 14 and 15 (5, 12 April 2019). Due to the limited quantity of seed, four accessions (59 seeds each) were planted by hand in each 288 plug tray, leaving an empty row between accessions to prevent contamination. For all breeding populations, n ≤ 288 seeds/genotype were sown using a vacuum seeder [E-Z Seeder, E-Z Seeder, Inc., WI]. All plug trays were placed in a mist house for 4 h to moisten the soilless medium using an intermittent mist system (St. Paul MN Plant Growth Facility, University of Minnesota; 44°59’17.8” N, -93°10’51.6” W) at a mist frequency of 10 min intervals (mist nozzles,

reverse osmosis water) during 0600-2200 h with a 7 s duration (21/21 °C, day/night, 16 h; 0600–2200 h) with lighting supplied by high pressure sodium high intensity discharge (HID) lamps at a minimum set point of 150 μmol m^{-2} s^{-1}. Once watered in, the trays were covered with plastic dome lids [Super Sprouter Standard Vented Humidity Dome 7", Hawthorne Gardening Company, Vancouver, WA] and transferred to a walk-in cooler for 2 wk at 4/4 °C day/night in darkness to break seed dormancy (cold stratification), which is recommended for most wild *Linum* species (K. Betts, personal communication, 2018; Barbara Atkins, STA laboratories, Longmont, CO). Trays were uncovered and misted by hand, as needed, over this 2 wk period to maintain adequate moisture levels in the soilless medium. After the 2 wk stratification, the dome lids were removed, and the trays were returned to the mist house for an additional 3 wk. During this 5 wk (total) germination period, the number of seeds germinated per week was recorded using different colored toothpicks inserted into the media (Anderson, 2019; Anderson et al., 2021). Plug trays were then moved onto capillary mats in a greenhouse at 16.7/15.5 °C day/night daily integral and a 16 h photoperiod (0600–2200 h; long days) on wk 19 and 20 (10, 17 May 2019). Supplemental lighting was supplied during cloudy days by 400 w high pressure sodium high intensity discharge (HPS-HID) lamps, at a minimum of 150 μmol m^{-2} s^{-1} at plant level. Fertigation (Mondays-Fridays) provided nutrients at a constant liquid feed (CLF) rate of 125 ppm N from water soluble 20-10-20 fertilizer. Accessions remained in the greenhouse until transplanting in wk. 24 (13 June 2019).

During wk 15 and 16, 100 vegetative cuttings from the top CF and OS selections (indicated by 'clone'; Table 2-1) were harvested to bulk up these genotypes for field

trials. The propagation protocol was identical to the one outlined above, except that cuttings were sourced from greenhouse stock plants. In wk 21 the rooted cuttings were moved to the identical greenhouse as the seedlings with the same fertigation regime; these also remained in the greenhouse until transplanting in wk 24.

The common garden nursery was located at the Rosemount Research and Outreach Center, Rosemount, MN (44°42’58.2" N, -93°5’54.9” W). Accessions and seed propagated selections were randomized. Twenty seedlings and/or ten rooted cuttings per genotype were transplanted, with selection for early germination within genotypes. Planting spacing was 45.7 cm O.C. within rows with 1.83 m row widths. The field was irrigated post-planting with 2.54 cm water. Irrigation continued throughout the summer to maintain a minimum of 2.54 cm water per week when there was insufficient rainfall. Weed control consisted of weekly mechanical tillage between rows, pre-emergent herbicide applications [Fortress®, OHP Inc., Bluffton, SC] at the recommended rates, and bi-weekly hand weeding within rows.

Flowering and survival notes

Each individual plant in the nursery was monitored weekly for flowering (≥ 1 open flower). Flowering data collection began in wk 27 through wk 43, or 17 wk total. The total number of weeks in flower was used to compare the flowering periods. Transplant survival was also recorded on a per-plant basis in wk 43. Winter survival was recorded the following spring in wk 19 (2020).

Measurements of plant size, flower, and stem characteristics

Given the size of the nursery (n = 2949 plants) it was not practical to carry out detailed phenotyping of every plant. Therefore, within each seed-propagated genotype, five plants were randomly selected for evaluation with the range of phenotypic variation for plant size, growth habit, and floral characteristics. For clonal selections, only three plants were selected per genotype due to genetic uniformity as clonal ramets.

Plant width was measured at the widest point (width 1). The meter stick was rotated 90° from the width 1 axis to measure the shorter width (width 2). Height was measured as the distance from the soil line to the highest point on the plant without stretching out stems or holding up any part of the plant. A semi-ellipsoid volume, which combines all three size measurements into a geometrical shape most representative of the plant growth habit and is visualized as a dome with unequal horizontal axes, was calculated using the formula:

$$Semi-ellipsoid\ volume\ (cm^3) = \frac{2}{3} \times \pi \times Height \times \left(\frac{1}{2} \times Width\ 1\right) \times \left(\frac{1}{2} \times Width\ 2\right)$$

The base area of an ellipse was calculated to obtain an overall measure of plant width, irrespective of height, using the formula:

$$Base\ area\ (cm^2) = \pi \times Width\ 1 \times Width\ 2$$

Circumference was calculated using the average width, according to the formula:

$$Circumference\ (cm) = \pi \times \left(\frac{Width\ 1 + Width\ 2}{2}\right)$$

The height to width ratio was calculated using the average width to determine whether plants were wider than they were tall, or vice versa, with the formula:

$$Height\ to\ width\ ratio = Height\ \div \left(\frac{Width\ 1 + Width\ 2}{2}\right)$$

Finally, the eccentricity of an ellipse (*e*) was calculated to quantify the shape of each plant measured, using the formula:

$$e\ = \sqrt{1 - \frac{(Width\ 2)^2}{(Width\ 1)^2}}$$

Eccentricity is a mathematical calculation with a range from 0-1 used to characterize the shape of a conical section. The eccentricity of a perfect circle is zero. Thus, values of *e* approaching zero indicate a more circular shape, while values approaching one indicate an increasingly elliptical shape. In addition to geometry, eccentricity calculations are commonly applied in astronomy to describe the shape of an object's orbit around another body (Limbach and Turner, 2015). However, the applications of eccentricity are far-ranging and have been used to characterize the shape of tumor cells in brain scans (Szczepankiewicz et al., 2016), yeast colonies (Prado et al., 2014), arterial stents (Kim et al., 2010), and the anomalous growth of tree rings following a landslide (Šilhán, 2019). Cells of varying eccentricities have even been created to study the effect of cell shape on plasma membrane signaling (Rangamani et al., 2013). In the present study, eccentricity values are used to estimate the shape of plant growth along the

horizontal axis, as determined by measurements of width. Eccentricity values closer to zero, indicating more uniform growth, are desired.

Flower data were measured on three flowers per plant. Flower diameter (mm) and flower morph (classified as approach herkogamous [pin], reverse herkogamous [thrum], or homostylous) were recorded. Pin flowers are classified as having styles which are longer than the stamens, while thrum flowers are the reverse. Homostylous flowers are those with styles and stamens of equal length. These data were used to estimate whether a population was monomorphic for one flower type or distylous (possessing both pin and thrum). Flowers were also assigned ratings for petal overlap (1 = >50% overlap, 2 = <50% overlap) and flower shape (tube, funnel, or bowl) (Diederichsen and Richards, 2003). Tube-shaped flowers have a narrow corolla, often with overlapping petals, resulting in a small flower diameter. Funnel flowers are intermediate. Bowl flowers are those in which the corolla appears to open fully to almost at a 90° angle to the pedicel, resulting in the largest flower diameter possible (Diederichsen and Richards, 2003). For ornamental purposes, a bowl-shaped flower is desired to maximize flower diameter and showiness. Stem traits were recorded for three stems per plant and included the overall stem length (cm) measured from the crown to the stem apex, as well as the stem diameter (mm; recorded 30 cm from the apex of the stem).

Postharvest traits

Plants with mature seed pods were harvested on a per plant basis in wk 43, 44, and 45. Plants were clipped at the base using pruners, then placed into labeled harvest bags for transport back to campus where they were dried for 5 d at 32.2 °C and moved into storage. Before each sample was cleaned, the capsule diameter (mm) and number of seeds per capsule were recorded for five randomly selected mature capsules. Samples were cleaned using a belt thresher [fabricated at the UMN], followed by a fractionating aspirator [CFZ1 and CFZ2 Fractionating Aspirator Test Models, Carter Day International Inc., Minneapolis, MN]. If additional cleaning was required, sieves of various sizes were used before picking out the remaining chaff by hand. Once cleaned, the total weight of the sample was recorded to measure total seed yield (g). A seed counter [DATA Count S-JR, DATA Detection Technologies Ltd., Jerusalem, Israel] was used to subsample 1000 seeds and the 1000 seed wt. (g) was recorded. If a sample has < 1000 seeds total, the total number was recorded and used to calculate an estimated 1000 seed weight using the equation:

$$1000\ seed\ wt.estimate\ (g) = \left(\frac{Yield\ (g)}{Total\ \#\ seeds}\right) \times 1000$$

Statistical analysis

Initially, separate two-way ANOVAs were used to compare 1000 seed weight methodologies (actual vs estimate) and whether there were population x methodology or genotype x methodology interactions. Once the validity of the 1000 seed weight estimate was confirmed, the effect of population and genotype factors on weeks in flower,

width 1, width 2, height, semi-ellipsoid volume, base area, circumference, height to width ratio, eccentricity, flower diameter, stem length, stem diameter, yield, 1000 seed weight (estimate), number of seeds per capsule, and capsule diameter were analyzed using independent, one-way analysis of variance (ANOVA) and mean separations (5% Tukey's Honestly Significant Difference, HSD) using the Statistical Package for Social Sciences (SPSS, v. 25 for Windows, SPSS, Inc., Chicago, IL). For population comparison analysis, phenotypic data were pooled by population. There was large variability in the sample size of each population as constrained by seed availability. To maintain statistical power, any species with $n < 10$ observations was dropped from the analysis. For analysis of genotypic differences, the chosen cutoff was $n = 3$ observations per genotype, which captured the majority of genotypes tested. Pearson correlations and descriptive statistics were calculated using SPSS to compare all traits analyzed by ANOVA. Flower morph was analyzed using a 1:1 chi-square (χ^2) test, as well as observations made in the present study. Petal overlap was analyzed using a 1:1 χ^2. Flower shape was analyzed using a 1:1:1 χ^2.

Covid-19 impacts and traits dropped from analysis

Due to Covid-19 restrictions, the same phenotyping was not conducted in year two of growth (Y2; 2020). Seed samples were harvested, but are still being processed, therefore year two yield data are currently unavailable. In lieu of this, a second experiment is presented which includes 2018-2019 yield data from the restart nursery

planted in spring 2018. These data are intended to provide an example of the yield differences which may be observed between years 1-2 of growth, to provide expectations for the Y2 forage hill yield data.

In addition to the traits mentioned above, several other traits were recorded which were either too subjective or not informative. Flower color was assigned according to the Royal Horticultural Society (RHS) color chart (Royal Horticultural Society, 2015). However, this rating was highly dependent on lighting conditions and individual differences in color perception and was therefore not included in the main results. Additionally, converting the RHS colors to a digital format is challenging, as no official conversion is published by the RHS, and different unofficial sources for the RHS to hexRGB conversion yield obvious differences in color intensity/saturation (Figure A 6; Figure A 7). Additional postharvest (oilseed) traits included a capsule dehiscence rating (1-3; fully dehiscent to non-shattering; Figure A 8a) and the number of immature seeds per capsule (partially developed or aborted seeds; Figure A 8b). Additional cut flower traits included the length to the first branch > 5 cm (Figure A 9a) and the proportion of stem unbranched (Figure A 9b). Additional plant size/shape traits included a rating of stature (1-3; prostrate to upright; Figure A 10a) and the average width (cm; Figure A 10b). ANOVA results for all additional quantitative traits are presented in Table A 1. Finally, the presence of horizontal laterals (y/n) was also recorded for every plant in the nursery and used to calculate the percentage of plants exhibiting this trait in a given population (Table A 2).

Experiment 2

Plant material

Initial evaluations of perennial flax began in 2005 and consisted of a randomized common garden containing *Linum altaicum, L. austriacum, L. baicalense, L. bienne, L. campanulatum, L. flavum, L. hirsutum, L. lewisii, L. perenne, L. sulcatum, L. tauricum, L. tenuifolium,* and *L. thracicum*. However, L. *altaicum, L. campanulatum, L sulcatum, L. tauricum, L. tenuifolium,* and *L. thracium* were not vigorous and it was noted that many of these plants did not reach reproductive maturity (K. Betts, personal communication, 2021). The open pollinated seed of the highest yielding plants (2005) were grown in 2006-7 to establish a second generation, i.e. the Broad Based 1 "BB1" population (Table 2-2). This procedure was repeated in 2008 to generate "BB2" as well as two additional populations, "KJ1" and "KJ2," which were selected for 'tuft' (upright, high branching) and 'bush' (spherical, low branching) habits, respectively (Table 2-2). Since these populations were open pollinated, the specific ancestry of the current populations is unknown.

Remnant seed from initial pilot evaluations of perennial flax (2005-2008) was planted at the Rosemount Research and Outreach Center, Rosemount, MN (44°42'58.2" N, -93°5'54.9" W) in spring 2017 (week 17; 24 April 2017) to restart the perennial flax breeding program. A single-row cone seeder was used to plant 2 x 5' row plots with 1.5 g seed/plot. (~8.5 lb/a) with 20" between rows, replicated 1-3x depending on the availability of seed and arranged using a completely randomized design. The plots were

fertilized with urea (50 lb/a actual N), and chemical weed control was provided by pendimethalin (2 pt/a rate) [Prowl®, BASF Corporation, Ludwigshafen, DE].

The most vigorous plants from each population in the restart nursery were harvested and planted in an adjacent field in week 41 (13 Oct 2017) to establish an "elite restart" nursery using the same planting design, fertilizer rates, and weed control as the original restart nursery (K. Betts, personal communication, 2021). In total, this population consisted of fifty-three genotypes (seed lots) from four breeding populations, plus two check genotypes (Table 2-2). In week 39 (2018), the two most vigorous plants per plot were flagged out of this population for harvest and freezing study tests.

Harvest

In 2018, plants from the elite restart nursery were harvested in wk 42. In 2019, plants matured much earlier and were harvested in wk 33. Plants were clipped at the base using pruners, then placed into labeled harvest bags. These were then dried for 5 days at 32.2 °C and moved into storage.

Traits measured

Seed cleaning followed the same procedure as in Experiment 1. In both years, the number of seeds per capsule, yield (g), and 1000 seed weight (g) were measured. Capsule diameter (mm) was also recorded in 2019. In 2018, the number of seeds per capsule was determined from the average of ten capsules. In 2019, the number of seeds per capsule

and capsule diameter (mm) were instead recorded for five capsules per sample, due to the large number of samples needing to be processed that year.

Statistical analysis

The effect of year, population and genotype factors on yield, 1000 seed weight, number of seeds per capsule, and capsule diameter were analyzed using independent one-way ANOVA; mean separations (5% Tukey's Honestly Significant Difference, HSD) using the Statistical Package for Social Sciences (SPSS, v. 25 for Windows, SPSS, Inc., Chicago, IL). For population comparison analysis, genotype data were pooled together for each population. To maintain statistical power, any genotype with $n < 2$ observations was dropped from the analysis. Pearson correlations and descriptive statistics were calculated using SPSS to compare all traits analyzed by ANOVA. Independent Welch's *t*-tests ($\alpha \leq .05$; two tailed) were also performed using excel to determine if there were statistically significant differences in mean yield and 1000 seed weight between Experiment 1 and Experiment 2. The CF and OS populations did not differ significantly from each other for yield and 1000 seed weight in Experiment 1 and were therefore pooled for this analysis.

Results and Discussion

Experiment 1

There were no significant differences between actual vs estimated 1000 seed weight values, nor any interaction between methodology and population or genotype (Table 2-3). The lack of significance between the two calculation methods or their interactions indicates that the 1000 seed wt. estimate is accurate for samples with < 1000 total seeds. Thus, the 1000 seed weight data in Table 2-4 includes estimated 1000 seed weight values, which greatly increased the sample size since 71.2% (659/926) of the genotypes yielded < 1000 seeds total. The main effects of population and genotype were very highly significant ($p \leq .001$; Table 2-4) for all traits evaluated. Thus, there are unique species and selection differences among populations.

Oilseed potential of wild and selected flax populations

Traits relevant to oilseed potential include yield (g), seed size (1000 seed weight; g), shattering measured as the number of seeds per capsule, and capsule diameter (mm). Since main effects are significant for all oilseed traits (Table 2-4), means separations show differences between the populations tested. Oilseed selections exhibited the highest mean per plant yield of any population, i.e. significantly greater than *L. altaicum*, *L. baicalense*, and *L. bienne*, although not differing significantly from any of the other populations (Figure 2-1a). Of the wild species tested, *L. austriacum* had the greatest yield. The CF selections population had the third highest yield, on average, even though this trait was not selected. This was most likely related to the improved vigor of the CF selections relative to the species populations (discussed in 'Plant size and shape'). Annual

domesticated flax, *L. usitatissimum*, had notably low yield, most likely due to the atypical growth conditions of a space planted nursery. *Linum usitatissimum* has been adapted over millennia to thrive in densely seeded fields (Fu et al., 2012), so it is not surprising that low per plant yield was observed in a common garden environment. The oilseed components of the perennial flax breeding program will transition to plot-based evaluations over time, which will enable researchers to test the more informative comparison of yield per area for perennial vs annual species.

Within each population tested, a range of values were observed for mean genotypic yield, indicating that there is still high variability within these populations (Figure A 1). The high level of variability present across all genotypes is reflective of the fact that most species tested are obligate outcrossers. Both selection populations had considerable overlap in terms of mean genotypic yield values. For example, the fifth highest yielding genotype overall was derived from the CF selections, while the sixth lowest yielding genotype came from the OS selections. This reinforces the overlapping means observed between the CF and OS selections (Figure 2-1a) and supports the notion that both OS and CF selections should be tested for yield in these early stages of breeding.

In contrast to yield, the seed size (1000 seed weight, g) of the CF and OS selection populations was significantly less than *L. baicalense*, *L. grandiflorum*, *L. lewisii*, and *L. usitatissimum* (Figure 2-1b). Oilseed and CF selections had relatively small seeds, and did not differ significantly from each other, or the wild species *L. altaicum*, *L. austriacum*, *L. bienne*, *L. hirsutum*, *L. pallescens*, and *L. perenne*. Perennial

L. baicalense had significantly larger seeds than any of the aforementioned populations, comparable in size to the large-flowered annual *L. grandiflorum*; both of which were still significantly smaller compared to domesticated *L. usitatissimum*. The smallest seeds were observed for *L. bienne* followed by *L. hirsutum*, both of which were significantly smaller than *L. baicalense*, *L. grandiflorum*, *L. lewisii*, *L. pallescens*, and *L. usitatissimum*. Although *L. usitatissimum* had the largest seeds on both a population and genotype mean basis (Figure A 2), it was less than reported values for the species, which range from 4-13 g per 1000 seeds (Diederichsen and Richards, 2003)

Level of seed shattering was estimated by recording the number of seeds per capsule. All flax species possess a five-chambered capsule, which can produce, at maximum, two seeds per chamber, resulting in an upper limit of ten seeds per capsule (Diederichsen and Richards, 2003). Previous reports for *L. usitatissimum* list the average number of seeds per capsule at around 6 (Oplinger et al., 1997; You et al., 2017). This is considerably higher than the number observed in this study for *L. usitatissimum* (Figure 2-1c). Like with yield, this is likely related to the atypical growth environment of the common garden nursery, which would have caused increased stress and lowered reproductive capacity of *L. usitatissimum*.

The wild species with the least amount of shattering was *L. baicalense*, which had a significantly greater number of seeds per capsule when compared with all populations except for *L. usitatissimum*. The next highest number of seeds per capsule was observed for the wild species *L. pallescens*, although this was not significantly different from any of the populations with lower values. Both the CF and OS selections had a proclivity for

shattering, which was surprising given their observed seed yield (Figure 2-1a,c). The only wild species with slightly more shattering than the CF and OS populations was *L. bienne*.

As with yield and 1000 seed weight, results on a genotype mean basis shows a high amount of variability within each population for shattering and there are exceptions to the population-level generalizations (Figure A 3). For example, genotype 'PI 231886' had the third greatest number of seeds per capsule observed, though it belonged to *L. bienne*, which had the lowest overall average. Additionally, in populations with a low number of replications, any generalizations based on population mean values would be strengthened by replicating the present study across years. A prime example of this is *L. baicalense*, which had a total of only n = 12 plants tested from across two genotypes, due to lack of available seed and poor summer survival. While the low level of shattering observed in *L. baicalense* makes it a promising candidate for interspecific crosses, the genotypes tested likely represent only a small fraction of total variation existing among wild populations.

Similar capsule diameters were observed for *L. baicalense* and *L. grandiflorum* and these were both significantly larger compared to all other populations (Figure 2-1d). Likewise, similar capsule diameters were observed for *L. usitatissimum* and *L. pallescens*, which both possessed significantly larger capsules compared to *L. altaicum*, *L. bienne*, *L. hirsutum*, and the CF and OS selections. The CF and OS selections mean capsule diameter was nearly identical, and small relative to other populations, exceeding only *L. hirsutum*, which had the smallest capsules observed in the study.

Cut flower potential of wild and selected flax populations

Traits relevant to cut flower potential include flower diameter (mm), weeks in flower, stem length (cm), and stem diameter (mm) (Figure 2-2). Since main effects are significant for all cut flower traits, means separations show differences between the wild species, as well as the OS and CF selections. Flower diameter differed greatly among populations. The largest flowers, on average, were observed for the species *L. lewisii*, and were significantly larger than all other populations besides *L. grandiflorum* and *L. austriacum* (Figure 2-2a). The flowers of *L. bienne* and *L. usitatissimum* were comparable in size and significantly smaller than any of the other populations. Given their shared evolutionary history (McDill et al., 2009; Wang et al., 2012), flower size may not have undergone strong selective pressure over time as *L. bienne* gave rise to domesticated *L. usitatissimum*. Among breeding populations, intentional selection for larger flower diameter had little impact, as the CF selections mean flower diameter was only slightly greater than that of the OS selections. Neither selection population had significantly different flower diameters compared to *L. perenne, L. austriacum, L. altaicum,* and *L. hirsutum,* and both selections had significantly larger flowers compared to *L. baicalense, L. bienne, L. pallescens*, and *L. usitatissimum*. On a genotype mean basis, a large range of flower diameters is observed for both selection populations, ranging from ~20-30 mm (Figure A 4). The CF selections alone exhibit a range of genotype mean values which encompasses all genotypes within populations of *L. altaicum, L. austriacum, L. grandiflorum, L. hirsutum, L. lewisii, L. narbonense,* and *L.*

perenne. Such wide-ranging variation highlights the opportunities for selection within this population, as well as the challenge of achieving a consistent response to selection within a highly outcrossing species.

A significantly greater number of weeks in flower was observed for the CF and OS selections compared to all wild species besides *L. grandiflorum* and *L. austriacum* (Figure 2-2b). The shortest flowering periods were observed for *L. altaicum*, *L. baicalense*, and *L. lewisii*, all of which were significantly less than the other populations. The remaining populations were intermediate to these extremes. As with flower diameter, *L. usitatissimum* and *L. bienne* exhibited similar phenotypes for the number of weeks in flower. Across all genotypes tested, a wide range of flowering periods was observed on a genotype mean basis, ranging from 0-12.8 wk (Figure A 5). Viewing genotype mean differences also revealed a few key trends regarding the level of variation present within populations. Most notably, with the exception of two OS genotypes, all of the CF and OS selections exceeded the grand mean of 6.9 wk in flower. This contrasts with *L. austriacum*, *L. perenne*, and *L. bienne*, which had genotypes in the 0-3 range, but also genotypes with > 10 wk in flower, on average. A distinct cluster of *L. lewisii* is observed on the low end of the range, and all but two *L. lewisii* genotypes were below the grand mean (Figure A 5).

The greatest mean stem length was observed for *L. pallescens*; this was significantly greater than all other populations besides *L. lewisii* and the CF and OS selections (Figure 2-2c). Overall, less variation was observed for stem length relative to other traits, with many populations exhibiting overlapping means separations such as *L.*

altaicum, L. austriacum, L. grandiflorum, L. hirsutum, L. lewisii, L. perenne, L. usitatissimum, and OS selections. Visual selection for improved stem length was evident for the CF selections, which had significantly longer stems compared to *L. baicalense, L. bienne, L. grandiflorum, L. hirsutum,* and *L. usitatissimum.*

Stem diameter (mm) is a complex trait affecting cut flower, oilseed, and fiber flax. Large diameter stems are stronger, largely due to their greater cross-sectional area, but large diameter stems have also been found to produce lower quality fibers with reduced tensile strength (Alcock et al., 2018). Alcock et al. (2018) demonstrated that stems with smaller diameters must compensate for unfavorable geometry (less cross-sectional area) by producing stronger fibers which keep the stem aloft. The implications for the present study are many. For cut flower flax, Alcock et al. (2018) supports our previous hypothesis that larger stem diameters would be desired for cut flower flax, as the stronger stems would hold up better for use in floral designs (Tork et al., 2019). However, for oilseed objectives, Alcock et al. (2018) may contradict the hypothesis that thinner, weaker stems would increase ease of mechanical harvest. If thinner stems indeed possess fibers of greater tensile strength, then these may put more strain on harvesting equipment. However, further study is required to confirm that the findings in *L. usitatissimum* apply to perennial species of flax. Bormaud et al. (2016) contradicted the findings of Alcock et al. (2018), although the objectives of their study, the genotypes tested, and the method of recording stem diameter all differed. This further illustrates that future work is necessary to characterize relationships between traits such as stem diameter, fiber strength, stem length, harvestability, and lodging. Planting density and

timing of harvest can also influence fiber quality, with denser planting resulting in reduced branching and longer, higher quality fibers. Harvesting early while the stem is still green can produce a more fine, light linen fabric, while late harvest tends to yield coarse, strong fabric, as well as seed used to re-plant the crop (Vaisey-Genser and Morris, 2003). With so many factors influencing stem diameter, a more detailed method of study may be warranted in the future, especially if interest grows for a perennial flax fiber breeding program.

Several species in the present study stand out for their increased stem diameters relative to other populations. The largest stem diameters, on average, were observed for *L. usitatissimum* and were significantly greater than all other populations besides *L. baicalense* and *L. grandiflorum* (Figure 2-2d). These latter two populations were observed to have larger diameters compared to all except *L. bienne*. While the species *L. pallescens* had notably thin stems, it did not differ significantly from the majority of populations tested. Both selection populations were very similar in terms of diameter, although OS selections were observed to have slightly thicker stems.

Plant size and shape

The CF selections population had a width 1 (cm) which was significantly greater than all populations besides the OS selections, which exhibited similar, though slightly smaller, width 1 values (Figure 2-3a). The OS selections were also significantly greater than *L. altaicum*, *L. baicalense*, *L. bienne*, *L. hirsutum*, *L. lewisii*, and *L. usitatissimum*

for mean width 1. The greatest width 1 observed among wild species was in *L. perenne* and *L. austriacum*, which had comparable mean values that were both significantly greater than *L. baicalense*, *L. hirsutum*, and *L. usitatissimum*. The smallest width 1 belonged to *L. baicalense* (Figure 2-3a).

The CF selections were also the largest by measurement of width 2 (cm) and were significantly greater than all populations besides the OS selections, which were not much smaller in size (Figure 2-3b). *Linum baicalense* was observed to have a significantly smaller width 2 than most species, excluding only *L. hirsutum* and *L. usitatissimum*. Among the wild species, *L. perenne* had the greatest width 2 measurements, on average, being significantly greater than *L. baicalense*, *L. hirsutum*, *L. lewisii*, and *L. usitatissimum*.

The greatest average height was observed in the CF selections population, followed by the OS selections (Figure 2-3c). Both *L. usitatissimum* and the CF and OS selections were significantly taller than *L. baicalense*, *L. bienne*, *L. hirsutum*, and *L. lewisii*. The remaining species *L. altaicum*, *L. austriacum*, *L. grandiflorum*, *L. pallescens*, and *L. perenne* were all similar in height.

The integration of all three size measurements (width 1, width 2, height) into the calculation of semi-ellipsoid volume (cm^3) illustrates the large differences in the size of the populations tested (Figure 2-3d). By this measurement, the CF selections are more than double the overall size of all species populations, besides *L. austriacum* and *L. perenne*, and they are significantly larger in volume than all populations besides the OS

selections (Figure 2-3d). The OS selections are similarly large, at nearly twice the average size of *L. grandiflorum*; significantly larger than all populations besides *L. austriacum* and *L. perenne*. These results highlight the significant impact that even 1-3 generations of selection can have on vigor and adaptation to a new environment.

The small plant volume of *L. lewisii* is surprising (Fig. 3d), given its close phylogenetic relationship with *L. perenne* and *L. austriacum* (McDill et al., 2009; Pendleton et al., 2008). One possibility is that the climate and soil type of Minnesota is unfavorable for this species, as Minnesota lies on the eastern border of its native range (Ogle et al., 2006). *Linum lewisii* is often found in alpine regions, although it is also known to be a native inhabitant of prairie grasslands. The wide range of biomes included in its native range suggests that adapting *L. lewisii* to MN for the purposes of developing a native perennial crop may still be feasible, if a collection can be located from a region similar to the target environment.

A larger plant volume than was observed for *L. grandiflorum* was expected given its annual life cycle and reported ornamental value. While it did flower profusely throughout the season (Figure 2-2b,Figure A 5), its volume did not differ significantly compared to most of the other species studied. This could indicate that the genotypes tested were poorly adapted to the local environment.

For evaluating ornamental value, it is also informative to consider the base area and circumference, which exclude plant height from the calculation (Figure 2-4a,b). In general, the same relative pattern as semi-ellipsoid volume is observed except that *L.*

lewisii and *L. bienne* appear larger, suggesting that their size comes more from their width than their height. As with volume, the largest base area and circumference belongs to the CF selections population, which is significantly larger than all other populations except the OS selections. It is also notable that the visual differences between populations appear greatest for semi-ellipsoid volume compared to base area and circumference, yet the circumference shows a greater number of significant mean separations between populations. For example, *L. baicalense* has a significantly smaller circumference compared to *L. altaicum*, *L. austriacum*, *L. bienne*, *L. grandiflorum*, *L. pallescens*, *L. perenne*, CF selections, and OS selections. A similar outcome occurs in *L. usitatissimum*, which has a significantly smaller circumference and base area relative to *L. austriacum*, *L. grandiflorum*, *L. perenne*, CF selections, but a semi-ellipsoid volume which only differs significantly from the CF and OS selection populations.

Ultimately, the choice of size measurement must relate to the breeding and selection goals. If the goal is to select for the largest or most compact plants overall, then semi-ellipsoid volume is superior, since it integrates all three size measurements. If the goal is a wide or narrow base, irrespective of height, then base area or circumference are the measurement(s) of choice. Base area may be a superior calculation criterion, as it integrates both width 1 and width 2, rather than using an average width like circumference. However, based on this study, circumference may be more effective at showing statistical differences between populations.

Measurements characterizing plant shape may also prove useful for selecting for ornamental quality and growth vigor. The height to width ratio observed for *L.*

usitatissimum was significantly larger than all other populations in the study, indicating that this species was much taller than it was wide (Figure 2-4c). In contrast, *L. bienne* grew much wider than it was tall, with a height to width ratio that was significantly less than *L. altaicum, L. hirsutum, L. lewisii, L. pallescens*, and *L. usitatissimum*. Anecdotally, *L. bienne* was often observed to have nearly horizontal growth, although a few genotypes did grow into an upright cushion shape. The height to width ratio of CF and OS selections was also low relative to the other populations tested, although these populations did not differ significantly from any others besides *L. usitatissimum*. *Linum perenne* is the wild species with the most similar height to width ratio as the selections. The remaining species had height to width ratios in the range of 0.6-0.8. The optimal height to width ratio depends largely on the selection objectives. For a compact bedding plant, a uniform cushion shape is desired, so the ideal height to width ratio would approximate 0.8-1.2. For a larger, bush type habit that appears more like a small shrub, a wider base may be acceptable to cover a larger footprint, therefore target values might instead range from 0.5-0.7, similar to the observed values for the CF and OS selections. In contrast, CF or fiber types would ideally be much taller than they are wide, so values ranging from 1.0-2.0 would be ideal. The only category where height to width ratio goals remain ambiguous is for oilseed selections, for these depend heavily on the production setting and harvesting mechanics, namely row spacing and seeding rate. At a high seeding rate and tight row spacing, tall, narrow plants would be desired to avoid overcrowding, resulting in greater height to width ratios. However, if wider row spacing and/or lower seeding rates are desired, so that individual plants may reach a greater final

size, a lower height to width ratio may be preferable so that plants grow wide enough to fill gaps between rows and shade out weeds, especially in the first year of growth. In domesticated flax, seeding tall cultivars at a high seeding rate showed greater competitive ability compared to short cultivars and low seeding rates (Kurtenbach et al., 2019). Whether this trend holds true for perennial flax would be an excellent research question for future agronomic studies.

The goal of testing eccentricity is to determine which population has the most spherical footprint. This calculation is most relevant to ornamental garden uses where compact, 'cushion' shaped plants are generally the goal. It may also be relevant to oilseed breeding, as a uniform growth habit would be more effective at shading weeds when plants are grown in tightly spaced rows. Eccentricity values approaching zero indicate a nearly perfect sphere, while values approaching one indicate an increasingly elliptic shape. The most spherical, on average, out of all the populations were the CF selections, which had significantly lower eccentricity values compared to *L. baicalense*, *L. hirsutum*, *L. lewisii*, *L. pallescens*, and *L. usitatissimum* (Figure 2-4d). The same occurs for the OS selections, except that this population did not differ significantly from *L. hirsutum*. Overall, in addition to being larger in size, both selection populations exhibited a more uniform and cushion shaped growth habit which would be desirable for the bedding plant industry. Of all the wild species, *L. perenne* exhibited the most uniform growth habit with a mean eccentricity value of 0.5, which was significantly less than *L. baicalense*, *L. lewisii*, and *L. pallescens*. Of these latter three species, *L. baicalense* was the most elliptic, due to an average width 1 that was nearly double the average of its width 2.

Trait correlations

The number of weeks in flower had highly significant positive correlations with several traits, including yield, height, width 1, width 2, semi-ellipsoid volume, circumference, and base area (Table 2-5). Of these, the highest correlation coefficient observed was for width 2 (r = .432). Additionally, there was a significant ($p \leq .05$) correlation with flower diameter. Weeks in flower showed highly significant negative correlations with 1000 seed weight and eccentricity; significant ($p \leq .01$) negative correlation with number of seeds per capsule and height to width ratio; significant ($p \leq .05$) negative correlation with stem diameter.

Yield showed highly significant positive correlations with capsule diameter, number of seeds per capsule, height, width 1, width 2, semi-ellipsoid volume, circumference, and base area (Table 2-5). Of these, the highest correlation coefficient was for circumference (r = .229). A possible explanation for the high correlations observed between size measurements, yield, and weeks in flower is that more vigorous plants enabled more resources to be devoted to flowering, thus creating greater chance of reproductive success, leading to higher seed yield.

One thousand seed weight showed highly significant positive correlations with capsule diameter, number of seeds per capsule, height to width ratio, and stem diameter; as well as a significant ($p \leq .01$) positive correlation with eccentricity (Table 2-5). Of these, the highest correlation coefficient observed was for capsule diameter (r = .383),

which makes sense since a larger capsule would be needed to accommodate a larger sized seed. This trend is clearly illustrated by the CF and OS selections populations, which were observed to have both small seeds and small capsule diameters, on average. Correlation between capsule width and seed size has been previously reported for *L. usitatissimum* (Diederichsen and Richards, 2003) and this trend is consistent across multiple *Linum* species. There were also several negative correlations with 1000 seed weight, for which width 2 was highly significant, circumference and base area were significant ($p \leq .01$), and width 1 was significant ($p \leq .05$). This relationship between seed size and width may be related to the fact that both selection populations had relatively small seeds despite their large plant size.

Capsule diameter also displayed a highly significant positive correlation ($p \leq .001$) with number of seeds per capsule, and a significant ($p \leq .01$) positive correlation with stem diameter (Table 2-5). The correlations between number of seeds per capsule, capsule diameter, and 1000 seed weight can likely be explained by the fact that the species with the least shattering, *L. usitatissimum* and *L. baicalense,* also had some of the largest seeds and, therefore, larger than average capsule diameters. The significant correlations between capsule diameter, 1000 seed weight, and stem diameter (Table 2-5) may suggest that stems must be thicker 30 cm from the apex to support larger reproductive structures, although this association is speculative and would require future testing to make any definite conclusion.

Not surprisingly, height showed a highly significant positive correlation with other size measurements, including width 1, width 2, height to width ratio, semi-ellipsoid

volume, circumference, and base area (Table 2-5). Of these, the highest correlation coefficient was observed for semi-ellipsoid volume (r = .692). A highly significant positive correlation was also observed between height and stem length (r = .506). This correlation coefficient would be expected to increase as progress is made in selecting for upright growth habit. A significant ($p \leq .01$) positive correlation between flower diameter and height was observed, as well as a highly significant negative correlation between height and eccentricity.

Similar to height, both width 1 and width 2 had highly significant positive correlations with all other plant size measurements, with the exception of height to width ratio and eccentricity, both of which displayed highly significant negative correlations (Table 2-5). The highest correlation coefficients observed for both widths 1 and 2 were for circumference (r = .969 and r = .968, respectively). This is not surprising, as the circumference measurement was calculated using the average of widths 1 and 2. Additionally, there were highly significant positive correlations between widths 1 and 2, stem length, and flower diameter; as well as negative correlations between widths 1 and 2 and stem diameter.

Height to width ratio had highly significant negative correlations with circumference and base area (Table 2-5), indicating that, as height increased, width also tended to decrease. There was also a significant ($p \leq .05$) negative correlation with semi-ellipsoid volume. A significant ($p \leq .01$) positive correlation was observed between height to width ratio and eccentricity, as well as a significant ($p \leq .05$) positive correlation with stem length.

There was a highly significant negative correlation observed between semi-ellipsoid volume and eccentricity (Table 2-5), possibly due to larger selection populations, which also had the lowest eccentricity values. Semi-ellipsoid volume also displayed highly significant positive correlations with base area, circumference, and stem length, as well as a significant ($p \leq .05$) positive correlation with flower diameter (Table 2-5). A significant ($p \leq .01$) negative correlation between semi-ellipsoid volume and stem diameter was observed.

Highly significant negative correlations were found between eccentricity, base area, and circumference (Table 2-5), potentially for the same reasons as correlation of eccentricity and semi-ellipsoid volume. Interestingly, there was also a significant ($p \leq .05$) positive correlation between stem diameter and eccentricity, indicating that more elliptic plants tended to have thicker stems.

Circumference had a highly positive correlation with base area ($r = .970$; Table 2-5), indicating that these are largely duplicative metrics for plant size irrespective of height. Both circumference and base area also showed highly significant positive correlations with stem length and flower diameter, and highly significant negative correlations with stem diameter. There was also a significant ($p \leq .01$) negative correlation between stem diameter and flower diameter observed. Finally, stem length had a significant ($p \leq .05$) negative correlation with stem diameter, and a highly significant positive correlation with flower diameter. As cut flower selection progresses, an even higher correlation between these traits should be expected. The degree of positive

correlation between stem length and flower diameter could potentially be a good method of measuring progress towards the cut flower ideotype (Tork et al., 2019).

Germination and plant survival

Seed germination rates varied greatly among the populations tested (Table 2-6). The lowest germination rate was observed for the CF selections (46.5%), of which most seeds germinated in G3 (wk 3; [Anderson, 2019; Anderson et al., 2021]). Among OS selections only 50.7% of seeds germinated, on average. In contrast, the highest germination rate of 78.8% was observed for *L. altaicum*, which also had the majority germinate in G3, but was unique among the perennial species in that some germination was observed in G2. The fastest germinating species was, to no surprise, domesticated *L. usitatissimum*, which had nearly as many seeds germinating in G1 as in G3. This reinforces the lack of dormancy in *L. usitatissimum* 25-30 d after harvest (Dandoti et al., 2017; Dexter et al., 2011). Additionally, it was surprising that the wild progenitor of domesticated flax, *L. bienne*, had a higher percent germination than *L. usitatissimum*. It also had the greatest percent germination during G1-G2 of any wild species (Table 2-6). One possible explanation for the germination rates of *L. usitatissimum* observed was that temperature, moisture, and light conditions were not optimal for the genotypes tested. These appear to be at least somewhat genotype-dependent, as conflicting reports exist for the optimal combination of these factors (Kurt, 2010; Kurt and Bozkurt, 2006). For the other annual species tested, *L. grandiflorum* had a small number of seeds germinating in

G1-G2, and the majority of germination in G3 (Table 2-6). Among the other perennials tested, most of the germination happened in G3 following cold-moist stratification, with germination decreasing during G4-G5. The species *L. baicalense* had high percent germination relative to other perennial species, although this was more delayed than average, with nearly identical germination rates in G3 and G4 (Table 2-6). The wild perennials *L. lewisii*, *L. pallescens*, and *L. perenne* had germination rates intermediate to the CF and OS selections. The perennials *L. altaicum*, *L. austriacum*, *L. baicalense*, and *L. hirsutum* germinated at higher rates than the OS selections (Table 2-6).

The highest percent summer survival was observed for the OS selections (93.2%; Table 2-6), which confirms that these selections are already becoming locally adapted. The next highest percent summer survival was observed for wild *L. perenne*, followed by the CF selections. *Linum austriacum* also had 81.5% summer survival (Table 2-6). Likewise, *L. pallescens* and *L. altaicum* showed potential for local adaptation, with 72.3% and 70.7% survival, respectively. The lowest percent summer survival was observed for *L. usitatissimum*, at 24.4% (Table 2-6). This was not due to senescence, as plants were considered to have survived the summer once seed set had occurred. The most likely cause for this high rate of mortality was transplant shock. *Linum baicalense* and *L. grandiflorum* also showed relatively poor summer survival, suggesting that significant effort would be required to adapt these species to this environment. However, it would be interesting to study whether the same outcome was observed through direct seeding. For *L. bienne*, *L. hirsutum*, and *L. lewisii* summer survival was between 50-60%

(Table 2-6), indicating that these are poorly adapted, but may be improved through several cycles of breeding.

Winter survival also varied greatly among the populations tested (Table 2-6). The greatest percent winter survival was observed for the OS selections (97.4%), which confirms that these genotypes are Zone 4 (Z4) hardy and well adapted to MN winters. *Linum perenne* exhibited similar winter hardiness (94.3%), followed by CF selections at 91.5% (Table 2-6). The species *L. altaicum*, *L. austriacum*, and *L. hirsutum* all had winter survival > 70%, which suggests that they have the potential to be fully Z4 hardy following additional selection. The low winter hardiness observed for *L. lewisii* was surprising given its wide distribution over alpine and plains regions, which stretches as far north as Alaska and Canada (Ogle et al., 2006). This result may be due to the relatively poor vigor of the genotypes tested, as evidenced by their small semi-ellipsoid volume (Figure 2-3d) and poor summer survival (Table 2-6). Likewise, year to year differences in snow cover could have impacted the survival of the species. Of the species native to the steppes of Asia and Siberia (Diederichsen, 2007), *L. pallescens* performed better than *L. baicalense*; the latter of which exhibited only 6.9% survival (Table 2-6), indicating that *L. baicalense* is only marginally hardy to USDA Z4. Despite its specific epithet, *L. bienne* behaved more like an annual in Z4, flowering and setting seed in the first year of growth and exhibiting only 0.5% winter survival (Table 6). As expected, the annual species *L. grandiflorum* and *L. usitatissimum* had 0% winter survival (Table 2-6).

Style morph

The observed style morph for most species matched previous reports, as applicable (Table 2-7). This was the case for *L. austriacum*, *L. bienne*, *L. hirsutum*, *L. pallescens*, *L. perenne*, and *L. usitatissimum*, as confirmed by observations and χ^2 tests. Mismatches in style morph occurred for *L. grandiflorum* and *L. lewisii*. The style morph for *L. grandiflorum* had been identified as polymorphic distylous (Table 2-7). Only homostylous flowers were observed in the present study, but homostylous, pin, and thrum flowers have been detected (Figure 2-5)**.** To the best of our knowledge, this is the first known report of a homostylous *L. grandiflorum.* Further study into the cross-compatibility of the three flower morphs is needed using precise measurement of style and stamen lengths (Pendleton et al., 2008; Ruiz-Martín et al., 2018).

The second inconsistency observed for style morph was in *L. lewisii*. Multiple reports have identified that *L. lewisii* is monomorphic approach herkogamous, possessing pin flowers (Table 2-7). In dichotomous keys, style morph is one of the main distinguishing features between *L. perenne* and *L. lewisii*, which are otherwise almost identical in appearance (Pendleton et al., 2008). For this reason, it was surprising to find that 14/46 observations in *L. lewisii* were 'thrum' flowers. This either means previous misidentification and/or questions the purity of *L. lewisii* germplasm examined herein which will need to be addressed in future studies. In the future, every genotype in the nursery should be phenotyped for stylar condition upon first flowering to provide additional confirmation of species identity.

Linum altaicum and *L. baicalense* have not been previously studied for style morphs. No strong conclusions could be drawn about either of these due to the low number of observations.

While the species background of the CF and OS selections is unknown, these populations appear to be polymorphic distylous (Table 2-7). Data did not deviate from a 1:1 χ^2, and no homostylous flowers were observed.

Petal overlap

Petal overlap ("gappiness") did not differ significantly from a 1:1 χ^2 for *L. altaicum* , *L. grandiflorum*, *L. pallescens*, and OS selections (Table 2-8), possibly due to low sample sizes. In cases where petal overlap differed significantly from the 1:1 ratio, only *L. bienne* had a majority of flowers with < 50% overlap. For populations with a majority of flowers with > 50% overlap, the two highest test statistics were observed for CF selections and *L. hirsutum*. For *L. austriacum* and *L. perenne*, even though the majority of flowers had > 50% overlap, both phenotypes were observed, and *L. perenne* was just under the threshold of significance. Most importantly, the improved ornamental quality of the CF selections relative to the OS selections is demonstrated.

Flower shape

Bowl shaped flowers were the most frequently observed flower shape and all populations differed significantly from the 1:1:1 χ^2 except for *L. baicalense* and *L. bienne*

(Table 2-9). *Linum bienne* is the only species tested with an equal distribution of flower shapes. Overall, tube-shaped flowers were the rarest shape, which can generally be considered a positive finding, as such a tightly bound corolla would have little ornamental value. Tube shaped flowers occurred only in *L. austriacum, L. bienne*, *L. usitatissimum*, and CF selections. Bowl shaped flowers, the most desirable ornamental phenotype, were generally the most common, especially among *L. austriacum* and OS selections. Other populations, including *L. grandiflorum*, *L. perenne*, and CF selections had a relatively large proportion of funnel-shaped flowers.

Comparison with previous research on the ornamental potential of Linum

As noted by Cullis (2011) and Fu (2019), there is a lack of formal research characterizing the ornamental potential of wild flax species. Aside from taxonomic descriptions (Ockendon, 1971), only one other study could be found with a similar objective of comparing trait values across several wild *Linum* species (Poliakova and Lyakh, 2017). This study evaluated five perennial (*L. austriacum*, *L. hirsutum*, *L. narbonense*, *L. perenne*, *L. thracicum*) and six annual (*L. angustifolium*, *L. bienne, L. hispanicum*, *L. crepitans*, *L. grandiflorum*, *L. pubescens*) for a suite of traits including, plant height, number of flowering stems, bush diameter (width), leaf area, flower diameter, capsule diameter, 1000 seed weight, and fatty acid composition (Poliakova and Lyakh, 2017). The results obtained by Poliakova and Lyakh (2017) are compared with the present study in cases where there was overlap between the traits and species tested,

namely *L. austriacum*, *L. hirsutum*, *L. perenne*, *L. bienne*, and *L. grandiflorum*. Flower diameters observed herein differed slightly from those reported by (Poliakova and Lyakh, 2017), particularly for *L. grandiflorum* and *L. hirsutum*, but also for *L. austriacum*, *L. perenne*, and, to a lesser extent, *L. bienne*. In general, the flower diameter measurements by Poliakova and Lyakh (2017) exceed those reported in the present study (Figure 2-2a). In general, the height values reported herein were lower (Figure 2-3c) and the width values greater (Figure 2-3a,b), compared to those reported by Poliakova and Lyakh (2017). For capsule diameter, there were some differences between the two studies, but the relative order of the species was generally the same (Figure 2-1d). The 1000 seed weight values reported herein were lower than those measured by Poliakova and Lyakh (2017), with the exception of *L. hirsutum* (Figure 2-1b). An important piece of data presented by Poliakova and Lyakh (2017) not obtained in the present study is oil content of the seeds. For convenience, their results for the species tested herein are presented ranked by highest percent oil content: *L. narbonense* (34.9%), *L. bienne* (33.1%), *L. austriacum* (32.0%), *L. perenne* (26.9%), *L. grandiflorum* (26.5%), and *L. hirsutum* (24.6%) (Poliakova and Lyakh, 2017).

The differences between the two studies might be attributed to the source of germplasm. Most of the germplasm tested by Poliakova and Lyakh (2017) was sourced from N.I. Vavilov Research Institute of Plant Industry (VIR) and All-Russian Research Institute for Flax (VNIIL), with the exception of *L. austriacum* and *L. hirsutum*, which were collected from their native range in the southern steppe of Ukraine. The authors also report carrying out several years of breeding work on *L. grandiflorum* at Zaporozhye

National University to develop varieties with different flower colors and shapes (Lyakh et al., 2018; Poliakova and Lyakh, 2017). The studies also differed by the age of germplasm tested, as the present study evaluated only year one trait values, while the study by Poliakova and Lyakh (2017) reported the average of three years of growth. Altogether, these differences in accession origin, testing environment, and year probably explain the differences in trait values between Poliakova and Lyakh (2017) and the present study. Still, the results raise questions about the range of genetic and phenotypic diversity captured by wild flax collections at VIR, VNIIL, USDA-GRIN, and GRIN-CA. As the UMN breeding program expands, it would be interesting to pursue collaboration with flax researchers in Ukraine and Russia to study the extent to which trait differences are attributable to environmental or genetic variation.

Experiment 2

Although the experimental design and germplasm tested differed from Experiment 1, the results herein demonstrate expectations for oilseed traits in the second year of growth. There was a highly significant effect of year on both yield and 1000 seed weight when data are analyzed on a population and genotype basis (Table 2-10). The factors of population and genotype, nor any of the interactions were significant for yield and 1000 seed weight (Table 2-10).

Yield and 1000 seed weight data by year have large differences between the mean trait values (Table 2-11). This is especially true for yield, where even the minimum value

in Y2 exceeded the Y1 mean yield. In general, Y2 plants were more vigorous, especially in the early season, compared to Y1 plants. Year 2 plants grew much faster, reaching maturity over two months earlier than Y1 plants. It was theorized that two harvests per year might be possible in Y2, but in Experiment 2, the Y2 plants never grew back or flowered after harvest in wk 33 (14 August 2019). However, two harvests per year were achieved in 2020 among some of the genotypes from Experiment 1, with some plants yielding both a midsummer and a fall harvest (D. Tork, unpublished data, 2020). Thus, selection for two harvests per year represents a realistic opportunity for rapidly increasing yield per year in perennial flax, and should, thus, be pursued with great priority in future breeding cycles. It is unknown why this trait did not appear in Experiment 2, but it is likely related to differences in plant spacing, location, or weather. Future production studies should optimize for consistently achieving two harvests per year.

A Welch's *t*-test showed that mean Y1 (2018) yield in Experiment 2 was significantly less ($p \leq .001$) compared to the mean year one (2019) yield of the CF and OS selection populations (pooled) in Experiment 1 (Table 2-11; Figure 2-1a). The range of mean genotypic values for Y1 yield was also much greater in Experiment 1 (Figure A 1), compared to the range of Y1 yield values in Experiment 2 (Table 2-11). One explanation is that the 2018 harvest occurred later than was ideal, possibly causing greater losses to shattering. However, the mean trait values for number of seeds per capsule did not differ significantly from year to year (Table 2-10; Figure 2-6), so there is no quantitative evidence to support this hypothesis. It is more likely that the yield differences observed between Experiments 1 and 2 were due to a combination of

genotypic differences, plant spacing, location, and/or weather. Regardless, these findings highlight the need to repeat the present experiments over years and locations for confirmation. The difference in mean Y1 yield between Experiments 1 and 2 also highlights the need for a study of row spacing and planting density in perennial flax, as per plant yield was much greater in a common garden environment.

As with yield, mean 1000 seed weight was significantly greater in Y2 (Table 2-11; Experiment 2). Despite significant mean differences, the range of seed weights between years was similar (Table 2-11). With such low yields in Y1, it is possible that a greater percentage of immature seed was present when recording seed weight, which could have caused a drop in mean 1000 seed weight values. This is supported by Welch's *t*-test results, which show that the mean Y1 (2018) 1000 seed weight in Experiment 2 (Table 2-11) differed significantly ($p \leq .001$) from the mean Y1 (2019) 1000 seed weight for the pooled CF and OS selections in Experiment 1 (Figure 2-1b); yet, the mean Y2 (2019) 1000 seed weight in Experiment 2 (Table 2-11) was statistically similar ($p = .290$) to the mean Y1 (2019) 1000 seed weight for the CF and OS selections (pooled) in Experiment 1 (Figure 2-1b). These data suggest that the low mean 1000 seed weight observed in Y1 of Experiment 1 may be an outlier, but further study will be needed to clarify the causes and relative frequency of variation in seed size among years. In the future, laboratory germination tests, as well as seed viability tests, could be used to quantify the relationship between seed size and maturity.

A small but significant effect of population on number of seeds per capsule occurred (Table 2-10), although mean separations could not be differentiated.

Additionally, a highly significant genotype and genotype x year interaction was observed (Table 2-10). The grand mean did not change significantly based on year and a wide range of values were observed across genotypes, with some genotypes having > 5 seeds per capsule, on average (Figure 2-6). However, the relative order of genotypes significantly changes between Y1-2 (Table 2-10). This may indicate that the number of seeds per capsule is an unreliable method for estimating shattering that is not consistent across growth years.

Capsule diameter data was only collected in Y2, but a highly significant effect of genotype was observed (Table 2-12). Means separations revealed significant differences between genotypes, despite all observations falling within a narrow range of 4.5-6.5 mm (Figure 2-7). No significant differences between populations were found.

Pearson correlations for most traits in Experiment 2 were quite different compared to Experiment 1 (Table 2-13). The only exception was a significantly positive correlation between capsule diameter and 1000 seed weight (r = .232, Table 2-13; r = .383, Table 2-5). The only other significant positive correlation in Experiment 2 was yield and 1000 seed weight (Table 2-13); this was not found in Experiment 1 (Table 2-5). This correlation between yield and 1000 seed weight was likely related to the significant effect of year on yield and 1000 seed weight. Additionally, all non-significant correlations observed in Experiment 2 (Table 2-13) have significantly positive correlations in Experiment 1 (Table 2-5). This may be attributed to the germplasm tested, as no significant population differences were found in Experiment 2, while large and

significant variation was observed for most traits in Experiment 1, due to the large number of species tested.

Implications for breeding

Oilseed potential of wild species

One of the primary objectives of this study was to determine the most promising wild species for future breeding efforts. Among the wild species tested, *L. austriacum* appears to be an ideal candidate for oilseed selection overall, as it has high mean yield (Figure 2-1a) as well as oil content (Lyakh et al., 2017). For the purposes of trait introgression, *L. baicalense* is the most promising, as it has high seed weight and low shattering compared to the other species (Figure 2-1b,c). However, the small size (Figure 2-3) and short flowering period (Figure 2-2b) of *L. baicalense* suggests that the germplasm would have little value otherwise once traits are introgressed into progeny, if that proves to be possible. Similarly, but to a lesser extent, the same is true for *L. pallescens*, which has relatively large seeds and low shattering (Figure 2-1b,c). Unlike *L. baicalense, L. pallescens* grew to moderate size (Figure 2-3) and had the longest stems of any population tested (Figure 2-2c). Arguably, there is more value to be gained by attempting wide crosses between breeding lines and *L. pallescens*. Finally, although *L. baicalense* had a higher germination rate compared to *L. pallescens,* the latter species had much better summer and winter survival, suggesting that it is better suited to growing conditions in Minnesota. Altogether, future evaluations of wild flax species for oilseed

potential should focus efforts on *L. austriacum* and making wide crosses with *L. pallescens* or *L. baicalense*.

Cut flower potential of wild species

It is much more difficult to determine the best wild species overall for cut flower potential, as no one species was superior for all traits of interest. When considering flower diameter and stem length together, *L. lewisii* is the best overall (Figure 2-2a,c). However, the short flowering period of *L. lewisii* is a significant drawback, as stem yield would be quite low if the plants only flower for 4 wk throughout the summer (Figure 2-2b). Given these constraints, *L. austriacum* is the most promising species overall, as it has large flower and stem diameters (Figure 2-2a,d), the longest flowering time of any wild perennial (Figure 2-2b), and relatively long stem length (Figure 2-2c). As previously discussed, *L. pallescens* has some favorable cut flower traits, but it lacks adequate flower and stem diameters (Figure 2-2a,d). *Linum grandiflorum* also has cut flower potential, as it has large flowers, a long flowering time, acceptable stem length, and thick stems (Figure 2-2a-d).

Garden potential of wild species

This trait will have increasing importance moving forward with the breeding program and it has already received greater focus throughout 2019 and 2020. In advanced trials, the UMN breeding lines have shown greater ornamental potential and more robust

growth in Minnesota compared to existing cultivars (D. Tork, unpublished data, 2018-20). Throughout 2019 and 2020, a majority of the garden selections were identified visually in the field. In the future, selection for garden potential will also be guided by traits such as weeks in flower, plant size, plant shape, and flower diameter. Based on 2019 data for these traits, either *L. austriacum* or *L. perenne* would be considered the top perennial species in terms of garden potential. *Linum austriacum* had the longest flowering time, on average, of all the wild perennials tested, and its volume was second only to *L. perenne*, indicating that it has good first year vigor. However, the height, height to width ratio, and eccentricity of *L. austriacum* indicate that this species is taller and more elliptic in shape compared to *L. perenne* (Figure 2-3c; Figure 2-4c,d), so it may appear wilder in appearance. In contrast, although *L. perenne* had the fourth longest flowering time of any wild species, it grew wider, and in a more uniform shape compared to *L. austriacum*. *Linum perenne* also had improved winter survival compared to *L. austriacum* (Table 2-6). In general, neither species is superior across all traits of interest, so both should be considered in future breeding for ornamental garden varieties, especially if interspecific crosses are viable. Finally, *L. grandiflorum* may provide added value to the breeding program, as it had a long flowering time, unique flower colors, and a size and shape which is comparable with *L. austriacum* and *L. perenne*.

Ideotype modifications for future breeding efforts

Based on these findings, we propose several changes to improve the ideotype models. The most significant change would be to drop subjective traits from evaluation, which would both simplify the models and ensure more consistency across years. Traits proposed in Tork et al. (2019) which were measured in 2019 but dropped from analysis include: rating of plant stature (1-3; Figure A 10a), rating of spontaneous capsule opening (shattering; 1-3; Figure A 8a), and flower color (based on RHS color swatches; Figure A 6, Figure A 7). These were too subjective, based on the number of individuals who participated in recording these measurements, and besides flower color, all of these traits were described by other quantitative data. For example, the height to width ratio could be referenced instead of stature rating and number of seeds per capsule and capsule diameter can be used in place of capsular dehiscence rating for characterizing shattering. In the future, digital imaging could be implemented as a non-subjective means of describing flower color, in addition to floral patterns and UV signals, which may aid understanding of plant-pollinator interactions (Chaki and Dey, 2021; Garcia et al., 2014).

The length to the first side branch measurement was also eliminated from the present study and therefore the variance in technical stem length was not considered as planned (Tork et al., 2019). Within a single stem, many of the individuals possessed dozens or more branch points in a range of sizes and stages of development. One option would have been to measure the lowest reproductive branch, which defines the technical stem length (Diederichsen and Richards, 2003), but this would make the measurement useless for cut flower selection, as many large, non-flowering branches were often observed below this point. Other studies utilizing technical stem length do not define

what constitutes the first branchpoint (Sharma and Faughey, 1999). Therefore, the measurement was changed to define a side branch as any shoot > 5 cm, which is the target length for shoot-tip cuttings. However, there were many cases where a stem had few branches except for a cluster of 5-10 cm shoots near the crown, thus resulting in a very short length to first side branch measurement. Due to the difficulty in deciding a useful and objective method of measurement, this trait was dropped entirely. Stem measurements are very time consuming, so for the time being, length to the first side branch should be dropped from the ideotype model. It would be quicker to simply flag non-branching types visually from within the cut flower selections population.

Stem diameter measurements provided limited utility in the present study, but they should be included in future iterations of the ideotype model, as these data would be useful in selecting for fiber potential, which is a future product of interest for the FGI breeding program. It may be worthwhile to revisit the method of determining stem diameter to find out which method is the most useful for breeding. In the present study, stem diameter was measured with calipers 30 cm below the stem apex, although several different methods of measuring stem diameter are reported in the literature. Average stem diameter was measured by photographing several cross sections per stem using an optical microscope and recording their diameter using ImageJ (Bourmaud et al., 2016, 2015). Undoubtedly, this method provided high accuracy measurements, but it would not be practical for quickly screening a large number of breeding lines. An alternative method used by (Alcock et al., 2018) was to measure the stem diameter with calipers 15 cm above the soil line, and in (Couture et al., 2002) the measurement was recorded 2 cm

above the soil line. Other researchers measured from the midpoint of the stem (Sharma and Faughey, 1999), and in some studies, the method of determining diameter is not defined (Couture et al., 2004; Yang et al., 2013). Based on this review, it appears that no attempt has been made to standardize stem diameter measurements in flax. Therefore, for the purposes of the FGI breeding program, it may be necessary to attempt several methods simultaneously and attempt to determine which method is the most informative based on the resulting data.

The year x genotype interaction (Experiment 2) for the number of seeds per capsule (Figure 2-6) raises questions about the accuracy of this shattering measurement. The level of shattering within a single genotype is not stable from year to year. One possible explanation is that this trait is weather dependent, but this is not supported by the similarity in grand mean values (Figure 2-6). If all lines are harvested on the same day (e.g. Experiment 2), then it is more likely that this measurement is affected by the relative rate of maturity, which could differ between growth years. For example, if the fastest maturing genotype in Y1 becomes the slowest maturing genotype in Y2, then greater shattering would be expected in Y1 relative to Y2, since there would be more time between maturity and harvest in which seeds could shatter. Future experiments would be required to test this hypothesis. In the meantime, the best option may simply be to increase the number of capsules evaluated per plant in order to reduce measurement error. Rather than evaluating the average of 5-10 capsules, this number could be increased to 15, or even 20, at the expense of the speed of postharvest processing.

Other than the trait modifications discussed above, it is recommended that the same suite of ideotype traits be measured in the future as a means of quantifying the effect of selection on target and non-target traits over time. Unfortunately, there were not sufficient resources available to measure seed quality traits, such as oil composition and the presence of antinutritional compounds, but these should be prioritized in the future as resources become available. Finally, the traits chosen for measurement in this study are neither final nor exhaustive. Consistency across years should be the goal, but as breeding goals change, or new information is generated, it may be necessary to add, modify, or remove certain traits from the ideotype models, keeping in mind the impact this will have on the interpretation of year-to-year trends.

Conclusions

At least for the initial 1-3 generations of domestication, simultaneous selection for multiple traits can be achieved. The most important outcome of the early stage domestication is increased vigor through adaptation to the local environment, as evidenced by increases in mean weeks in flower, plant size traits, and percent survival for both selection populations relative to wild species. Meanwhile, selection for oilseed and cut flower traits within the same population produced differential outcomes for traits of interest. The mean yield increase observed for the oilseed selections relative to the cut flower selections is the best example of this process. Both populations had comparable increases for size and weeks in flower, but only the oilseed selections outperformed all

wild species for Y1 seed yield. For cut flower selections, the impact of selection for flower diameter measurements and visual observations of stem length were not as pronounced. In general, there is still considerable similarity among the two selection populations, suggesting that simultaneous selection should continue for at least several more generations. Within each selection population there also were genotypes with trait values favoring the opposite criteria, such as CF selection 'S-290-2-DT' with high yield (Figure A 1), OS selections 'S-293-5-DT - clone' with large flower diameter (Figure A 4), and 'S-292-2-DT clone' with a long flowering period (Figure A 5).

It may be necessary to separate different ideotype populations into isolation nurseries which would be justified by referencing multi-year trends in ideotype trait values. For example, if gain from selection reaches a multi-year plateau for local adaptation traits, such as plant size or survival, then the combination of CF and OS selections in the same nursery could slow selection progress for traits of interest that are specific to each category. Likewise, if a trait that is important for one population begins to move in an unfavorable direction in another population over time, then there may be reason to separate the nurseries. For example, this could occur if selection for cut flower traits such as flower diameter or stem length began to negatively impact yield, or vice versa. For now, in these early stages of selection, our results indicate that multiple ideotypes can be selected out of the same diverse pool of germplasm, which will conserve time, resources, and prevent genetic bottlenecks from occurring early in the domestication program. As progress towards the goal of perennial flax domestication advances, measurement of the ideotype traits should be conducted at a standard interval

so that future trends in target and non-target traits can be recognized and responded to. As more data is collected, the practical application of these ideotype models could be greatly enhanced by the development of effective visualization tools to compare trends, trait correlations, and population differences over time. Therefore, continued utilization of this ideotype approach is not only an opportunity to study the process of crop domestication in real time, it is also an actionable decision-making tool for domestication programs which need to balance many goals at once.

While this study does not address the challenge of selection across multiple growth years, major yield differences were observed between Y1-2 in Experiment 2. The majority of this study focused on the opportunity to improve Y1 phenotypes. This was partly due to the interruptions caused by covid-19, which prevented the same detailed phenotyping in Experiment 1 from occurring in 2020. However, it was also noted at the start of the project that plants generally performed well in Y2, and that Y1 vigor was the area most in need of improvement (K. Betts, personal communication, 2018).

Based on the size of the samples harvested in 2020, the yield per plant will likely be much greater than the Y1 values reported herein. Therefore, the greatest challenge is to achieve acceptable stand establishment and yield in Y1. This is especially true of the oilseed selections, which will need to be capable of establishing from direct sowing in the fall or the spring. The quantity of seed generated between 2018-2020 might prove sufficient to begin answering some of the associated agronomic questions, such as determining the optimal row spacing, planting time, and planting density. For future breeders of perennial flax, the greatest opportunities and questions still ahead involve

examining trait changes across Y1-3 of growth, determining the genetic basis for traits of interest, and finding effective ways of working around the high level of genetic diversity inherent in an obligate outcrosser. There is also the opportunity to spin off a fiber breeding program from the long-stemmed cut flower selections currently available. Finally, to fully realize the potential perennial flax, ecosystem services should be quantified, such as reductions in nutrient leaching, and the ability to uptake heavy metals for bioremediation projects (Angelova et al., 2004; Griga and Bjelková, 2013; Havel et al., 2010; Saleem et al., 2020; Smykalova et al., 2010). Pollinator services could also be integrated into the breeding pipeline by measuring pollinator visitation, and identifying factors such as nectar production, flower color, or other traits that influence visitation (Jevtić et al., 2014; Suso et al., 2016). The vast and varied opportunities for utilizing perennial flax make this a prime target for continued crop domestication efforts.

Tables

Table 2-1. *Linum* species and populations (cut flower or CF; oilseed or OS selections), accession code, seed source, location and/or collection site of sources seeds used in Experiment 1 analysis.

Species	Accession	Seed source, location; (collection site)
L. alatum	532378	Kew Millennium Seed Bank, Royal Botanic Gardens, Wisley, Woking, Surrey, UK
L. altaicum	CN 107286	GRIN-CA, Plant Gene Resources of Canada, Saskatoon, Canada
	CN 19182	GRIN-CA
	PI 522277	USDA-GRIN, Ames, IA
	PI 650292	USDA-GRIN (Baden-Wurttemberg, Germany)
	202723	Kew Millennium Seed Bank
L. austriacum	Ames 29749	USDA-GRIN (Ukraine)
	CN 107255	GRIN-CA
	CN 107268	GRIN-CA
	CN 107288	GRIN-CA
	PI 440472	USDA-GRIN (Russian Federation)
	PI 440473	USDA-GRIN (Russian Federation)
	PI 502405	USDA-GRIN (Ukraine)
	PI 502410	USDA-GRIN (Russian Federation)
	PI 650293	USDA-GRIN (Hungary)
	PI 650294	USDA-GRIN (Stavropol, Russian Federation)
	PI 650295	USDA-GRIN (Poland)
	PI 650296	USDA-GRIN (Tolbukhin, Bulgaria)
	PI 650297	USDA-GRIN (Germany)
	PI 650298	USDA-GRIN (Plovdiv, Bulgaria)
	PI 650299	USDA-GRIN (Tolbukhin, Bulgaria)
	PI 650300	USDA-GRIN (Bacs-Kiskun, Hungary)
	PI 650301	USDA-GRIN (Armenia)
	PI 650302	USDA-GRIN (Krym, Ukraine)
L. baicalense	PI 650303	USDA-GRIN (Mongolia)
	PI 650304	USDA-GRIN (Mongolia)
	PI 650305	USDA-GRIN (Mongolia)
	PI 650306	USDA-GRIN (Mongolia)
	PI 650307	USDA-GRIN (Mongolia)
L. bienne	806822	Kew Millennium Seed Bank
	Ames 19348	USDA-GRIN (Portugal)
	CN 107257	GRIN-CA
	CN 107258	GRIN-CA

Species	Accession	Seed source, location; (collection site)
	CN 107299	GRIN-CA
	CN 113641	GRIN-CA
	CN 113642	GRIN-CA
	PI 231886	USDA-GRIN (Belgium)
	PI 253971	USDA-GRIN (Iraq)
	PI 254371	USDA-GRIN (Delhi, India)
	PI 650308	USDA-GRIN (Coimbra, Portugal)
	PI 522285	USDA-GRIN (IA, USA)
	PI 522286	USDA-GRIN (IA, USA)
	PI 522287	USDA-GRIN (IA, USA)
	PI 522288	USDA-GRIN (IA, USA)
	PI 522289	USDA-GRIN (IA, USA)
	PI 522291	USDA-GRIN (IA, USA)
	PI 522290	USDA-GRIN (IA, USA)
	PI 633835	USDA-GRIN (Delhi, India)
L. decumbens	CN 19028	GRIN-CA
L. flavum	CN 107281	GRIN-CA
	PI 650314	USDA-GRIN (Germany)
	PI 650315	USDA-GRIN (Denmark)
L. grandiflorum	'Bright Eyes'	Outsidepride Seed Source, LLC, 915 North Main Street, Independence, OR 97351
	CN 107259	GRIN-CA
	CN 107260	GRIN-CA
	CN 107263	GRIN-CA
	CN 107287	GRIN-CA
	CN 19026	GRIN-CA
	CN 19027	GRIN-CA
	'Scarlet Flax'	Botanical Interests, Inc, 660 Compton Street, Broomfield, CO 80020
L. hirsutum	Ames 23759	USDA-GRIN (Pest, Hungary)
	CN 107271	GRIN-CA
	PI 502406	USDA-GRIN (Russian Federation)
	PI 502407	USDA-GRIN (Russian Federation)
	PI 502408	USDA-GRIN (Russian Federation)
	PI 502409	USDA-GRIN (Russian Federation)
	PI 650316	USDA-GRIN (Hungary)
	PI 650317	USDA-GRIN (Poland)
	PI 650318	USDA-GRIN (Romania)
L. hudsonoides	172989	Kew Millennium Seed Bank
L. leonii	Ames 23152	USDA-GRIN (Germany)

Species	Accession	Seed source, location; (collection site)
L. lewisii	'Maple Grove' Ames 27614	USDA-GRIN (UT, USA)
	Ames 29912	USDA-GRIN (USA, OR)
	Ames 31360	USDA-GRIN (USA)
	Ames 31361	USDA-GRIN (USA)
	Ames 31364	USDA-GRIN (USA)
	Ames 31365	USDA-GRIN (USA)
	Ames 31366	USDA-GRIN (USA)
	Ames 31368	USDA-GRIN (USA)
	Ames 31369	USDA-GRIN (USA)
	Ames 31370	USDA-GRIN (USA)
	Ames 31371	USDA-GRIN (USA)
	Ames 31372	USDA-GRIN (USA)
	Ames 32565*	USDA-GRIN (USA)
	Ames 32566	USDA-GRIN (USA)
	Ames 32568	USDA-GRIN (USA)
	Ames 33352	USDA-GRIN (NV, USA)
	Ames 33353	USDA-GRIN (AZ, USA)
	Ames 33354	USDA-GRIN (USA)
	CN 107266*	GRIN-CA
	CN 107279	GRIN-CA
	CN 107290	GRIN-CA
	CN 107292	GRIN-CA
	CN 107300	GRIN-CA
	CN 19186	GRIN-CA
	PI 452487	USDA-GRIN (MT, USA)
	PI 522305*	USDA-GRIN (IA, USA)
	PI 522306*	USDA-GRIN (IA, USA)
	PI 650320*	USDA-GRIN (UT, USA)
L. narbonense	CN 107265	GRIN-CA
L. pallescens	774464	Kew Millennium Seed Bank
	Ames 21727	USDA-GRIN (Asia)
L. perenne	Ames 21222	USDA-GRIN (Moldova)
	Ames 31374	USDA-GRIN (USA)
	Ames 32570	USDA-GRIN (USA)
	'Blue Flax'	Livingston Seed, 202 S Washington St, Norton, MA 02766
	'Blue Flax'	AK Wildflowers, Denali Seed Company, 6237 S Pere Marquette Highway, Pentwater, MI 49449
	CN 107256	GRIN-CA

Species	Accession	Seed source, location; (collection site)
	CN 107261	GRIN-CA
	CN 107270	GRIN-CA
	CN 107282	GRIN-CA
	CN 107283	GRIN-CA
	'Himmelszelt' CN 19024	GRIN-CA
	CN 19025	GRIN-CA
	CN 19179	GRIN-CA
	'Appar' PI 445972	USDA-GRIN (SD, USA)
	PI 650323	USDA-GRIN (Hungary)
	PI 650324	USDA-GRIN (Hungary)
	PI 650325	USDA-GRIN (Hungary)
	PI 650326	USDA-GRIN (Hungary)
	PI 650327	USDA-GRIN (Hungary)
	PI 650328	USDA-GRIN (ID, USA)
	PI 650329	USDA-GRIN (ID, USA)
L. stelleroides	CN 107274	GRIN-CA
L. strictum	Ames 26261	USDA-GRIN (Belgium)
	PI 650331	USDA-GRIN (Portugal)
L. sulcatum	354862	Kew Millennium Seed Bank
L. usitatissimum	'Bison' CN 33399	GRIN-CA
	'Linko de Riga' CN 97295	GRIN-CA
	'N.D. Resistant 52' CN 97375	GRIN-CA
	'Tammes #7 Pink' CN 97427	GRIN-CA
	'Novelty' PI 522557	USDA-GRIN (Ontario, Canada)
	'Bison' PI 522606	USDA-GRIN (ND, USA)
	'Raja' PI 523232	USDA-GRIN (Ontario, Canada)
	'Flanders' PI 539916	USDA-GRIN (Saskatchewan, Canada)
L. virgultoum	CN 113624	GRIN-CA
L. viscosum	766777	Kew Millennium Seed Bank
Selection Populations		
Selections - CF	R-14.3-3-DT clone	Breeding program
	R-2.2-2-DT	Breeding program
	R-2.2-2-DT clone	Breeding program
	R-2.6-1-DT clone	Breeding program
	R-6.10-2-DT clone	Breeding program
	R-7.6-2-DT	Breeding program

Species	Accession	Seed source, location; (collection site)
	R-7.6-2-DT clone	Breeding program
	R-9.3-1-DT	Breeding program
	R-LBR5-DT clone	Breeding program
	S-105-1-DT	Breeding program
	S-272-5-DT clone	Breeding program
	S-290-2-DT	Breeding program
	S-290-2-DT clone	Breeding program
	S-293-4-DT	Breeding program
	S-293-4-DT clone	Breeding program
	S-294-3-DT clone	Breeding program
	S-297-3-DT	Breeding program
Selections - OS	R-10.2-2-DT	Breeding program
	R-10.2-2-DT clone	Breeding program
	R-11.1-1-DT	Breeding program
	R-3.2-1-DT	Breeding program
	R-3.2-1-DT clone	Breeding program
	R-5.1-1-DT	Breeding program
	R-5.1-1-DT clone	Breeding program
	R-8.3-2-DT	Breeding program
	R-9.4-1-DT	Breeding program
	S-111-1-DT	Breeding program
	S-121-1-DT	Breeding program
	S-121-1-DT clone	Breeding program
	S-291-1-DT	Breeding program
	S-291-1-DT clone	Breeding program
	S-291-2-DT	Breeding program
	S-292-1-DT	Breeding program
	S-292-2-DT	Breeding program
	S-292-2-DT clone	Breeding program
	S-292-5-DT	Breeding program
	S-293-2-DT	Breeding program
	S-293-2-DT clone	Breeding program
	S-293-5-DT	Breeding program
	S-293-5-DT clone	Breeding program
	S-297-1-DT	Breeding program
	S-297-1-DT clone	Breeding program

*Indicates ‘thrum’ flower types were observed for indicated *L. lewisii* genotype

Table 2-2. Background of *Linum spp.* populations and genotypes tested in Experiment 2

Population	Genotype
BB1[a]	3-12N
	3-14N
	3-16N
	4-16N
	4-1N
	4-6N
	5-14N
	5-2N
	5-8N
	6-14N
	6-19N
	6-22N
	7-18N
	7-20N
	7-24N
	8-12N
	8-13N
	8-17N
	8-24N
	8-25N
	9-10N
	9-1N
	9-2N
BB2[b]	1-11S
	1-20S
	1-21S
	1-7S
Check	APP ('Appar'; *cf.* Table 2-1)
	MG ('Maple Grove'; *cf.* Table 2-1)
KJ1[c]	3-11S
	3-12S
	3-16S
	3-17S
	3-2S
	4-10S
	4-11S
	4-15S
	4-16S
	4-18S
	4-19S
	4-20S
	4-21S
	4-9S
	5-13S
	5-14S

Population	Genotype
	5-6S
	5-7S
	5-8S
KJ2[c]	1-2N
	1-6N
	2-10N
	2-11N
	2-4N
	2-5N
	2-7N

[a] The BB1, or "broad based 1" population was established from the highest yielding plants from a 2005 common garden nursery containing thirteen randomly mated species
[b] BB2 is a breeding population established in 2009 from the highest yielding plants in BB1
[c] The KJ1 and KJ2 breeding populations were selected from BB1 in 2009 for plant habit

Table 2-3. ANOVA (df, F ratio, prob > F) used to confirm validity of 1000 seed weight estimates. When the total # seeds < 1000, the total yield was divided by the number of seeds and multiplied by 1000 to obtain an estimated 1000 seed wt.

Effect	**df**	***F* ratio**	**Prob > *F***
Population	11	57.710	≤.001
Methodology	1	0.253	.615
Population x methodology	8	0.994	.439
Genotype	114	8.940	≤.001
Methodology	1	0.475	.491
Genotype x methodology	62	0.541	.541

Table 2-4. ANOVA (df, F ratio, Prob > F) for the effects of population (*Linum* species, selections) and genotype on yield (g), 1000 seed weight (g; including estimated values), weeks in flower, width 1 (cm), width 2 (cm), height (cm), # seeds per capsule, capsule diameter (mm), stem length (cm), stem diameter (mm), flower diameter (mm), height:width ratio, semi-ellipsoid volume (cm^3), eccentricity, circumference (cm), base area (cm^2).

		Population			Genotype	
Trait	**df**	***F* ratio**	**Prob > *F***	**df**	***F* ratio**	**Prob > *F***
Yield (g)	11	8.634	≤.001	114	3.652	≤.001
1000 seed wt. (g)	11	51.710	≤.001	114	8.940	≤.001
Weeks in flower	11	66.743	≤.001	150	19.996	≤.001
Width 1 (cm)	11	19.019	≤.001	148	6.938	≤.001
Width 2 (cm)	11	29.230	≤.001	148	7.956	≤.001
Height (cm)	11	24.438	≤.001	148	8.158	≤.001
# seeds per capsule	11	6.497	≤.001	114	3.930	≤.001
Capsule diameter (mm)	11	22.924	≤.001	114	9.456	≤.001
Stem length (cm)	11	20.078	≤.001	129	13.763	≤.001
Stem diameter (mm)	11	7.930	≤.001	123	3.126	≤.001
Flower diameter (mm)	11	116.550	≤.001	93	32.953	≤.001
Height:width ratio	11	9.185	≤.001	148	5.232	≤.001
Semi-ellipsoid volume (cm^3)	11	22.446	≤.001	148	5.557	≤.001
Eccentricity	11	12.401	≤.001	148	2.114	≤.001
Circumference (cm)	11	25.363	≤.001	148	8.305	≤.001
Base area (cm^2)	11	22.114	≤.001	148	6.454	≤.001

Table 2-5. Pearson correlations (r) for weeks in flower, yield (g), 1000 seed weight (g; including estimated values), capsule diameter (mm), # seeds per capsule, height(cm), width 1 (cm), width 2 (cm), height:width ratio, semi-ellipsoid volume (cm^3), eccentricity, circumference (cm), base area (cm^2), stem length (cm), stem diameter (mm), flower diameter (mm) for flax populations of *L. altaicum*, *L. austriacum*, *L. baicalense*, *L. bienne*, *L. grandiflorum*, *L. hirsutum*, *L. lewisii*, *L. pallescens*, *L. perenne*, *L. usitatissimum*, cut flower (CF) selections, and oilseed (OS) selections.

Trait	Weeks in flower	Yield (g)	1000 seed wt. (g)	Capsule diameter (mm)[a]	# seeds per capsule[a]	Height (cm)	Width 1 (cm)	Width 2 (cm)	Height:width ratio	Semi-ellipsoid volume (cm^3)	Eccentricity	Circumference (cm)	Base area (cm^2)	Stem length (cm)[b]	Stem diameter (mm)[b]
Yield (g)	.273***	1													
1000 seed wt. (g)	-.227***	.024	1												
Capsule diameter (mm)[a]	-.017	.135***	.383***	1											
# seeds per capsule[a]	-.089**	.165***	.217***	.210***	1										
Height (cm)	.373***	.215***	.045	-.018	-.024	1									
Width 1 (cm)	.393***	.217***	-.116*	.037	-.002	.386***	1								
Width 2 (cm)	.432***	.225***	-.162***	-.007	-.007	.408***	.876***	1							
Height:width ratio	-.136**	-.026	.253***	.007	.023	.467***	-.474***	-.431***	1						
Semi-ellipsoid volume (cm^3)	.343***	.214***	-.087	.006	-.004	.692***	.754***	.775***	-.084*	1					
Eccentricity	-.226***	-.081	.125**	.030	.029	-.188***	-.163***	-.564***	.130**	-.292***	1				
Circumference (cm)	.426***	.229***	-.144**	.016	-.005	.410***	.969***	.968***	-.467***	.789***	-.373***	1			
Base area (cm^2)	.349***	.205***	-.126**	.020	.009	.388***	.925***	.954**	-.399***	.843***	-.378***	.970***	1		
Stem length (cm)[b]	.039	.071	.029	-.027	-.075	.506***	.506***	.402***	.092*	.482***	.001	.471***	.433***	1	
Stem diameter (mm)[b]	-.106*	.076	.205***	.124**	.074	-.080	-.157***	-.179***	.038	-.121**	.118**	-.174***	-.160***	-.103*	1
Flower diameter (mm)[b]	.107*	.079	.009	-.014	-.062	.159**	.203***	.184***	.006	.121*	-.080	.201***	.164**	.227***	-.168**

***. Correlation is significant at the 0.001 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

[a] Pearson correlations were calculated on a genotype mean basis for the average of five capsules

[b] Pearson correlations were calculated on a genotype mean basis for the average of three stems/flowers.

Table 2-6. Percent germination by week (weeks G1-G5), total % germination, % summer survival, and % winter survival for flax species and cut flower (CF) and oilseed (OS) selections investigated for domestication potential. Percent summer survival is defined as the total number of transplants which survived the growing season. Using the number of plants that survived the growing season, % winter survival was calculated based on the number that survived the winter.

	% Germination							
Population	G1*	G2*	G3	G4	G5	**% Germination**	**% Summer Survival**	**% Winter Survival**
L. altaicum	0.0	7.8	52.9	11.4	6.7	78.8	70.7	71.7
L. austriacum	0.0	0.0	38.9	16.7	2.8	58.3	81.5	76.5
L. baicalense	0.0	0.0	30.2	28.1	4.4	62.7	29.0	6.9
L. bienne	0.1	15.5	52.5	5.1	0.7	73.9	53.7	0.5
L. grandiflorum	1.3	2.9	47.4	2.6	1.6	55.8	37.2	0.0
L. hirsutum	0.0	0.0	33.9	18.5	1.0	53.4	56.3	70.8
L. lewisii	0.0	0.0	38.0	9.9	2.1	50.1	57.7	66.1
L. pallescens	0.0	0.0	42.7	1.8	2.7	47.3	72.3	55.9
L. perenne	0.0	0.0	30.8	13.5	3.3	47.5	90.5	94.3
L. usitatissimum	24.2	11.2	29.7	4.0	0.4	69.5	24.4	0.0
Selections - CF	0.0	0.0	39.7	5.1	1.8	46.5	88.7	91.5
Selections - OS	0.0	0.0	43.6	4.5	2.6	50.7	93.2	97.4

*Seedlings were held in a dark cooler at 4°C during Weeks 1-2 for cold moist stratification, after which they were germinated in a mist greenhouse (see text).

Table 2-7. Reported style morphs for flax species, field observations of style morphs (# pin, # thrum, # homostylous), 1:1 Chi-square (χ^2) test (df = 1), p-value, and whether observations match previous reports of stylar condition (y/n). Pin or approach herkogamous flowers are those in which the stigma protrudes above the anther height. Thrum or reverse herkogamous flowers have stigmas recessed below the anther height. Homostylous flowers are those in which the stigma and anthers are equal in height. Polymorphic species exhibit two (distylous) or three (tristylous) conditions within a single population and compatible interactions require flowers of different types to cross pollinate, promoting outcrossing. Monomorphic species exhibit one flower type only.

Population	Style morph	Reference	# Pin	# Thrum	# Homostylous	X^2 test	Test statistic	P-value	Match reports (y/n)
L. altaicum	N/A	N/A	2	4	0	1:1	0.667	.414	N/A
L. austriacum	Polymorphic distylous	(Murray, 1986, p. 198; Ruiz-Martín et al., 2018)	31	41	1	1:1	1.39	.238	Yes
L. baicalense	N/A	N/A	3	1	6				N/A
L. bienne	Monomorphic homostylous	(Murray, 1986; Ruiz-Martín et al., 2018)	0	0	25				Yes
L. grandiflorum	Polymorphic distylous	(Murray, 1986; Ruiz-Martín et al., 2018)	0	0	17				No
L. hirsutum	Polymorphic distylous	(Murray, 1986; Ruiz-Martín et al., 2018)	8	9	2	1:1	0.06	.808	Yes
L. lewisii	Monomorphic approach herkogamous (pin)	(Ruiz-Martín et al., 2018)	32	14	0				No
L. pallescens	Monomorphic homostylous	(Ruiz-Martín et al., 2018)	0	0	8				Yes

Population	Style morph	Reference	# Pin	# Thrum	# Homostylous	X^2 test	Test statistic	P-value	Match reports (y/n)
L. perenne	Polymorphic distylous	(Murray, 1986; Ruiz-Martín et al., 2018)	39	27	0	1:1	2.18	.139	Yes
L. usitatissimum	Monomorphic homostylous	(Murray, 1986; Ruiz-Martín et al., 2018)	0	0	11				Yes
Selections – CF*	N/A	N/A	24	36	0	1:1	2.40	.121	N/A
Selections – OS*	N/A	N/A	47	49	0	1:1	0.04	.838	N/A

* Cut flower (CF) and oilseed (OS) selections of unknown species background were tested under the assumption that these populations were polymorphic distylous

Table 2-8. 1:1 Chi-square tests (1:1; test statistics, p-values, df = 1) for petal overlap in all flax species and cut flower (CF) and oilseed (OS) selection populations. For ornamental value, petal overlap > 50% is desired to give the appearance of a larger, more full-looking corolla. Significant p-values ($\alpha \leq 0.05$) are highlighted in bold.

Population	Overlap > 50%	Overlap < 50%	Test statistic	P-value
L. altaicum	4	1	1.80	.180
L. austriacum	48	25	7.25	**.007**
L. baicalense	9	0	9.00	**.003**
L. bienne	6	19	6.76	**.009**
L. grandiflorum	10	7	0.52	.467
L. hirsutum	18	1	15.21	**≤.001**
L. lewisii	33	12	9.80	**.002**
L. pallescens	5	3	0.50	.480
L. perenne	41	25	3.88	**.049**
L. usitatissimum	11	0	11.00	**≤.001**
Selections - CF	46	14	17.07	**≤.001**
Selections - OS	53	43	1.04	.307

Table 2-9. Chi-square tests (1:1:1; test statistics, p-values, df = 2) for flax flower shapes in all populations and cut flower (CF) and oilseed (OS) selections tested. Flower shapes are classified as tube, funnel, or bowl according to Diederichsen and Richards (2003). P-values of $\alpha \leq 0.05$ are highlighted in bold.

Population	Tube	Funnel	Bowl	Test statistic	P-value
L. altaicum	0	0	5	10.00	**.007**
L. austriacum	1	9	63	93.48	**≤.001**
L. baicalense	0	3	6	6.00	.050
L. bienne	6	9	10	1.04	.595
L. grandiflorum	0	11	6	10.71	**.005**
L. hirsutum	0	4	15	19.05	**≤.001**
L. lewisii	0	2	43	78.53	**≤.001**
L. pallescens	0	1	7	10.75	**.005**
L. perenne	0	19	47	50.82	**≤.001**
L. usitatissimum	3	8	0	8.91	**.012**
Selections - CF	3	10	47	55.90	**≤.001**
Selections - OS	0	13	83	124.56	**≤.001**

Table 2-10. ANOVA for the main effects (year, population, genotype) and their interactions for traits measured in both years, including yield (g), 1000 seed weight (g), and number of seeds per capsule. The effects of population and genotype were tested in separate ANOVAs.

		Yield (g)		1000 seed weight (g)		# seeds per capsule	
Effect	**df**	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***
Population	4	0.412	.800	0.128	.972	3.263	.011
Year	1	207.240	≤.001	43.924	≤.001	1.998	.158
Population x year	4	0.376	.826	0.365	.833	0.463	.763
Genotype	54	1.076	.341	0.952	.574	2.414	≤.001
Year	1	311.363	≤.001	56.007	≤.001	3.324	.068
Genotype x year	54	0.997	.486	0.665	.966	2.477	≤.001

Table 2-11. Mean ± SE and range for flax yield (g) and 1000 seed weight (g) across years in Experiment 2. For both traits, year two observations were significantly greater than year one.

Trait	Year 1 (2018)		Year 2 (2019)		Significance
	Mean ± SE	Range	Mean ± SE	Range	
Yield (g)	0.20 ± 0.37	0.00–1.86	7.57 ± 0.36	0.21–33.43	***
1000 seed weight (g)	1.06 ± 0.02	0.72–1.52	1.22 ± 0.02	0.88–1.57	***

Table 2-12. ANOVA (df, F ratio, Prob > F) for the effects of population and genotype on capsule diameter (mm) in year 2.

		Population			**Genotype**	
Trait	df	*F* ratio	Prob > *F*	df	*F* ratio	Prob > *F*
Capsule diameter (mm)	4	1.392	.235	54	2.491	≤.001

Table 2-13. Pearson correlations (r) for all phenotypic traits tested in flax (Experiment 2).

	Yield (g)	1000 seed wt. (g)	# seeds per capsule[a]
1000 seed wt. (g)	.343***	1	
# seeds per capsule[a]	.077	.057	1
Capsule diameter (mm)[ab]	-.048	.232***	-.089

*** Correlation is significant at the 0.001 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

[a] Pearson correlations calculated on a genotype mean basis for the average of five capsules

[b] Pearson correlations calculated for year 2 data only

Figures

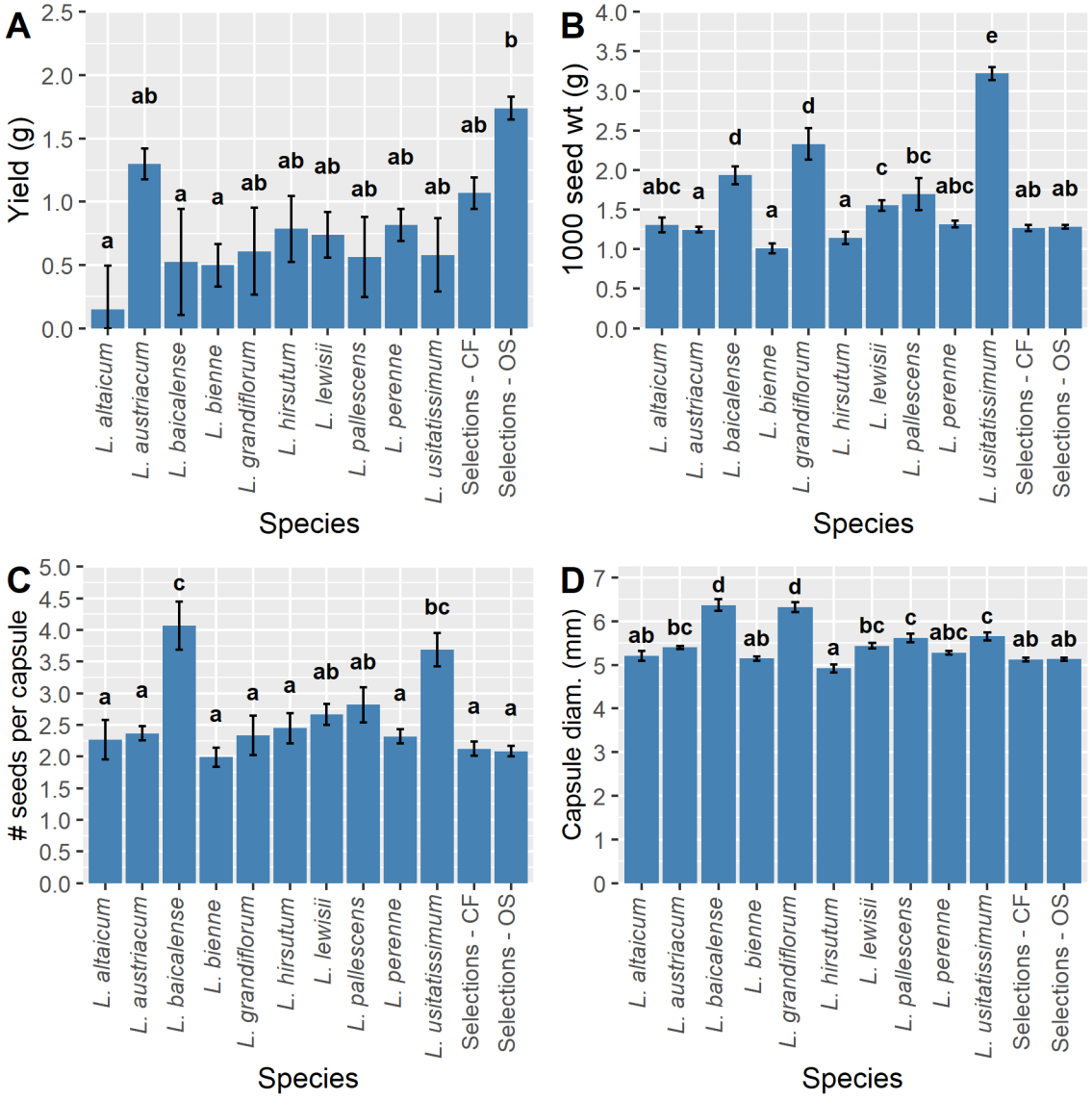

Figure 2-1. Comparison of wild flax species with cut flower (CF) and oilseed (OS) selections for mean ± S.E. year one trait values related to oilseed ideotype: (a) yield (g), (b) 1000 seed weight (g), (c) # seeds per capsule, and (d) capsule diameter (mm). Mean separations (5% HSD) are displayed as letters above the columns denoting significance.

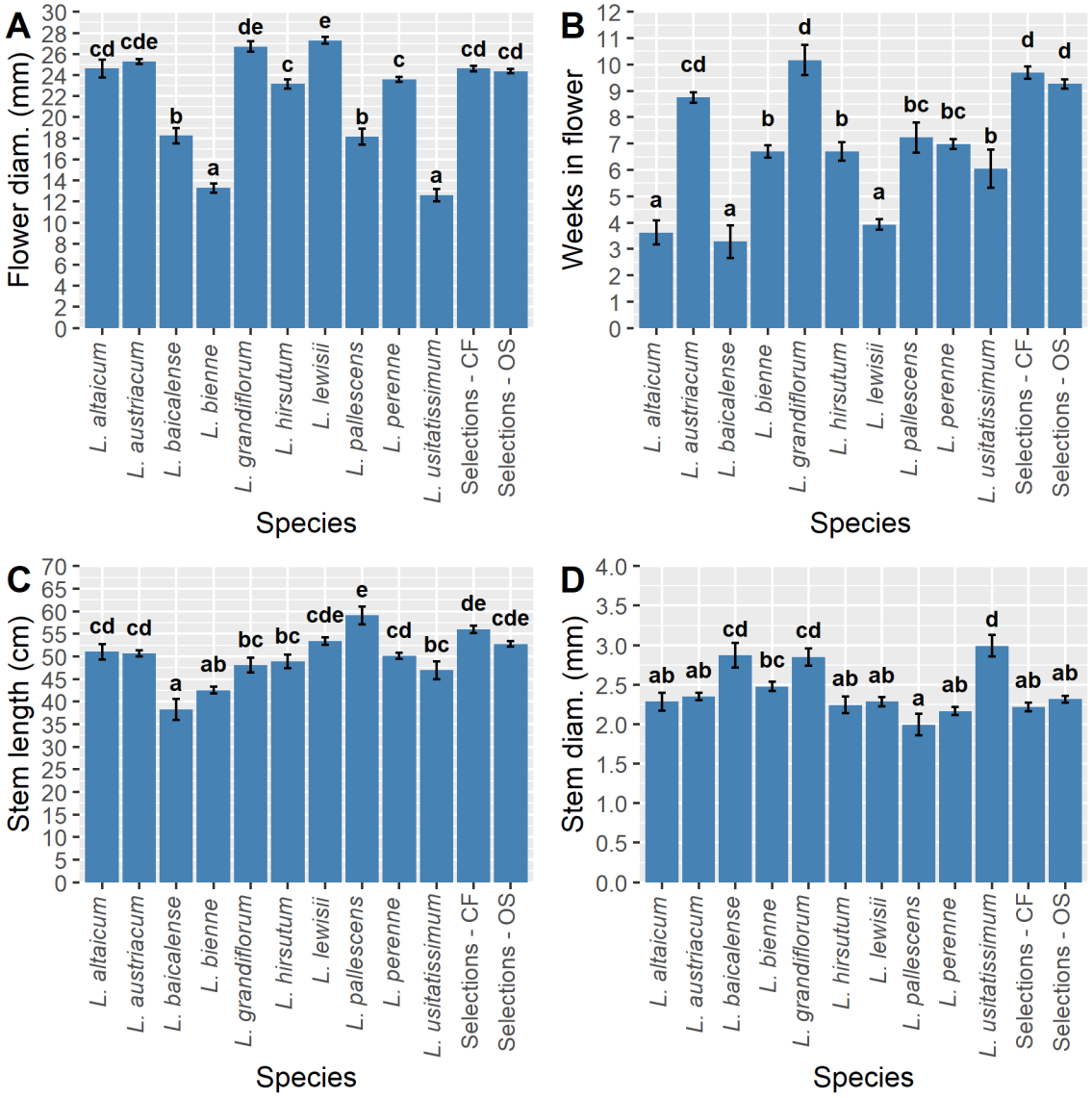

Figure 2-2. Comparison of wild flax species with cut flower (CF) and oilseed (OS) selections for mean ± S.E. year one trait values related to the cut flower ideotype: (a) flower diameter (mm), (b) number of weeks in flower, (c) stem length (cm), and (d) stem diameter (mm). Mean separations (5% HSD) are displayed as letters above the columns denoting significance

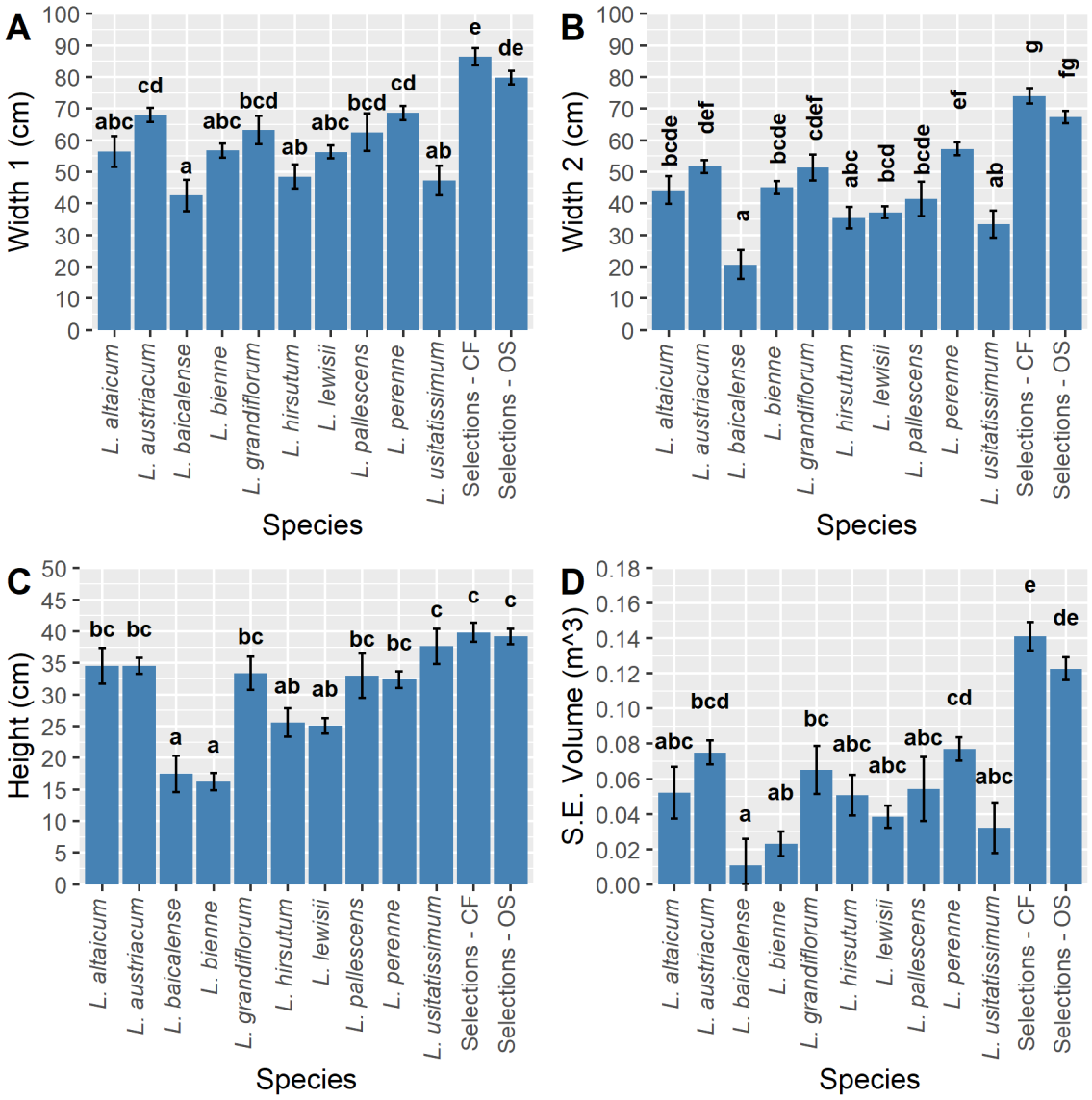

Figure 2-3. Comparison of wild flax species with cut flower (CF) and oilseed (OS) selections for mean ± S.E. year one plant size trait values: (a) width 1 (cm), (b) width 2 (cm), (c) height (cm), and (d) semi-ellipsoid (S.E.) volume (m^3). Means separations (5% HSD) are displayed as letters above the columns denoting significance.

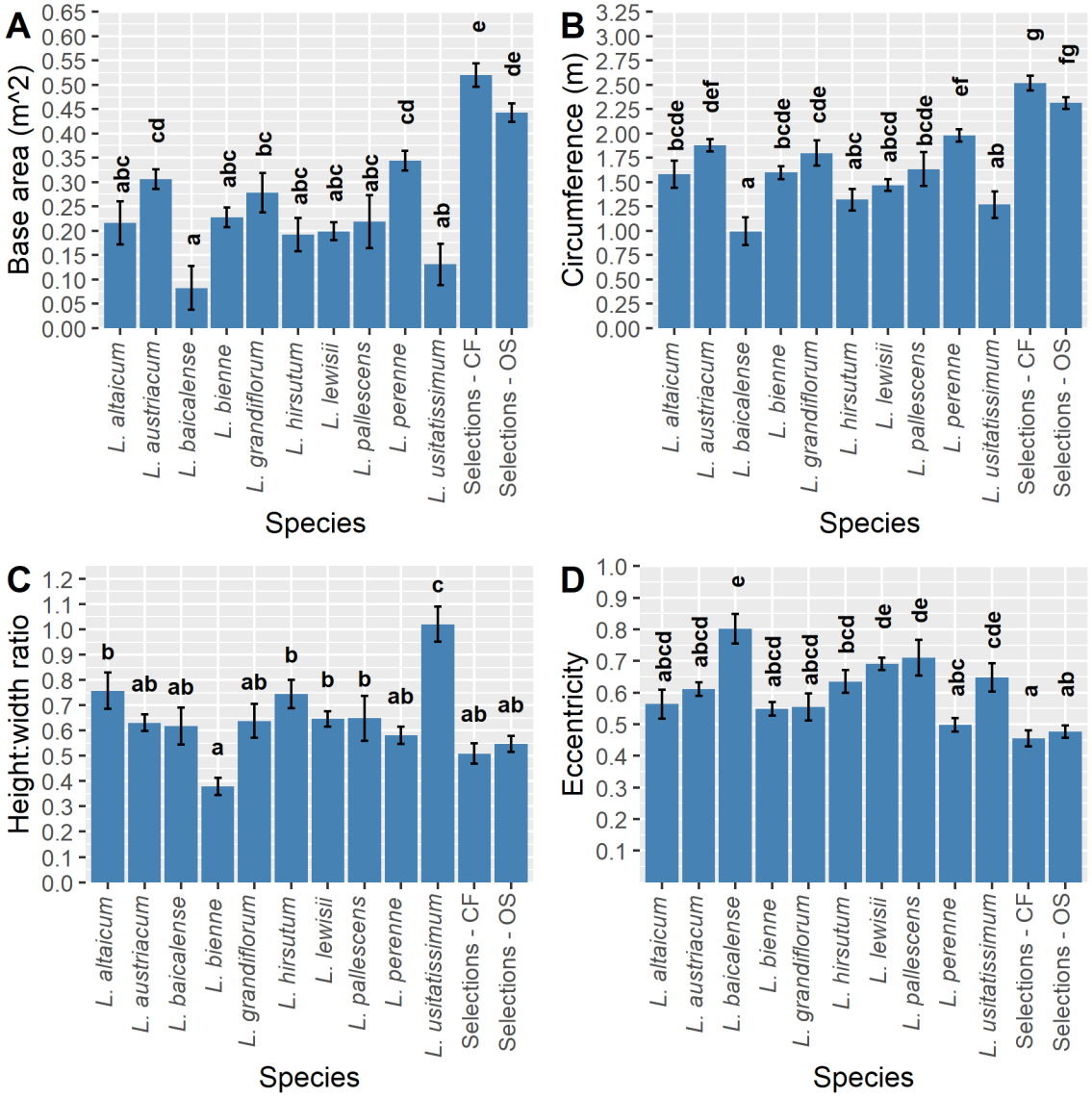

Figure 2-4. Comparison of wild flax species with cut flower (CF) and oilseed (OS) selections for mean ± S.E. year one plant size and shape trait values: (a) base area (m^2), (b) circumference (m), (c) height:width ratio, and (d) eccentricity (*e*). Mean separations are displayed as letters above the columns denoting significance.

Figure 2-5. Flower colors and floral morphs of *Linum grandiflorum*: (a) red and pink-flowered progeny of a red x white cross (white flowered type not shown) (b) From left to right: thrum (short-styled), homostylous (equal length), and pin (long-styled) flower morphs. Arrows indicate relative style (S) and anther (A) lengths. Note that sepals are partially removed in the two leftmost flowers to expose the floral organs.

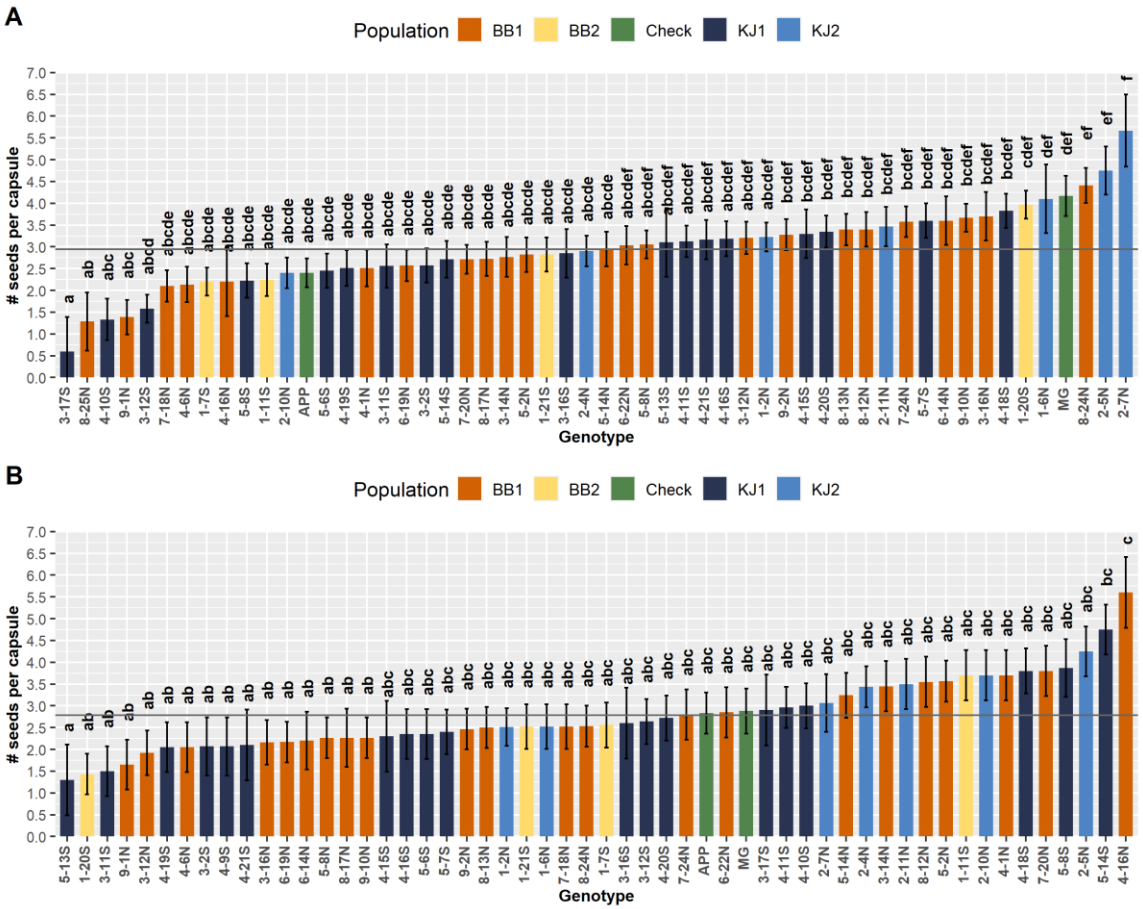

Figure 2-6. Mean ± S.E. number of seeds per capsule for genotype x year interactions demonstrating flax shattering which changes the relative order of genotypes across years: a) year one (2018) data b) year two (2019) data. Means separations (5% HSD) are displayed as letters above the columns denoting significance within each year. The gray horizontal line indicates the grand mean for each year.

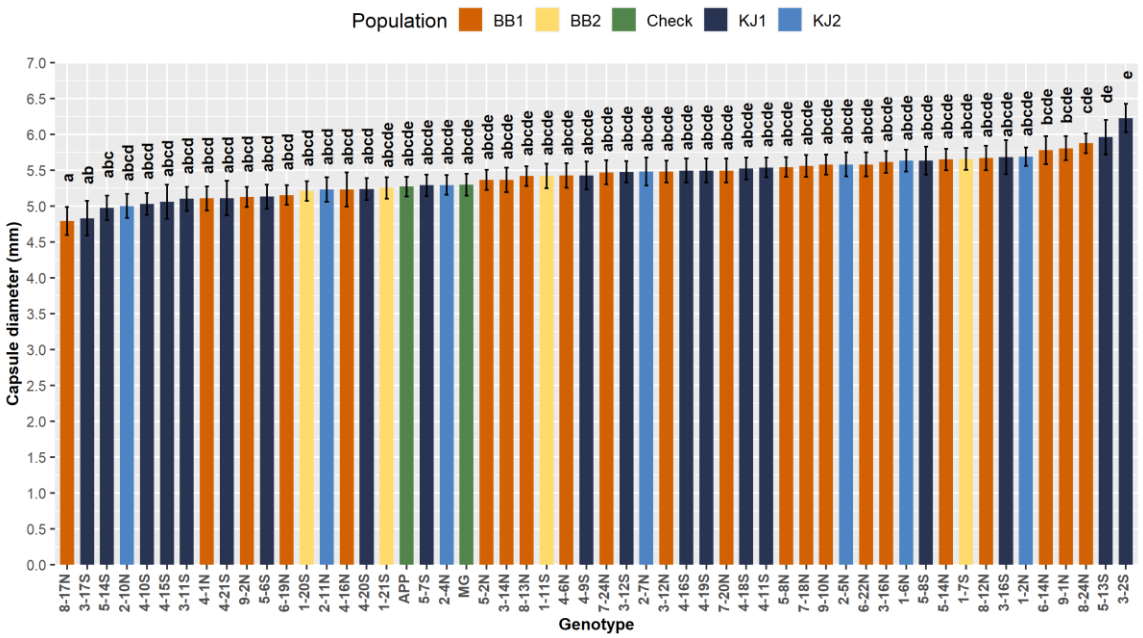

Figure 2-7. Mean ± S.E. capsule diameter (mm) by flax genotype for Experiment 2, year 2. Means separations (5% HSD) are displayed as letters above the columns denoting significance within each year.

Chapter 3

Perennial flax: A Potential Cut Flower Crop

Manuscript to be submitted to *HortScience.*

The genus *Linum* L. contains ~200 species, including several ornamentals, yet no reports exist regarding the cut flower potential of this genus. The aim of this study was to evaluate the cut flower potential of perennial flax cultivars (*L. perenne L.* 'Blue Flax' and 'Sapphire'; Experiment 1, 2018) and accessions (*L. austriacum* L., *L lewisii* Pursh., and *L. perenne*; Experiment 2, 2019), and to record traits that will enable breeding and selection for improved cut flower performance. In both experiments, vase solution treatments included a deionized (DI) water control and a floral preservative (Floralife 300). Vase life, number of flowers, percent of initial buds opened, flower diameter, individual flower longevity, and average daily water loss were recorded. In Experiment 2, additional stem phenotypic traits were recorded, including stem length, stem diameter, number of branches, length to first branch, number of previous flowers, and number of seed capsules. The mean vase life across both cultivars in Experiment 1 was 9.22 d. In Experiment 2, *L. perenne* had the longest average vase life (9.25 d), followed by *L. austriacum* (9.07 d) and *L. lewisii* (8.32 d). The floral preservative significantly increased vase life by an average of 1.67 d in Experiment 1, and 1.63 d in Experiment 2, and resulted in a significantly greater number of flowers (~2x) in both experiments. Significant variation was observed among genotypes for most traits, including vase life and number of flowers, highlighting the opportunities for improving the potential of cut flower perennial flax through breeding.

Introduction

The genus *Linum* contains approximately 180-200 species (Bolsheva et al., 2017; McDill et al., 2009). The most well-known of these is domesticated annual flax, *L. usitatissimum* L., common flax or linseed. Originally domesticated in the fertile crescent, this species has been cultivated since ~8,000 B.C.E, making it one of the earliest domesticated plants (McDill et al., 2009; Vaisey-Genser and Morris, 2003). Throughout history, flax has been highly valued as a multi-use crop for fiber, feed, and industrial applications (Vaisey-Genser and Morris, 2003). *Linum* also contains a large number of wild perennial species which are distributed throughout the temperate and subtropical regions of Europe, Asia, and North America (Bolsheva et al., 2017; McDill et al., 2009). Several species of *Linum* have a history of cultivation as ornamentals, including *L. perenne* L., *L. austriacum* L., *L. narbonense* L., *L grandiflorum* Desf., and *L. flavum* L., although few reports are available on the variation available for ornamental breeding, including for cut flower uses (Cullis, 2019; Diederichsen and Richards, 2003; Fu, 2019).

In 2018, a perennial flax breeding program was established at the University of Minnesota as part of the Forever Green Initiative (FGI), with the goal of providing a new high-value perennial crop to Minnesota producers, with added environmental benefits of pollinator services, improved water quality, and reduced soil erosion (Betts et al., 2008). The long-term goal of the FGI breeding program is to develop a perennial version of oilseed flax, however, this could take years or even decades. For context, the perennial grain Intermediate Wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey subsp. *intermedium*), took ~16 yr of breeding and agronomic development before

the first variety ‘MN-Clearwater’ was released to the public in 2019 (Bajgain et al., 2020). To accelerate variety development, perennial flax breeders are simultaneously pursuing ornamental applications of perennial flax, including breeding for cut flower and garden performance using an ideotype model. The rationale and selection goals for oilseed, garden, and cut flower ideotypes are outlined in our recent review of the subject (Tork et al., 2019).

Perennial flax has the potential to become a new specialty cut flower for cold climates like Minnesota, but to the best of our knowledge, there are no existing reports on vase life performance of any *Linum* species. An integral part of the cut flower industry is the introduction of new species and unique or rare flower colors which can drive consumer interest and increase sales (Dole et al., 2009). However, a lack of information on expected vase life and specific post-harvest recommendations pose a barrier to adoption of new cut flower crops. For example, the use of floral preservative, which is typically composed of sucrose (plant food) and a biocide to reduce bacterial growth, often results in an extended vase life, greater postharvest bud opening, flower size, flower longevity, and improved color (Dole et al., 2009; Pun and Ichimura, 2003; Reid and Jiang, 2012). Floral preservatives can also cause adverse effects in some species, such as increased rates of leaf chlorosis in oriental lily, although this can be alleviated by the addition of gibberellic acid (GA_3) (Han, 2003; Rabiza-Świder et al., 2015). Response to vase solution treatments is often genotype- or species-specific, and can vary significantly, even among cultivars from the same breeding program (Clark et al., 2010; Janowska and Jerzy, 2004; Reid and Jiang, 2012).

Vase life studies typically involve harvesting flower stems from field- or greenhouse-grown plants. Immediately following harvest, stems are hydrated using unamended water or a commercial hydrator to maintain floral quality during short term (< 24 h) transport, after which they are placed in a long-term holding solution such as a floral preservative (Clark et al., 2010). Vase life experiments evaluate commercially relevant factors, such as the type of vase solution, harvest timing, storage conditions (wet, dry), environmental conditions (temperature, light, humidity), etc. (Fanourakis et al., 2013; Redman et al., 2002; Reid and Jiang, 2012; Skutnik et al., 2020). The goal of these evaluations is to extend vase life and improve postharvest floral development and quality. The results are then used to develop specific handling recommendations for cut flower growers, wholesalers, and florists to help drive demand and widespread adoption of the species or cultivar tested (Dole et al., 2009).

Initial surveys of *Linum* have identified *L. austriacum* L., *L. lewisii* Pursh, and *L. perenne* L. as the top perennial species of interest for the various breeding objectives of the FGI program (D. Tork, unpublished data, 2019). The species *L. austriacum* and *L. perenne* are native to Europe and western Asia with overlapping native ranges, and are identical in appearance except for a difference in pedicel angle (Ockendon, 1971). Additionally, hybridization between these species has been reported (Jhala et al., 2008; Ockendon, 1968; Seetharam, 1972). In contrast, *L. lewisii* is native to the western half of North America, and is reproductively isolated from *L. austriacum* and *L. perenne* (Pendleton et al., 2008; USDA-NRCS, n.d.). Despite being geographically isolated across continents for most of their evolutionary history, *Linum perenne* and *L. lewisii* are

difficult to distinguish morphologically, with the primary taxonomic distinction being the presence (*L. perenne*; distylous, self-incompatible) or absence (*L. lewisii*; monomorphic approach herkogamous; self-compatible) of heterostyly (Pendleton et al., 2008; Ruiz-Martín et al., 2018). Both *L. perenne*, and to a lesser extent, *L. austriacum*, have also been naturalized throughout North America (USDA-NRCS, n.d.). The native status and self-compatibility of *L. lewisii* make it a favorable choice for the perennial flax breeding program, but it has lacked vigor compared with *L. austriacum* and *L. perenne* in field trials in Minnesota, posing a significant barrier to adoption (D. Tork, unpublished data, 2019-2020).

The objectives of this study were to characterize the vase life of perennial flax with and without the use of floral preservative and to record traits that will enable breeding and selection for improved cut flower performance. This study aims to facilitate the adoption of perennial flax as a new cut flower crop for cold climate regions by developing recommendations for optimal postharvest handling. Perennial flax can be used as filler flowers contributing color and texture to vase arrangements. In floral design, filler flowers are small flowers used to accent the larger, primary flowers by filling empty spaces and adding accents of complementary color or texture (Hunter, 2013). Flax possesses relatively small, but striking blue flowers, along with finely textured foliage, making it well suited as a filler material. In this study, commercially relevant traits such as total vase life, total number of flowers, percentage of buds opening, flower longevity, and water loss will be compared across the floral preservative and DI water treatment groups for cultivars of *L. perenne* (‘Blue Flax’; ‘Sapphire’), and

accessions of *L. austriacum*, *L. lewisii*, and *L. perenne*. Several stem phenotypic traits relevant to cut flower breeding were also recorded for species accessions, including stem length, length to the first side branch, stem diameter, number of previous flowers, number of seed pods, number of flower buds, and number of branches. This study serves the dual purpose of generating guidelines for the postharvest handling of perennial flax as a cut flower, while informing breeding work by comparing postharvest performance across the three perennial flax species, and potential correlations between morphological and postharvest traits.

Materials and Methods

Experiment 1

Experiment 1, in year one (Y1; fall 2018), tested two commercial *L. perenne* cultivars grown at University Research and Outreach Centers (ROCs) in Grand Rapids (47° 14' 50.604" N, 93° 32' 39.983" W), Morris (45° 37' 41.520" N, 95° 53' 20.688" W), and Waseca, MN (43° 54' 24.084" N, 93° 26' 0.167" W). Plug trays (72s) of 'Sapphire' and 'Blue Flax' rooted liners were obtained from The Nursery Stock Market, Inc. (Presswood, KY). Ten clones per cultivar were planted for each cultivar in spaced rows (45.2 cm O.C. within rows and 60.96 cm between rows) at each site. The Waseca site was later dropped from the experiment due to insufficient weed control. Thus, cut flowers from only two sites were tested.

Harvest

Ideally, cut flower harvest would be confined to a short time period in the early morning between approximately 0700-1000 h (Clark et al., 2010); however, this was not possible in this experiment due to the distance between sites. Harvest occurred in a single day at each site during wk 37 (13 September 2018) with the Grand Rapids, MN site being harvested first (0800-1000 h), followed by Morris, MN later that day (1400-1600 h). Six to eight flowering stems were harvested from each plant for both cultivars at both locations. Stems were cut at the base using hand clippers, which were sterilized in ethanol (70% EtOH) in between plants. Immediately after cutting, stems from the same plant were grouped and wrapped in a moist paper towel, then placed in a plastic bag (Ziploc® Freezer Gallon) to hold in moisture. Bags containing harvested stems were immediately placed on ice in a cooler for transport. The ice was covered with a towel prior to loading the samples into the cooler to prevent cold damage to the stems. After both sites were harvested, stems were transported immediately back to St. Paul, MN for overnight storage in a 4 ºC walk-in cooler located at the Plant Growth Facilities, St. Paul Campus, University of Minnesota, St. Paul, MN (44°59’17.8” N, -93°10’51.6” W). All stems were in storage by 2130 h on the harvest day.

Experimental design

Experimentation took place in the laboratory (277 Alderman Hall, St. Paul Campus) under 24 h light with an average intensity of 10.86 μmol m^{-2} sec^{-1} and an

ambient air temperature of 21 °C day/night. Two vase solutions were tested: a) deionized, distilled water (control) and b) a floral preservative solution of FloraLife Crystal Clear® Flower Food 300 (Floralife, Walterboro, SC) mixed according to recommendation (10 g/1 L water). Each vase was filled with 200 ml of either solution and arranged using a completely randomized design (CRD) with one stem per vase. This minimized the effect of location of the vase on the laboratory bench top.

The stems were prepared after all vases had been filled with solutions. Stems were stripped of the lower half of leaves and 2.5 cm (1 in) was cut from the base of the stem using a sterilized scalpel blade to prevent debris from contaminating the stem solution. After cutting, each stem was immediately placed in the assigned vase to begin the experiment.

Experiment 2

Experiment 2, in year two (Y2; fall 2019), compared accessions from three perennial species: *L. perenne, L. austriacum,* and *L. lewisii*. These were grown in a common garden nursery located at Rosemount Research and Outreach Center, Rosemount, MN (44°42’58.2” N, -93°5’54.9” W) with the same spacing within rows (45.7 cm O.C.) as Experiment 1 but with differing row spacing (1.52 m), determined by the cultivation equipment available at each site. Seeds were sown in 288 plug trays [Landmark Plastic, Akron, OH] in Berger BM2 Seed Germination and Propagation Mix (Berger, Saint-Modeste, QC) and covered with fine vermiculite [Palmetto Vermiculite

Medium A-2, Palmetto Vermiculite, Woodruff, SC] in wk 14 and 15 (5, 12 April 2019). All plug trays were placed in a mist house for 4 h to moisten the soilless medium using an intermittent mist system (St. Paul MN Plant Growth Facility, University of Minnesota; 44°59'17.8" N, -93°10'51.6" W) at a mist frequency of 10 min intervals (mist nozzles, reverse osmosis water) during 0600-2200 h with a 7 s duration (21/21 °C, day/night, 16 h; 0600–2200 h) with lighting supplied by high pressure sodium high intensity discharge (HID) lamps at a minimum set point of 150 μmol m^{-2} s^{-1}. Once watered in, the trays were covered with plastic dome lids [Super Sprouter Standard Vented Humidity Dome 7", Hawthorne Gardening Company, Vancouver, WA] and transferred to a walk-in cooler for two weeks at 4/4 °C day/night in darkness to break seed dormancy (cold stratification), which is recommended for most wild *Linum* species (K. Betts, personal communication, 2018; Barbara Atkins, STA laboratories, Longmont, CO). Trays were uncovered and misted by hand, as needed, over this 2 wk period to maintain adequate moisture levels in the soilless medium. After the 2 wk stratification, the dome lids were removed, and the trays were returned to the mist house for an additional 3 wk. Plug trays were then moved onto capillary mats in a greenhouse at 16.7/15.5 °C day/night daily integral and a 16 h photoperiod (0600–2200 h; long days). Supplemental lighting was supplied during cloudy days by 400 w high pressure sodium high intensity discharge (HPS-HID) lamps, at a minimum of 150 μmol m^{-2} s^{-1} at plant level. Fertigation (Mondays-Fridays) provided nutrients at a constant liquid feed (CLF) rate of 125 ppm N from water soluble 20-10-20 fertilizer. Accessions were placed in the greenhouse on wk 19 and 20 (10, 17 May 2019) until transplanting in wk 24 (13 June 2019). Accessions

were randomized for planting, and twenty seedlings per genotype were transplanted. The field was irrigated post-planting with 2.54 cm water. Irrigation continued throughout the summer to maintain a minimum of 2.54 cm water per week when there was insufficient rainfall. Weed control consisted of weekly mechanical tillage between rows, pre-emergent herbicide applications [Fortress®, OHP Inc., Bluffton, SC] at the recommended rates, and bi-weekly hand weeding within rows.

Selection of genotypes for testing

All accessions for Experiment 2 were obtained as seed from the United States Department of Agriculture Germplasm Resources Information Network (USDA-GRIN) and Canada's Plant Gene Resources of Canada (GRIN-CA). Prior to selecting genotypes for testing, individual plants were identified that met three criteria: 1) the plant had sufficient number of stems (6) in bloom, 2) stems were ≥ 30 cm in length, and 3) first branching occurred no less than 20 cm from base of stem. These criteria ensured that stems were morphologically similar and could fit easily in the bud vases used for testing. Genotypes were randomly selected for testing (Table 3-1).

The CRD experimental design was originally balanced, with nine genotypes per species; however, it was later discovered that accession PI 522305 was mistakenly labeled as *L. perenne* on nursery inventory documents. Following the GRIN classification, this accession was corrected to be in the *L. lewisii* group (Table 3-1).

Harvest

The harvest in Experiment 2 followed the same protocol used in Experiment 1 except for the timing of harvest and the amount of time in storage. Due to the closer proximity of Rosemount to Saint Paul, MN, stems were harvested during week 38 (16 Sept. 2019) between 0700-1100 h, transported to St. Paul and placed in cold storage (4 ºC walk-in cooler) by 1200 h that same day. In contrast to Experiment 1, in which the postharvest tests were initiated the day after harvest (following an overnight storage of stems in the cooler), Experiment 2 was initiated immediately after harvest. In this case, the cut flax stems only remained in cold storage for ~3 h until all the test vases were set up with solutions (~1500 h).

Experimental design

The same experimental protocols used in Experiment 1 were used in Experiment 2. The only differences were the germplasm tested (as noted above) as well as recording initial measurements before each stem was processed, including stem length (cm), length to first branch > 5 cm (cm), stem diameter at 30 cm from the apex (mm), number of seed pods (capsules), number of previous flowers (as indicated by the number of pedicels, which remain attached even after the flower bud has abscised), and the number of secondary branches before each stem was processed. After processing the stems, the number of viable flower buds remaining on each stem was recorded.

Data collection (Experiments 1 & 2)

Vases were checked every 24 h to record the number of flowers open and flower diameter (mm). Open flowers (floral organs visible) were tagged with colored yarn corresponding to each day of the experiment to enable measurement of flower longevity or petal holding capacity. One major difference between Experiments 1 and 2 was that in Experiment 1, disturbance of individual flowers was minimized when taking measurements. Only after > 50% petal drop or full flower abscission were individual flowers considered to be terminated. In contrast in Experiment 2, the stems were given three strong taps with a finger before recording flower drop each day. Flower diameter was recorded for any open flower in Experiment 1, whereas in Experiment 2, the flower diameter measurements were limited to flowers in which the petals were fully splayed open.

Vase solutions were changed on a weekly basis, during which time the volume lost (ml) was recorded. Vases containing solution, but no stem, were included as controls to measure the average water loss due to evaporation (ml) for each solution. Using this data, the total water loss (including that lost by evapotranspiration (ET), and from uptake by the stems) was calculated for each vase by subtracting the average rate of solution evaporation. The volume of solution from phloem unloading was not measured and constituted an additional volume into the measured water loss. Since each stem had a different vase life, the total water loss was averaged over total vase life to calculate the average daily water loss (ml):

$$Average\ daily\ water\ loss\ (ml)$$
$$= \frac{Vase\ total\ water\ loss\ (ml)\ -\ Control\ vase\ avg\ total\ water\ loss\ (ml)}{Total\ Vase\ Life\ (no.\ of\ days)}$$

Termination of vase life was based on factors which would cause the average consumer to discard the stem (retail vase life) (Clark et al., 2010). Based on pilot experiments (N. Anderson and D. Tork, unpublished data, 2018), reasons for termination commonly observed in perennial flax include: leaf wilt, leaf chlorosis, chlorosis localized at the flower buds, and wilt localized at the flower buds (bent neck).

Data analysis

Experiment 1

Independent factorial ANOVAs were conducted to compare the influence of location, cultivar, and treatment on the following dependent variables: total vase life (d), average number of flowers open, percent of initial buds opened, average daily water loss (ml), flower diameter (mm), and individual flower longevity (d). The factors of location (Morris, MN; Grand Rapids, MN), cultivar ('Blue Flax', 'Sapphire') and treatment (DI water, floral preservative) all consisted of two levels, therefore post-hoc tests were not conducted to compare differences between groups. Pearson correlations (r values) were also calculated for all phenotypic traits measured. These statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 25 for Windows, SPSS, Inc., Chicago, IL). Chi-square tests (χ^2) were used to evaluate whether the reasons

for termination had an equal frequency within (1:1:1:1 χ^2) and among (1:1 χ^2) treatments and cultivars.

Experiment 2

Data were analyzed using independent factorial ANOVAs and mean separations (5% Tukey's Honestly Significant Difference, HSD, $\alpha = 0.05$) to compare the influence of treatment, species, and genotype on total vase life (d), average number of flowers open, percent of initial buds opened, average daily water loss (ml), flower diameter (mm), and individual flower longevity (d). The factor of treatment consisted of two levels (DI water, floral preservative), therefore post-hoc tests were not conducted. Additional independent factorial ANOVAs, along with mean separations, were conducted to compare the effect of species and genotype on pre-test phenotypic data, which included stem length (cm), length to the first branch (cm), stem diameter (mm), number of previous flowers, number of seed pods, number of viable buds, and number of branches. Pearson correlations (r values) were also calculated for all phenotypic traits. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 25 for Windows, SPSS, Inc., Chicago, IL). Chi-square tests (χ^2) were used to evaluate whether the reasons for termination had an equal frequency within (1:1:1:1 χ^2) and among (1:1 χ^2) treatment groups, and among (1:1:1 χ^2) species.

Results and Discussion

Experiment 1

Vase life

For both cultivars, the floral preservative solution resulted in a longer vase life compared to the DI water control, and the mean vase life across all treatments was 9.22 d (Table 3-2). The effect of cultivar on vase life was not significant, although ‘Blue Flax’ had a slightly longer vase life for both treatments compared to ‘Sapphire’ (Table 3-2). The effect of treatment on total vase life was highly significant ($p \leq .001$), and the floral preservative extended the vase life of both cultivars by > 1 d, on average. No other factors, or their interactions, had a significant effect on vase life.

The pooled 1:1:1:1 χ^2 testing equal distribution of termination reason deviated significantly ($p \leq .001$) from the expected ratio, indicating that some reasons for termination of vase life were more frequently observed overall (Table 3-3). Evaluating the 1:1:1:1 χ^2 within cultivars also revealed significant deviation for both ‘Blue Flax’ ($p \leq .001$) and ‘Sapphire’ ($p \leq .01$) from the expected ratio (Table 3-3). However, the 1:1 χ^2 tests among cultivars show that ‘Blue Flax’ and ‘Sapphire’ show a similar distribution of reasons for termination, with the exception of leaf wilt ($p \leq .05$), which was more frequently observed in ‘Sapphire’ than in ‘Blue Flax’ (Table 3-3).

Within each cultivar, the 1:1:1:1 χ^2 shows that both treatments deviate significantly from the equal distribution (Table 3-3). An elevated incidence of flower bud chlorosis was observed for the DI water treatment for both cultivars relative to the floral

preservative treatment (Table 3-3). The 1:1 χ^2 comparing the incidence of flower bud chlorosis among treatments is highly significant ($p \leq .001$) for both 'Blue Flax' and 'Sapphire,' and only four floral preservative vases total were terminated because of flower bud chlorosis (Table 3-3). This result is likely due to the lack of nutrients in the DI water solution, as cut flowers commonly require a carbohydrate source for postharvest development of flower buds (Doorn, 1996; Reid and Jiang, 2012; Vehniwal and Abbey, 2019).

For both cultivars, the floral preservative treatment had a greater proportion of terminations due to flower bud wilt and leaf chlorosis compared to the DI water treatment (Table 3-3). For 'Blue Flax,' the 1:1 χ^2 tests for the incidence of flower bud wilt and leaf chlorosis deviated significantly ($p \leq .001$) from an equal distribution across treatments (Table 3-3). Combined, flower bud wilt and leaf chlorosis made up 87% of vase life terminations among the 'Blue Flax' floral preservative group. Within 'Sapphire,' significant deviations from the 1:1 χ^2 were observed for leaf chlorosis ($p \leq .001$) and flower bud wilt ($p \leq .05$), indicating that the incidence of these reasons for termination is dependent on treatment. Combined, leaf chlorosis and flower bud wilt accounted for 77% of all terminations among the 'Sapphire' floral preservative group.

Number of flowers

There was a significant effect of treatment ($p \leq .001$) on the total number of flowers observed (Table 3-2). Cultivar was not a significant factor, and 'Blue Flax' and

‘Sapphire’ had a statistically similar number of flowers, regardless of treatment (Table 3-2). Overall, vases with floral preservative solution resulted in > 2x the mean number of flowers compared to vases containing DI water (Table 3-2). No other factors, or their interactions, had a significant effect on the total number of flowers (Table 3-2).

Percent of flower buds opened

There was a significant effect of treatment ($p \leq .001$) on the percent of flower buds opened (Table 3-2). On average, the floral preservative treatment resulted in ~59% of buds opened, whereas only ~24% of buds opened in the DI water treatment (Table 3-2). No other factors, or their interactions, had a significant effect on the percentage of flower buds open (Table 3-2).

Average daily water loss

The average daily water loss was highly significantly different ($p \leq .001$) for cultivar, treatment, and their interaction (Table 3-2). ‘Sapphire’ had similar daily water loss across the treatments, whereas the ‘Blue Flax’ floral preservative treatment had, on average, > 2x the rate of water loss compared to the ‘Blue Flax’ DI water treatment (Table 3-2). It is difficult to discern what might be causing this significant ($p \leq .001$) cultivar by treatment interaction given the lack of correlation between average daily water loss and other traits, with the exception of a significant negative correlation

($r = .215$, $p \leq .01$) between average daily water loss and percent of flower buds opened (Table 3-4).

Flower diameter

A highly significant effect of cultivar ($p \leq .001$) on flower diameter was observed, and 'Sapphire' had a significantly smaller flower diameter across treatments compared to 'Blue Flax' (Table 3-2). Additionally, a significant effect of treatment ($p \leq .01$) on flower diameter was observed. Across cultivars, the floral preservative treatment resulted in larger flower diameter, on average, suggesting that the floral preservative encouraged healthy postharvest floral development. There was a significant effect of location ($p \leq .05$), and a significant cultivar by location interaction ($p \leq .01$) (Table 3-2). 'Blue Flax' stems from the Grand Rapids location were observed to have larger average flower diameter (M ± SE; 19.68 ± 0.32) compared to the Morris location (17.92 ± 0.33). Conversely, 'Sapphire' was observed to have slightly larger flower diameter for stems from Morris (16.07 ± 0.22) compared to Grand Rapids (15.27 ± 0.30).

Individual flower longevity

There was a highly significant ($p \leq .001$) effect of cultivar on individual flower longevity, and 'Sapphire' was observed to have an individual flower longevity ~2 d longer than 'Blue Flax,' on average (Table 3-2). No other factors, or their interactions, had a significant effect on flower longevity (Table 3-2). Finger taps were added to the experimental protocol in 2019 since the flower longevity observed in 2018 was not

representative of field observations (data not shown), in which petal drop occurred ~midday. Perennial flax are known to produce new flowers every morning, followed by petal drop in the afternoon (Addicott, 1977; Eastman, 1968; Vaisey-Genser and Morris, 2003). Additionally, flower longevity had a strong negative correlation with flower diameter in 2018 ($r = -.346$, $p \leq .001$) (Table 3-3). This link to flower diameter might partially explain the difference in flower longevity between the two cultivars. The larger petals of 'Blue Flax' had greater surface area for evapotranspiration which could have encouraged rapid petal drop. Further studies would be required to determine the exact cause(s) of this petal drop.

Highly significant correlations ($p \leq .001$) were observed between vase life and number of flowers ($r = 0.405$; Table 3-3), percent of initial buds opened ($r = 0.448$), and individual flower longevity ($r = 0.330$). This suggests a logical relationship between the overall health of the stem and postharvest floral development. The high correlation coefficient for average number of flowers and the percentage of initial buds opened ($r = 0.720$, $p \leq .001$) may be explained by the fact that total number of flowers is one of the inputs for the calculation of percent of flower buds opened. The significant ($p \leq .01$) correlation between flower diameter and percent of flower buds opened is more difficult to interpret but may be due to the greater average flower diameter and percent of flower buds opened for 'Blue Flax' compared to 'Sapphire,' or because of the increased mean values for both traits in the floral preservative treatment compared to the DI water treatment.

Experiment 2

Vase life

ANOVAs showed a highly significant ($p \leq .001$) effect of treatment on vase life (Table 3-5). Pooled across species, the floral preservative treatment resulted in a vase life increase of 1.63 d, on average (Table 3-6). Based on a two sample t-test (two-tailed $\alpha = 0.05$, $p = 0.88$), this was statistically similar to the 1.67 d increase observed among pooled cultivars in Experiment 1 (Table 3-2). A small but significant effect of species on vase life was observed ($p = 0.40$; Table 3-5) but means separations could not be differentiated (Table 6). Overall, *L. perenne* had the longest vase life (9.25 d, Table 3-6). Additionally, a small but significant species by treatment interaction was observed for vase life (Table 3-5), and differential responses to the floral preservative treatment were observed among species (Table 3-7). The greatest mean vase life difference between treatments was observed for *L. austriacum* (2.59 d), followed by *L. perenne* (1.66 d), and *L. lewisii* (0.83 d); the *L. austriacum* treatment groups had both the longest and shortest average vase life observed when comparing the results by species and treatment (Table 3-7).

A significant ($p \leq .001$) effect of genotype was also observed for vase life (Table 3-5), and means separations showed statistical differences among genotypes, suggesting that mean vase life could be improved through breeding and selection (Table 3-8). For example, although *L. lewisii* had the lowest average vase life of any species, it also contained genotype PI 522305 #3, which had the longest average vase observed in this

study (11.33 d; Table 3-8). This genotype had significantly greater vase life compared to *L. austriacum* genotypes Ames 29749 #12 and PI 502410 #3; *L. lewisii* genotypes Ames 31369 #15, Ames 32565 #17, CN 107266 #10, and PI 650320 #5; and it exhibited statistically comparable performance to all *L. perenne* genotypes. This result highlights the importance of considering mean genotypic and species differences when making selections for improved vase life. Of the species tested, *L. perenne* exhibited the most uniform performance on a genotype basis, as no significant mean differences were observed among *L. perenne* genotypes.

As with Experiment 1, the reasons for termination of vase life were categorized as either chlorosis or wilt observed in the flower buds (flower bud wilt/chlorosis) or leaf/stem tissues (leaf wilt/chlorosis). The 1:1:1:1 χ^2 testing equal distribution of termination reason deviated significantly ($p \leq .001$) from the expected ratio within all three species, and within the pooled group (Table 3-9). However, the 1:1:1 χ^2 tests evaluating the distribution of reason for termination among species were not significantly different except for flower bud wilt ($p \leq .05$), which had a lower rate of incidence within *L. perenne* compared to the other species (Table 3-9). Within *L. austriacum*, the DI water ($p \leq .01$) and floral preservative ($p \leq .001$) treatments deviated significantly from the 1:1:1:1 χ^2 ratio, although the 1:1 χ^2 tests for among treatment differences were all nonsignificant (Table 3-9). This indicates that some reasons for termination were observed more/less frequently within treatments, but that the relative frequency did not differ based on treatment. In contrast, within *L. lewisii*, significant deviations from the expected ratio were observed both within (1:1:1:1 χ^2; $p \leq .001$) and among (1:1 χ^2;

$p \leq .01$) treatments for flower bud and leaf chlorosis, but not for flower bud and leaf wilt (Table 3-9). A greater incidence of flower bud chlorosis was observed for the DI water treatment, whereas a greater incidence of leaf chlorosis was observed for the floral preservative treatment (Table 3-9). Similarly, within *L. perenne*, the DI water ($p \leq .01$) and floral preservative ($p \leq .001$) treatments deviated significantly from the 1:1:1:1 χ^2 ratio, and the 1:1 χ^2 tests for among treatment differences were significant ($p \leq .01$) for flower bud and leaf chlorosis, but not flower bud and leaf wilt (Table 3-9). Again, a greater incidence of flower bud chlorosis was observed in the DI water treatment, whereas a greater incidence of leaf chlorosis was observed in the floral preservative treatment (Table 3-9).

Number of flowers

ANOVAs showed a significant effect of species ($p \leq .01$) and treatment ($p \leq .001$) on the number of flowers per stem, and the species by treatment interaction was not significant (Table 3-5). On average, 5.70 flowers per stem were observed for the floral preservative treatment, whereas 3.03 flowers per stem were observed for DI water vases (Table 3-6). Among species, *L. perenne* had a significantly greater number of flowers per stem (5.56) compared to *L. lewisii* (3.32) (Table 3-6). *Linum austriacum* was intermediate between these (4.22) and did not differ significantly from either species (Table 3-6).

ANOVAs also showed a significant effect of genotype on the number of flowers per stem (Table 5), and significant differences among genotypes were observed (Table

3-8). These data suggest that overall cut flower performance cannot be evaluated based on vase life alone. For example, while *L. lewisii* genotype PI 522305#3 and *L. austriacum* genotype CN 107255 #17 were statistically greater than several other genotypes in terms of vase life, these genotypes also had significantly fewer flowers compared to several genotypes, including *L. austriacum* genotype PI 650295 #14, and *L. perenne* genotypes Ames 21222 #16, CN 19024 #8, and PI 445972 #3 (Table 3-8). Furthermore, the genotype with the greatest number of flowers, *L. perenne* CN 19024 #8, also had a mean vase life that was statistically greater than several genotypes including *L. austriacum* Ames 29749 #12, and *L. lewisii* Ames 32565 #15 and PI 650320 #5 (Table 3-8). Altogether, these results highlight the importance of selecting for improved postharvest floral development (measured as number of flowers), in addition to vase life.

Percent of initial buds opened

Similar to the total number of flowers per stem, the ANOVAs for percent of flower buds opened revealed significant effects of treatment ($p \leq .001$) and species ($p \leq .05$), with nonsignificant interaction effects (Table 3-5). The floral preservative treatment resulted in a greater percent of flower buds opened per stem (50.39%) compared to the DI water control (26.18%). Among species, *L. perenne* had a significantly greater percent of flower buds opened (44.86%) compared to *L. lewisii* (32.80%), and *L. austriacum* was once again intermediate (37.20%), not differing significantly from either species (Table 3-6). The ANOVA also showed a significant

effect of genotype on percent of flower buds opened (Table 3-5), and significant differences among genotypes for percent of flower buds opened were observed, although the majority of genotypes tested were statistically similar (Table 3-8). Most notably, the genotype with the greatest number of flowers, *L. perenne* CN 19024 #8, also had the highest percent of flower buds opened, indicating that the significantly greater number of flowers observed for this genotype was actually due to improved postharvest floral development, rather than a greater initial number of buds (Table 3-8). In terms of the percent of flower buds opened, genotype CN 19024 #8 was significantly greater than *L. austriacum* genotype Ames 29749 #12, and *L. lewisii* genotypes Ames 32565 #13 and Ames 32565 #17 (Table 3-8). These results suggest that percent of flower buds opened should be considered alongside the total number of flowers when selecting for improved postharvest floral development.

Average daily water loss

Analysis of variance showed a significant effect of species ($p \leq .001$), genotype ($p \leq .001$), and genotype x treatment interaction ($p \leq .05$) on average daily water loss (Table 3-5). The effects of treatment and treatment x species interaction were not significant (Table 3-5). Significantly greater daily water loss was observed for *L. austriacum* (3.19 ml/d) compared to *L. perenne* (2.24 ml/d) and *L. lewisii* (1.79 ml/d) (Table 3-6). Among genotypes, significant differences in daily water loss mean values

were observed, ranging from 0.61 ml/d (*L. lewisii*; Ames 32565 #13) to 4.78 ml/d (*L. austriacum*; PI 502410 #3) (Table 3-8).

Flower diameter

The effect of species, treatment, and species x treatment interaction on flower diameter were all nonsignificant (Table 3-5). However, when genotypes were tested independent of species, a significant ($p \leq .001$) effect of genotype and treatment were observed, as well as a significant ($p \leq .05$) genotype x treatment interaction, which shows genotype-specific responses to vase solution treatment (Table 3-5, Figure 3-1). The sample size (n) for flower diameter observations varied since flowers were only measured once fully open, and petal drop sometimes occurred before the measurement could be recorded (Table 3-8). Significant differences among genotypes were observed for mean flower diameter both within and among species. For example, *L. perenne* contained the genotypes with the largest (31.80 mm; PI 445972 #3) and the smallest (17.83 mm; CN 19024 #8) mean flower diameters observed in the study, which were significantly different from each other, as well as many of the other genotypes tested. Despite having the longest vase life and greatest number of flowers, the small flower diameter of CN19024 #8 detracts from the overall cut flower performance of this genotype. Depending on the goals for selection, it may instead be more advantageous to favor a genotype such as PI 445972 #3, which has a statistically similar vase life and number of flowers as CN 19024 #8, but a significantly larger flower diameter. This example further

illustrates the importance of considering multiple traits during selection for cut flower performance.

Individual flower longevity

Interestingly, individual flower longevity (d) was not significant for any factor (Table 3-5), and a grand mean of 1.36 d was observed (Table 3-6). Among species, *L. perenne* had slightly elevated flower longevity (1.47 d), on average, compared to *L. austriacum* (1.34 d) and *L. lewisii* (1.30 d), although the differences were not significant. Flower longevity data has not yet been measured in the field, but anecdotally, flowers of perennial flax tend to open in the morning around 0600-0700 h and drop by mid-afternoon, between 1300-1700 h, which is consistent with a previous report on flower abscission in *L. lewisii* (Addicott, 1977). Therefore, even with finger taps added to the protocol, the individual flower longevity is still longer than expected based on field observations. Several genotypes have been identified visually in the field which hold their petals late into the afternoon, so future vase life evaluations will compare whether these show significant improvement over previous observations. Overall, the lack of significant variation among genotypes for individual flower longevity poses one of the greatest challenges for selection of improved cut flower flax (Table 3-8).

Trait correlations

Vase life was highly significantly ($p \leq .001$) correlated with the number of flowers ($r = 0.325$), the percent of flower buds opened ($r = 0.476$), and individual flower longevity ($r = 0.367$; Table 3-10). The connection between vase life, number of flowers, and percent of buds open is probably because stems with a longer vase life have more time for floral development. Significant negative correlations ($p \leq .05$) were also found between vase life and both average daily water loss ($r = -0.170$) and flower diameter ($r = -0.212$; Table 3-10). Average daily water loss showed a significant positive correlation ($r = 0.234$; $p \leq .01$) with number of flowers, logically indicating that floral development increases water use and transpiration (Table 3-10). The number of flowers was also significantly correlated ($r = 0.674$, $p \leq .001$) with the percent of flower buds opened (Table 3-10). Interestingly, the only significant ($p \leq .001$) correlations with flower longevity were with total vase life ($r = 0.367$) and percent of initial buds open ($r = 0.304$, $p \leq .001$; Table 3-10), and there was not a correlation between flower diameter and flower longevity like in Experiment 1 (Table 3-6).

Indirect selection for postharvest traits

Attempts to find an easily recorded phenotypic trait which correlated with postharvest outcomes had mixed results. Of the traits measured at the start of the experiment, only the length to the first branch showed significant ($r = 0.166$; $p \leq .05$) correlation with vase life (Table 3-10). In contrast, several traits showed significant ($p \leq .001$) positive correlation with the number of flowers, including the number of

previous flowers (r = 0.281), the number of viable buds (r = 0.474), and the number of branches (r = 0.275); a significant positive correlation (r = 0.178; $p \leq .05$) was also found between the number of seed pods and the number of flowers (Table 3-10). Additionally, the number of branches and the number of viable buds were highly significantly correlated (r = 0.576; $p \leq .001$; Table 3-10). Taken together, these results suggest that selection for genotypes with a greater length to the first branch, but a larger number of branches would increase the number of flowers per stem, and perhaps even positively affect vase life.

Highly significant positive correlations ($p \leq .001$) with average daily water loss were observed for all traits measured at the start of the experiment except for the length to the first branch (Table 3-10). It makes logical sense that stems with greater length, diameter, number of seed pods, number of viable buds, and number of branches would require greater daily water intake. The significant positive correlation (r = 0.558; $p \leq .001$) between daily water loss and number of previous flowers is more difficult to interpret, since all that remains attached to the stem from previous flowers is a small ~1-2 cm pedicel. However, the significant positive correlations ($p \leq .001$) observed between number of previous flowers and stem length (r = 0.337); stem diameter (r = 0.325) suggest that number of previous flowers is related to the a size of the stem, which may explain the correlation with average daily water loss (Table 3-10). Alternatively, the significant positive correlation (r = 0.281; $p \leq .001$) between the number of previous flowers and number of flowers opened during the experiment may simply indicate that a greater number of previous flowers is reflective of a healthier, more

reproductive stem, which then has more postharvest floral development and therefore more water use.

Overall, the high degree of correlation between the initial morphological measurements indicates redundancy, which suggests that several of these initial measurements could be dropped in future experiments. For example, the number of previous flowers is highly significantly correlated with the number of branches ($r = 0.684$, $p \leq .001$), and both of these traits show similar correlations with average daily water loss, and number of flowers (Table 3-10). Similarly, the number of previous flowers and the number of seed pods show very similar correlation coefficients across all other traits and are themselves highly significantly correlated ($r = 0.911$, $p \leq .001$). Therefore, future experiments could benefit from including one of these initial counts, but not all three.

The effect of species and genotype on traits measured at the start of the experiment was significant for the number of branches ($p \leq .01$), stem diameter ($p \leq .05$) and number of previous flowers ($p \leq .05$) (Table 3-11). A highly significant ($p \leq .001$) effect of genotype was observed for stem length, length to the first branch, stem diameter, and number of viable buds; a significant ($p \leq .05$) effect on number of previous flowers, number of seed pods, and number of branches was also observed (Table 3-11). Among species, *L. austriacum* had a significantly greater number of branches compared to *L. lewisii*, and *L. perenne* was intermediate, not differing significantly from either species (Table 3-12). The same pattern was observed for stem diameter and number of viable

buds; for both of these, *L. austriacum* was significantly greater than *L. lewisii* (Table 3-12).

Among genotypes, significant differences were observed for all traits except for the number of previous flowers and the number of branches (Table 3-13). These data present an opportunity to select genotypes with favorable combinations of stem phenotype and cut flower performance. For example, *L. perenne* genotype CN 107270 #9 has a significantly greater stem length and length to the first branch compared to many of the other genotypes tested, making it superior for use in floral designs (Table 3-13); this genotype also has an average vase life that is 1.42 d greater than the mean vase life for *L. perenne* (Table 3-6). Altogether, the methods employed herein are able to distinguish statistical differences among genotypes for several traits important to cut flower performance, including stem phenotypes and postharvest traits. These will be used moving forward to screen elite breeding lines for overall cut flower performance.

Conclusions

Perennial flax has the potential to perform well as a cut flower crop that can be used as a filler material to add vibrant true blue color to floral arrangements (Figure 3-2). Based on this study, we recommend using bunches of stems of the species *L. perenne* or *L. austriacum* in a floral preservative that is changed 1-2x per week. If following this approach, the average expected vase life of ~9 d meets industry standards, and 4-5 flowers per stem can be expected to open over that time frame. It is important to note that

these results are for wild, unselected materials, and that performance improvements could be achieved through breeding. Based on the variation observed among genotypes in this study, it should be possible to increase the average vase life of perennial flax to over 11 d, with over 10 flowers per stem. Future experiments will also be needed to optimize postharvest storage conditions for shipping harvested flax stems. Cold treatment is used in some species to extend storage life, although adverse effects on post-treatment vase life are commonly observed (Redman et al., 2002; Skutnik et al., 2020). In both experiments, the stems exhibited no obvious signs of damage after short-term storage at 4 °C. Perennial flax exhibits excellent frost tolerance in the field, so even colder storage temperatures may be needed to prevent flower opening during multiple days of transit and storage, keeping in mind the tradeoffs between storage temperature, storage time, vase life (Redman et al., 2002; Skutnik et al., 2020).

The proclivity towards rapid petal drop introduces challenges for both retail and wholesale florists, who need to be sure that their products are in peak condition at the time of sale or presentation. Follow-up experiments are planned to test the ethylene sensitivity of perennial flax flowers using silver thiosulphate (STS) or 1-methylcyclopropene (MCP), which have been shown in some species to increase vase life, delay flower senescence, and enhance flower quality (Dole et al., 2009; Elhindi, 2012; Vehniwal and Abbey, 2019). Breeding efforts to select for improved petal holding capacity are also currently underway, and several genotypes have been identified in field trials with superior flower longevity. The issue of petal drop can also be entirely avoided by harvesting stems late in the growing season after seed set has occurred, but before

capsule maturity. In the first year of growth, this usually occurs from August-September; in the second year, the green boll stage may come as early as June. The round green seed capsules transform flax into a different kind of filler material, by contributing a unique texture rather than color (Figure 3-3).

The morphological similarities between *L. austriacum*, *L. perenne*, and *L. lewisii*, and their potential for hybridization, suggest that a cautious approach should be taken when interpreting species comparisons (Jhala et al., 2008; Ockendon, 1971, 1968; Pendleton et al., 2008; Seetharam, 1972). There could be significant error introduced if any of the accessions were misclassified during the curation process. Future studies will use SNP or other molecular markers to more conclusively delineate individual species. From a breeder's perspective, this potential to hybridize is still a positive attribute, as it introduces the possibility of capturing the best traits from all three species within a general perennial flax breeding program. The specific and genotypic differences observed in this study will enable selections for improved cut flower performance that advance the goal of developing new cut flower flax varieties. This study also contributes to the body of knowledge about the ornamental potential of various wild flax species, which should prove useful to future ornamental *Linum* breeders.

Tables

Table 3-1. *Linum* accessions tested in Experiment 2, obtained as seed from the United States Department of Agriculture Germplasm Resources Information Network (USDA-GRIN) or the Plant Gene Resources of Canada Genetic Resource Information Network – Canadian Version (GRIN-CA). Accession and plant # combined form the genotype codes referenced throughout (e.g. Ames 29749 #10).

Species	Accession	Plant #	Seed source
L. austriacum	Ames 29749	10	USDA-GRIN
	Ames 29749	12	USDA-GRIN
	CN 107255	4	GRIN-CA
	CN 107255	17	GRIN-CA
	PI 502410	3	USDA-GRIN
	PI 650293	20	USDA-GRIN
	PI 650294	14	USDA-GRIN
	PI 650295	14	USDA-GRIN
	PI 650299	16	USDA-GRIN
L. lewisii	Ames 31369	15	USDA-GRIN
	Ames 31371	4	USDA-GRIN
	Ames 32565	13	USDA-GRIN
	Ames 32565	17	USDA-GRIN
	CN 107266	5	GRIN-CA
	CN 107266	6	GRIN-CA
	CN 107266	10	GRIN-CA
	PI 650320	5	USDA-GRIN
	PI 650320	13	USDA-GRIN
	PI 522305	3	USDA-GRIN

Species	Accession	Plant #	Seed source
L. perenne	Ames 21222	16	USDA-GRIN
	CN 107270	5	GRIN-CA
	CN 107270	9	GRIN-CA
	CN 107283	1	GRIN-CA
	CN 19024	8	GRIN-CA
	PI 445972	3	USDA-GRIN
	PI 650328	2	USDA-GRIN
	PI 650328	15	USDA-GRIN

Table 3-2. Perennial flax cultivars tested (*L. perenne* 'Blue Flax' and 'Sapphire'), vase solution treatment (DI=deionized, distilled water; FP=floral preservative), and mean vase life (d), number of flowers, percent of initial flower buds opened, average daily water loss (ml), flower diameter (mm), and individual flower longevity (d). ANOVA results are presented at the base of the table directly below each trait. ANOVA results also include the factor of location, which was not included in the upper part of the table, as it was a nonsignificant factor for all traits besides flower diameter.

Cultivar	Tmt.	df	Vase life (d)	# flowers	% flower buds opened	Avg. daily water loss (ml)	Flower diam. (mm)	Indiv. flower longevity (d)
Blue Flax	DI		8.52	2.67	24.36	3.88	17.23	3.56
	FP		10.20	6.56	60.8	1.75	19.37	3.27
Sapphire	DI		8.21	2.65	22.8	1.82	15.14	5.30
	FP		9.86	6.74	56.5	1.70	16.23	5.25
Pooled	DI		8.38	2.66	23.66	2.96	16.23	4.35
	FP		10.05	6.64	58.91	1.73	17.98	4.15
Grand Mean			9.22	4.65	41.19	2.36	17.22	4.23
					ANOVA			
Source of Variation								
Cultivar (C)		1	NS†	NS	NS	***	***	***
Treatment (T)		1	***	***	***	***	**	NS
Location (L)		1	NS	NS	NS	NS	*	NS
C x T		1	NS	NS	NS	***	NS	NS
C x L		1	NS	NS	NS	NS	**	NS
T x L		1	NS	NS	NS	NS	NS	NS
C x T x L		1	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively
†NS, nonsignificant.

Table 3-3. Reason for termination of vase life by cultivar ('Blue Flax' and 'Sapphire') and treatment (DI = deionized water, FP = floral preservative). Chi square tests for equal distribution of termination reason, within (1:1:1:1 χ^2) and among (1:1 χ^2) treatments and cultivars.

Subset	Reason for termination of vase life				1:1:1:1 χ^2 (df = 3)
	Flower bud chlorosis	Flower bud wilt	Leaf chlorosis	Leaf wilt	
Blue Flax					
DI	45	6	2	1	99.04 ***
FP	3	29	18	4	34.15 ***
1:1 χ^2 among treatments (df = 1)	36.75 ***	15.11 ***	12.8 ***	1.8 NS†	
Sapphire					
DI	34	6	0	6	68.72 ***
FP	1	18	15	9	15.69 **
1:1 χ^2 among treatments (df = 1)	31.11 ***	6.00 *	15.00 ***	3.00 *	
Both cultivars (treatments pooled)					
Blue Flax	48	35	20	5	38.44 ***
Sapphire	35	24	15	12	14.93 **
Pooled	83	59	35	17	51.03 ***
1:1 χ^2 among cultivars (df = 1)	0.16 NS	0.32 NS	0.03 NS	4.74 *	

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively
† NS = not significant

Table 3-4. Pearson correlation coefficients (r) among all traits measured in Experiment 1 including, vase life (d), number of flowers, percent of initial flower buds opened, average daily water loss (ml), flower diameter (mm), and individual flower longevity (d). Two-tailed $\alpha = 0.05$.

Trait	Vase life	# flowers	% flower buds opened	Avg. daily water loss	Flower diam.
# flowers	.405 ***	1			
% flower buds opened	.448 ***	.720 ***	1		
Avg. daily water loss	-.048	-.109	-.215 **	1	
Flower diam.	.149	.125	.224 **	.006	1
Indiv. flower longevity	.330 ***	-.002	-.023	-.116	-.346 ***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively (two-tailed)

Table 3-5. ANOVA (df, F ratio, Prob > F) for the effects of species, genotype, treatment, and their interactions on vase life (d), total number of flowers, percent of initial flower buds opened during the experiment, average daily water loss (ml), flower diameter (mm), and individual flower longevity (d).

		Vase Life (d)		**# flowers**		**% flower buds opened**		**Avg. daily water loss (ml)**		**Flower diam. (mm)**		**Indiv. flower longevity (d)**	
Effect	**df**	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***
Species	2	3.29	.040	6.67	.002	4.20	.017	11.15	≤.001	1.00	.371	0.77	.463
Treatment	1	25.58	≤.001	28.30	≤.001	50.56	≤.001	0.58	.448	1.70	.196	1.31	.254
Species x treatment	2	3.28	.040	1.14	.322	1.06	.349	0.68	.507	2.74	.070	2.41	.094
Genotype	26	3.44	≤.001	6.47	≤.001	2.34	≤.001	4.69	≤.001	7.53	≤.001	1.51	.082
Treatment	1	33.62	≤.001	53.19	≤.001	58.40	≤.001	1.02	.314	12.77	≤.001	0.77	.381
Genotype x treatment	26*	1.30	.175	1.93	.010	1.04	.427	1.72	.029	2.29	.011	1.45	.104

*df = 23 for flower diameter ANOVA for genotypes dropped due to low # observations (n < 2) (see N/A; Table 3-8)

Table 3-6. Mean ± SE trait values by treatment (DI = deionized water; FP = floral preservative) and species for vase life (d), number of flowers, percent of initial flowered buds opened, average daily water loss (ml), flower diameter (mm), and individual flower longevity (d). Mean separations based on Tukey's 5% HSD results for significant main effects.

	n	Vase Life (d)	# flowers	% flower buds opened	Avg. daily water loss (ml)	n	Flower diam. (mm)	n	Indiv. flower longevity (d)
Treatment									
DI	81	8.07 ± 0.23	3.03 ± 0.36	26.18 ± 2.41	2.31 ± 0.18	35	24.33 ± 0.64	67	1.28 ± 0.10
FP	81	9.70 ± 0.23	5.70 ± 0.36	50.39 ± 2.41	2.50 ± 0.18	55	23.25 ± 0.52	75	1.43 ± 0.09
Species									
L. austriacum	54	9.07 ± 0.28 a	4.22 ± 0.43 ab	37.20 ± 2.94 ab	3.19 ± 0.218 a	32	24.50 ± 0.73	43	1.29 ± 0.12
L. lewisii	60	8.32 ± 0.26 a	3.32 ± 0.41 a	32.80 ± 2.79 a	1.79 ± 0.21 b	31	23.09 ± 0.69	55	1.30 ± 0.10
L. perenne	48	9.25 ± 0.30 a	5.56 ± 0.46 b	44.86 ± 3.12 b	2.24 ± 0.23 b	27	23.78 ± 0.75	44	1.47 ± 0.12
Grand Mean	162	8.88 ± 0.16	4.34 ± 0.25	38.29 ± 1.70	2.40 ± 0.126	90	23.79 ± 0.42	142	1.36 ± 0.07

Table 3-7. Mean ± SE values for vase life (d) showing the interaction of species and treatment (DI = deionized water; FP = floral preservative).

Species	Tmt.	Vase life (d)
L. austriacum	DI	7.78 ± 0.39
	FP	10.37 ± 0.39
L. lewisii	DI	8.00 ± 0.37
	FP	8.63 ± 0.37
L. perenne	DI	8.42 ± 0.42
	FP	10.08 ± 0.42

Table 3-8. Mean ± SE trait values on a genotype basis for each species for vase life (d), number of flowers, percent of initial flower buds opened, average daily water loss (ml), flower diameter (mm), and individual flower longevity (d). The sample numbers (n) varied for the traits examined. Mean separations based on Tukey's 5% HSD results for significant main effects.

Genotype	n	Vase Life (d)	# flowers	% flower buds opened	Avg. d wtr loss (ml)	n	Flower diam. (mm)	n	Indiv. flr long. (d)
L. austriacum									
Ames 29749 #10	6	9.50 ± 0.71 a-e	4.83 ± 0.94 a-c	35.46 ± 8.13 a-c	3.75 ± 0.53 c-e	4	24.13 ± 1.32 a-d	5	1.30 ± 0.33
Ames 29749 #12	6	7.00 ± 0.71 ab	1.33 ± 0.94 a	15.71 ± 8.13 a	4.50 ± 0.53 de	3	31.47 ± 1.32 e	4	0.92 ± 0.42
CN 107255 #4	6	10.00 ± 0.71 b-e	3.00 ± 0.94 a	47.26 ± 8.13 a-c	2.79 ± 0.53 a-e	0	N/A	4	2.50 ± 0.36
CN 107255 #17	6	11.17 ± 0.71 de	3.00 ± 0.94 a	62.73 ± 8.13 bc	1.15 ± 0.53 a-c	5	23.89 ± 1.04 a-d	6	1.57 ± 0.29
PI 502410 #3	6	7.33 ± 0.71 a-c	4.83 ± 0.94 a-c	28.80 ± 8.13 a-c	4.78 ± 0.53 e	4	27.73 ± 1.32 de	3	1.28 ± 0.44
PI 650293 #20	6	9.33 ± 0.71 a-e	3.17 ± 0.94 a	25.80 ± 8.13 a-c	2.38 ± 0.53 a-e	3	22.68 ± 1.40 a-d	6	0.99 ± 0.29
PI 650294 #14	6	9.33 ± 0.71 a-e	3.83 ± 0.94 ab	39.20 ± 8.13 a-c	3.10 ± 0.53 a-e	4	23.51 ± 1.32 a-d	5	1.50 ± 0.33
PI 650295 #14	6	8.67 ± 0.71 a-e	9.33 ± 0.94 cd	42.80 ± 8.13 a-c	4.25 ± 0.53 de	4	24.08 ± 1.32 a-d	5	1.01 ± 0.33
PI 650299 #16	6	9.33 ± 0.71 a-e	4.67 ± 0.94 a-c	37.02 ± 8.13 a-c	1.98 ± 0.53 a-e	5	23.50 ± 1.04 a-d	5	0.85 ± 0.33
L. lewisii									
Ames 31369 #15	6	7.33 ± 0.71 a-c	3.33 ± 0.94 ab	25.38 ± 8.13 a-c	3.49 ± 0.53 b-e	4	20.72 ± 1.14 a-d	4	2.28 ± 0.42
Ames 31371 #4	6	9.17 ± 0.71 a-e	4.50 ± 0.94 a-c	40.40 ± 8.13 a-c	3.06 ± 0.53 a-e	4	23.79 ± 1.32 a-d	6	1.51 ± 0.29
Ames 32565 #13	6	9.17 ± 0.71 a-e	2.50 ± 0.94 a	23.85 ± 8.13 ab	0.61 ± 0.53 a	2	22.30 ± 1.61 a-d	5	1.33 ± 0.33
Ames 32565 #17	6	7.00 ± 0.71 ab	1.83 ± 0.94 a	24.47 ± 8.13 ab	2.42 ± 0.53 a-e	3	19.85 ± 1.40 ab	6	0.83 ± 0.29
CN 107266 #5	6	8.50 ± 0.71 a-e	4.33 ± 0.94 a-c	29.88 ± 8.13 a-c	0.87 ± 0.53 ab	4	26.98 ± 1.14 c-e	6	1.55 ± 0.29
CN 107266 #6	6	9.33 ± 0.71 a-e	3.50 ± 0.94 ab	36.82 ± 8.13 a-c	2.47 ± 0.53 a-e	4	20.18 ± 1.14 a-c	4	1.55 ± 0.36
CN 107266 #10	6	7.50 ± 0.71 a-d	4.50 ± 0.94 a-c	39.32 ± 8.13 a-c	1.89 ± 0.53 a-d	5	25.40 ± 1.04 b-e	6	1.16 ± 0.29
PI 650320 #5	6	6.17 ± 0.71 a	2.00 ± 0.94 a	26.44 ± 8.13 a-c	1.04 ± 0.53 a-c	3	26.15 ± 1.40 a-d	6	1.27 ± 0.29
PI 650320 #13	6	7.67 ± 0.71 a-e	4.17 ± 0.94 ab	51.20 ± 8.13 a-c	1.37 ± 0.53 a-c	2	21.90 ± 1.61 a-d	6	0.97 ± 0.29
PI 522305 #3	6	11.33 ± 0.71 e	2.50 ± 0.94 a	30.21 ± 8.13 a-c	0.64 ± 0.53 a		N/A	6	1.14 ± 0.29
L. perenne									

Genotype	n	Vase Life (d)	# flowers	% flower buds opened	Avg. d wtr loss (ml)	n	Flower diam. (mm)	n	Indiv. flr long. (d)
Ames 21222 #16	6	8.33 ± 0.71 a-e	8.33 ± 0.94 b-d	57.70 ± 8.13 a-c	2.12 ± 0.53 a-e	3	23.84 ± 1.40 a-d	6	1.86 ± 0.29
CN 107270 #5	6	8.83 ± 0.71 a-e	1.50 ± 0.94 a	36.67 ± 8.13 a-c	2.57 ± 0.53 a-e	3	26.68 ± 1.40 b-e	3	0.94 ± 0.44
CN 107270 #9	6	10.67 ± 0.71 b-e	3.83 ± 0.94 ab	32.38 ± 8.13 a-c	3.04 ± 0.53 a-e		N/A	5	1.74 ± 0.33
CN 107283 #1	6	8.17 ± 0.71 a-e	3.17 ± 0.94 a	32.50 ± 8.13 a-c	2.09 ± 0.53 a-e	6	26.27 ± 0.93 b-e	6	1.84 ± 0.29
CN 19024 #8	6	10.83 ± 0.71 c-e	10.50 ± 0.94 d	68.89 ± 8.13 c	1.66 ± 0.53 a-d	6	17.83 ± 0.93 a	6	1.48 ± 0.29
PI 445972 #3	6	9.83 ± 0.71 a-e	9.33 ± 0.94 cd	46.84 ± 8.13 a-c	2.80 ± 0.53 a-e	2	31.80 ± 1.61 e	6	1.30 ± 0.29
PI 650328 #2	6	8.50 ± 0.71 a-e	3.67 ± 0.94 ab	36.85 ± 8.13 a-c	2.49 ± 0.53 a-e	2	21.78 ± 1.61 a-d	6	1.49 ± 0.29
PI 650328 #15	6	8.83 ± 0.71 a-e	4.17 ± 0.94 ab	47.04 ± 8.13 a-c	1.10 ± 0.53 a-c	4	25.10 ± 1.32 a-d	6	0.92 ± 0.29

Table 3-9. Reason for termination of vase life by species (*L. austriacum*, *L. lewisii*, *L. perenne*) and treatment (DI = deionized water, FP = floral preservative). Chi square tests for equal distribution of termination reason, within (1:1:1:1 χ^2) and among (1:1 χ^2) treatments and among (1:1:1 χ^2) species.

Subset	Reason for termination of vase life				1:1:1:1 χ^2 (df = 3)
	Flower bud chlorosis	Flower bud wilt	Leaf chlorosis	Leaf wilt	
L. austriacum					
DI	8	14	4	1	14.04 **
FP	3	15	8	1	17.30 ***
1:1 χ^2 among treatments (df = 1)	2.27 NS†	0.03 NS	1.33 NS	0.00 NS	
L. lewisii					
DI	11	14	5	0	15.60 ***
FP	2	7	21	0	35.87 ***
1:1 χ^2 among treatments (df = 1)	6.23 **	2.33 NS	9.85 **	N/A	
L. perenne					
DI	12	6	6	0	12.00 **
FP	1	4	18	1	33.00 ***
1:1 χ^2 among treatments (df = 1)	9.31 **	0.40 NS	6.00 **	1.00 NS	
All species (treatments pooled)					
L. austriacum	11	29	12	2	28.22 ***
L. lewisii	13	21	26	0	25.73 ***
L. perenne	13	10	24	1	22.50 ***
Pooled	37	60	62	3	55.83 ***
1:1:1 χ^2 among species (df = 1)	0.56 NS	7.52 *	5.76 NS	2.13 NS	

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively
† NS = not significant

Table 3-10. Pearson correlation coefficients (r) for all traits measured in Experiment 2 including, vase life (d), number of flowers, percent of initial flower buds opened, average daily water loss (ml), flower diameter (mm), individual flower longevity (d), stem length (cm), length to the first branch (cm), stem diameter (mm), number of previous flowers (based on pedicel count), number of seed pods, number of viable flower buds, and number of branches at the apex of the stem. Traits measured prior to the start of the experiment are below the dotted line. Two tailed $\alpha = 0.05$.

Trait	Vase life	# flowers	% flower buds opened	Avg. daily water loss	Flower diam.	Indiv. flower longevity	Stem length	Length to first branch	Stem diam.	# prev. flowers	# seed pods	# viable buds
# flowers	0.325 ***	1										
% flower buds opened	0.476 ***	0.674 ***	1									
Avg. daily water loss	-0.170 *	0.234 **	-0.039	1								
Flower diam.	-0.212 *	-0.202	-0.270 *	0.020	1							
Indiv. flower longevity	0.367 ***	0.131	0.304 ***	-0.032	0.042	1						
Stem length	-0.014	0.083	-0.016	0.422 ***	-0.027	0.113	1					
Length to first branch	0.166 *	-0.140	0.064	-0.033	-0.108	-0.062	0.660 ***	1				
Stem diam.	-0.019	0.147	-0.010	0.496 ***	-0.113	0.129	0.455 ***	0.188 *	1			
# prev. flowers	-0.146	0.281 ***	0.030	0.558 ***	-0.002	0.042	0.337 ***	-0.213 **	0.325 ***	1		
# seed pods	-0.154	0.178 *	-0.015	0.533 ***	0.026	-0.039	0.303 ***	-0.145	0.298 ***	0.911 ***	1	
# viable buds	-0.066	0.474 ***	-0.162 *	0.459 ***	0.057	-0.068	0.200 *	-0.282 ***	0.285 ***	0.397 ***	0.298 ***	1
# branches	-0.113	0.275 ***	-0.059	0.585 ***	0.048	0.073	0.296 ***	-0.231 **	0.365 ***	0.684 ***	0.550 ***	0.576 ***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively (two-tailed)

Table 3-11. ANOVA (df, F ratio, Prob > F) for the effects of species and genotype on stem length (cm), length to the first branch (cm), stem diameter (mm), number of previous flowers, number of seed pods, number of viable buds, and number of branches at the apex of the stem.

		Stem length (cm)		**Length to first branch (cm)**		**Stem diameter (mm)**		**# previous flowers**		**# seed pods**		**# viable buds**		**# branches**	
Effect	**df**	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***
Species	2	1.34	.266	1.01	.366	3.89	.022	2.55	.082	0.75	.476	3.66	.028	5.64	.004
Genotype	26	8.94	≤.001	12.26	≤.001	3.36	≤.001	1.79	.017	1.60	.044	4.27	≤.001	1.67	.033

Table 3-12. Mean ± SE trait values on a species basis for traits measured at the start of the experiment including stem length (cm), length to the first branch (cm), stem diameter (mm), number of previous flowers (based on pedicel count), number of seed pods, number of viable flower buds, and number of branches at the apex of the stem. The sample numbers (n) varied by species. Mean separations based on Tukey's 5% HSD for significant main effects.

Species	n	Stem length (cm)	Length to first branch (cm)	Stem diameter (mm)	# previous flowers	# seed pods	# viable buds	# branches at apex
L. austriacum	54	44.93 ± 1.18	32.80 ± 1.09	1.95 ± 0.04 b	10.91 ± 1.47	4.70 ± 0.96	13.65 ± 0.86 b	5.80 ± 0.34 b
L. lewisii	60	44.27 ± 1.12	33.37 ± 1.03	1.81 ± 0.04 a	8.42 ± 1.39	3.70 ± 0.91	10.43 ± 0.82 a	4.27 ± 0.32 a
L. perenne	48	46.93 ± 1.25	34.98 ± 1.15	1.90 ± 0.04 ab	13.10 ± 1.56	5.33 ± 1.02	12.13 ± 0.92 ab	5.23 ± 0.32 ab
Grand mean	162	45.38 ± 0.68	33.71 ± 0.62	1.89 ± 0.02	10.81 ± 0.85	4.58 ± 0.56	12.07 ± 0.50	5.10 ± 0.19

Table 3-13. Mean ± SE trait values on a genotype basis for traits measured at the start of the experiment including stem length (cm), length to the first branch (cm), stem diameter (mm), number of previous flowers (based on pedicel count), number of seed pods, number of viable flower buds, and number of branches at the apex of the stem. The sample number (n=6) was consistent across genotypes. Mean separations based on Tukey's 5% HSD for significant main effects.

Genotype	Stem length (cm)	Length to first branch (cm)	Stem diameter (mm)	# previous flowers	# seed pods	# viable buds	# branches
L. austriacum							
Ames 29749 #10	57.50 ± 2.34 gh	37.17 ± 1.94 c-f	1.97 ± 0.10 a-c	9.00 ± 4.19 a	4.00 ± 2.74 ab	18.83 ± 2.13 d-f	7.83 ± 0.98 a
Ames 29749 #12	45.50 ± 2.34 b-g	28.33 ± 1.94 a-c	1.78 ± 0.10 a-c	14.83 ± 4.19 a	3.17 ± 2.74 ab	12.00 ± 2.13 a-f	5.00 ± 0.98 a
CN 107255 #4	50.83 ± 2.34 d-h	36.83 ± 1.94 c-e	2.22 ± 0.10 c	12.50 ± 4.19 a	5.00 ± 2.74 ab	6.33 ± 2.13 a-c	6.67 ± 0.98 a
CN 107255 #17	50.33 ± 2.34 c-h	45.67 ± 1.94 e-g	1.77 ± 0.10 a-c	9.67 ± 4.19 a	5.67 ± 2.74 ab	5.50 ± 2.13 ab	4.00 ± 0.98 a
PI 502410 #3	40.17 ± 2.34 a-d	24.83 ± 1.94 a	1.90 ± 0.10 a-c	23.83 ± 4.19 a	15.33 ± 2.74 b	17.00 ± 2.13 c-f	7.17 ± 0.98 a
PI 650293 #20	41.17 ± 2.34 a-d	31.67 ± 1.94 a-d	1.95 ± 0.10 a-c	12.17 ± 4.19 a	5.50 ± 2.74 ab	12.33 ± 2.13 a-f	5.00 ± 0.98 a
PI 650294 #14	43.83 ± 2.34 a-f	35.33 ± 1.94 b-e	2.25 ± 0.10 c	3.67 ± 4.19 a	1.50 ± 2.74 ab	15.50 ± 2.13 a-f	4.00 ± 0.98 a
PI 650295 #14	42.17 ± 2.34 a-e	30.33 ± 1.94 a-d	2.07 ± 0.10 a-c	7.00 ± 4.19 a	1.50 ± 2.74 ab	22.17 ± 2.13 f	7.00 ± 0.98 a
PI 650299 #16	32.83 ± 2.34 a	25.00 ± 1.94 ab	1.65 ± 0.10 a	5.50 ± 4.19 a	0.67 ± 2.74 a	13.17 ± 2.13 a-f	5.50 ± 0.98 a
L. lewisii							
Ames 31369 #15	48.17 ± 2.34 c-h	35.83 ± 1.94 c-e	1.88 ± 0.10 a-c	6.50 ± 4.19 a	3.33 ± 2.74 ab	11.83 ± 2.13 a-f	4.50 ± 0.98 a
Ames 31371 #4	42.00 ± 2.34 a-e	24.17 ± 1.94 a	1.97 ± 0.10 a-c	15.00 ± 4.19 a	8.67 ± 2.74 ab	10.83 ± 2.13 a-f	3.33 ± 0.98 a
Ames 32565 #13	35.17 ± 2.34 ab	27.83 ± 1.94 a-c	1.63 ± 0.10 a	7.00 ± 4.19 a	2.33 ± 2.74 ab	10.17 ± 2.13 a-e	4.67 ± 0.98 a
Ames 32565 #17	43.50 ± 2.34 a-f	36.83 ± 1.94 c-e	1.93 ± 0.10 a-c	9.17 ± 4.19 a	5.17 ± 2.74 ab	7.83 ± 2.13 a-d	4.83 ± 0.98 a
CN 107266 #5	44.33 ± 2.34 a-f	32.00 ± 1.94 a-d	1.68 ± 0.10 a	2.33 ± 4.19 a	0.00 ± 2.74 a	14.67 ± 2.13 a-f	4.00 ± 0.98 a
CN 107266 #6	54.83 ± 2.34 f-h	47.50 ± 1.94 fg	1.98 ± 0.10 a-c	9.00 ± 4.19 a	5.83 ± 2.74 ab	9.00 ± 2.13 a-d	4.00 ± 0.98 a
CN 107266 #10	57.17 ± 2.34 gh	38.83 ± 1.94 d-f	1.70 ± 0.10 ab	11.50 ± 4.19 a	1.83 ± 2.74 ab	12.33 ± 2.13 a-f	5.67 ± 0.98 a
PI 650320 #5	40.17 ± 2.34 a-d	31.17 ± 1.94 a-d	1.67 ± 0.10 a	5.00 ± 4.19 a	1.67 ± 2.74 ab	9.00 ± 2.13 a-d	4.00 ± 0.98 a
PI 650320 #13	39.33 ± 2.34 a-d	30.50 ± 1.94 a-d	1.85 ± 0.10 a-c	11.17 ± 4.19 a	5.00 ± 2.74 ab	8.83 ± 2.13 a-d	3.83 ± 0.98 a
PI 522305 #3	38.00 ± 2.34 a-c	29.00 ± 1.94 a-d	1.80 ± 0.10 a-c	7.50 ± 4.19 a	3.17 ± 2.74 ab	9.83 ± 2.13 a-d	3.83 ± 0.98 a

Genotype	Stem length (cm)	Length to first branch (cm)	Stem diameter (mm)	# previous flowers	# seed pods	# viable buds	# branches
L. perenne							
Ames 21222 #16	45.17 ± 2.34 a-g	34.17 ± 1.94 a-d	1.93 ± 0.10 a-c	9.50 ± 4.19 a	1.17 ± 2.74 ab	14.00 ± 2.13 a-f	4.83 ± 0.98 a
CN 107270 #5	39.67 ± 2.34 a-d	33.50 ± 1.94 a-d	1.75 ± 0.10 a-c	8.17 ± 4.19 a	2.83 ± 2.74 ab	4.50 ± 2.13 a	5.83 ± 0.98 a
CN 107270 #9	60.50 ± 2.34 h	52.83 ± 1.94 g	2.20 ± 0.10 bc	9.17 ± 4.19 a	3.00 ± 2.74 ab	10.67 ± 2.13 a-e	4.50 ± 0.98 a
CN 107283 #1	54.33 ± 2.34 e-h	37.33 ± 1.94 c-f	2.02 ± 0.10 a-c	16.83 ± 4.19 a	8.83 ± 2.74 ab	10.33 ± 2.13 a-e	4.00 ± 0.98 a
CN 19024 #8	44.50 ± 2.34 a-f	29.00 ± 1.94 a-d	1.93 ± 0.10 a-c	21.83 ± 4.19 a	7.00 ± 2.74 ab	16.17 ± 2.13 b-f	7.00 ± 0.98 a
PI 445972 #3	48.33 ± 2.34 c-h	29.00 ± 1.94 a-d	1.93 ± 0.10 a-c	23.17 ± 4.19 a	11.17 ± 2.74 ab	21.33 ± 2.13 ef	7.00 ± 0.98 a
PI 650328 #2	43.17 ± 2.34 a-f	30.17 ± 1.94 a-d	1.87 ± 0.10 a-c	11.00 ± 4.19 a	6.50 ± 2.74 ab	10.83 ± 2.13 a-f	4.33 ± 0.98 a
PI 650328 #15	39.83 ± 2.34 a-d	33.83 ± 1.94 a-d	1.58 ± 0.10 a	5.17 ± 4.19 a	2.17 ± 2.74 ab	9.17 ± 2.13 a-d	4.33 ± 0.98 a

Figures

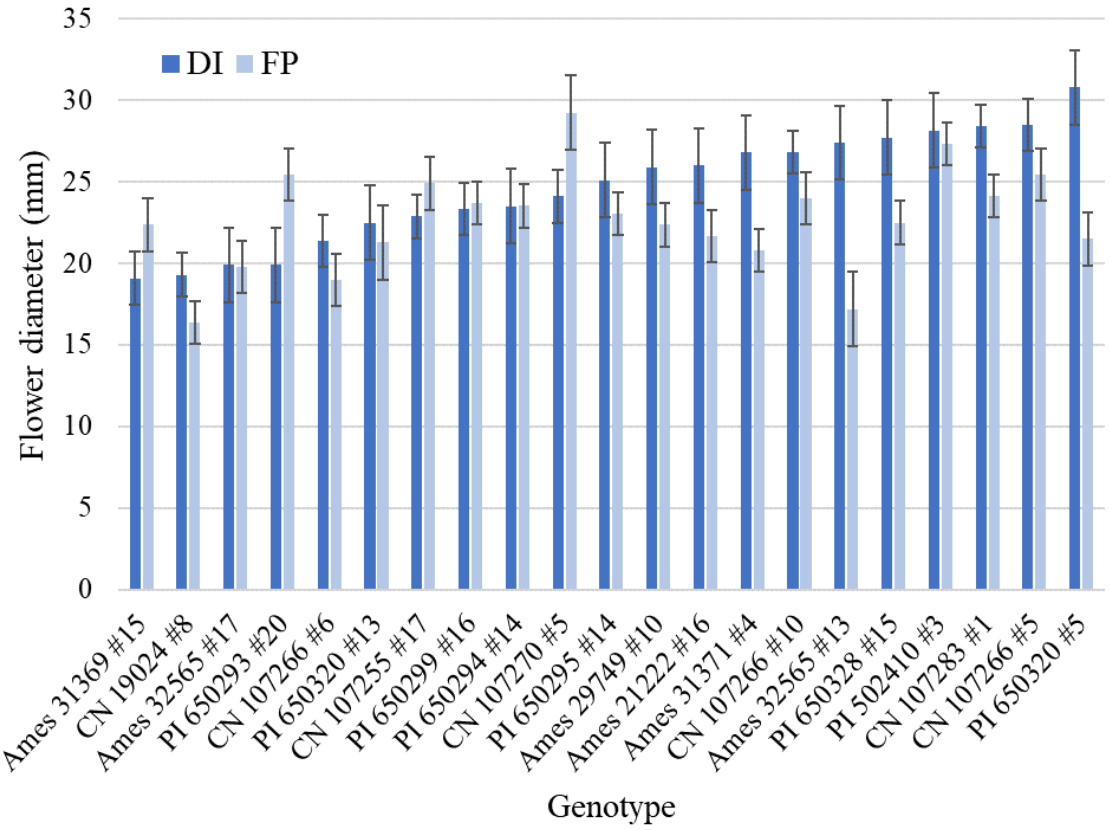

Figure 3-1. Mean ± SE flower diameter (mm) values showing interaction of treatment (DI = deionized water; FP = floral preservative) and genotype. Genotypes are sorted based on mean DI flower diameter.

Figure 3-2. Example floral design using flax (blue flowers; arrows) as a filler crop to add color. Floral design credit: N. Anderson; photo credit: D. Tork.

Figure 3-3. Example floral design using flax as a filler crop to add texture (round green seed capsules). Floral design credit: N. Anderson; photo credit: D. Tork.

Chapter 4

Controlled Freezing Studies as a Corollary Selection Method for Winter Hardiness in Perennial Flax (*Linum spp.*)

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Perennial flax breeding objectives at the University of Minnesota (UMN) are to develop agronomic (oilseed, fiber) and horticultural (cut flower, garden perennial) varieties that are hardy in Minnesota (USDA Plant Hardiness Zones 3 & 4). The objective of this research was to determine the range of cold hardiness in UMN perennial flax breeding populations compared with accessions of *L. austriacum*, *L. lewisii*, and *L. perenne*. Fifty-three genotypes from seven populations were subjected to low temperature acclimation, followed by controlled freezing using a programmable freezer. The observed LT_{50}s (lethal temperature for 50% plant kill) ranged from > 0 °C (0% survival) to < -12°C (100% survival), for the test temperatures of 0, -4, -8, -10, and -12 °C. Cold damage was measured after four weeks of regrowth as the proportion of alive shoots and a root damage rating. Significant negative correlations were observed between LT_{50} and proportion of alive shoots (r = -.918), root damage rating (r = -.935). Both *L. austriacum* and *L. perenne* had significantly less cold damage compared to *L. lewisii* and the breeding populations. A secondary objective was to determine if cold tolerance was related to the location of shoot regrowth after freezing. This study establishes methods of screening perennial flax cold tolerance that are more cost effective, rapid, and repeatable compared to field evaluations.

Introduction

Perennial flax (*Linum spp.*, Linaceae) domestication is being pursued at the University of Minnesota as part of the Forever Green Initiative, which aims to reduce soil erosion and nutrient runoff by increasing the amount of perennial cover on the landscape (Betts et al., 2008). The long-term goal of this project is to develop a perennial variety of oilseed flax that can be grown on a large acreage, analogous to the perennial grain intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey subsp. *intermedium*), otherwise known as Kernza™ (Bajgain et al., 2020). Development of economically viable perennial grain crops has been identified as a potential solution to the widespread soil erosion and nutrient runoff throughout Minnesota and the rest of the Mississippi river basin (Colson et al., 2005; Culman et al., 2013; MPCA, 2013). Ornamental perennial flax is simultaneously being developed for garden and cut flower purposes, as there is a lack of breeding/selection for this market. Perennial flax has the added benefit of supporting native and managed pollinator populations (Betts et al., 2008; USDA, n.d.). At such an early stage of domestication, these agronomic and ornamental objectives share many common goals, such as winter hardiness, upright growth habit, and lack of seed dormancy. Thus, these breeding objectives are being selected simultaneously, based on perennial flax crop ideotypes (Tork et al., 2019).

In northern latitudes, winter hardiness is a critical breeding objective for perennial crops. In USDA plant hardiness Zones 4 and 3, above-ground structures of woody plants

must be capable of surviving air temperatures as low as -34.4 °C and -40 °C, respectively (USDA, n.d.). However, woody and herbaceous perennials (most of which are deciduous and die back to the ground each fall), require root/crown systems that can survive -10 °C and -12 °C for USDA Z4 and Z3, respectively (Anderson et al., 2012; Du et al., 2013; Kim and Anderson, 2006). Field evaluation of winter hardiness is difficult due to annual variability of factors such as air and soil temperature, amount of snow cover, freeze/thaw cycles, diseases, etc. (Blum, 1988). For field evaluations to be useful, the winter conditions must also be severe enough to differentiate genotypes for winter survival (Rognli, 2013). These unpredictable environmental factors, in addition to the range of abiotic stressors (temperature, moisture, wind, light), require field evaluations of winter hardiness to be replicated over years and locations to demonstrate stability (Rognli, 2013; Waldron et al., 1998). Such experiments are often limited early in a breeding program due to the small amount of seed available and cost (Rognli, 2013).

Controlled laboratory freezing studies are an alternative means of screening winter hardiness that is more rapid and repeatable than field evaluations. Although winter hardiness is a complex trait influenced by the ability to withstand environmental stressors, freezing tolerance is the primary component which explains most of the variation in winter hardiness (Pearce, 2001; Wiering et al., 2018). Laboratory freezing studies typically use clonal genotypes which have been acclimated (Rognli, 2013). During acclimation, plants are exposed to gradual decreases in temperature to 2-5 ºC and length of photoperiod (8 h). This process induces cold-response genes which alter the physiological state of the plant, causing changes in carbohydrate composition, growth,

and leaf coloration (Hoffman et al., 2010; Pietsch et al., 2009; Rognli, 2013; Wiering et al., 2018). In total, the acclimation period is 6-8 wk (Rognli, 2013).

Upon acclimation, plants are tested in a programmable freezer (Anderson et al., 2012; Du et al., 2013; Kim and Anderson, 2006). Since whole plants are frozen in containers, pilot studies must be conducted to determine the length of time needed for the center of each container to reach the set temperature. Unlike plants in the field, the root systems of containerized plants are not insulated by the surrounding soil column; thus, this method tests the temperatures that the root system can tolerate. Previous studies have determined that the roots of all perennials (both herbaceous and woody) must tolerate temperatures of -10 °C and -12 °C for USDA Zones 4 and 3, respectively (Anderson et al., 2012; Du et al., 2013; Kim and Anderson, 2006). After freezing, plants are returned to acclimation temperatures (2-5 ºC) to thaw before returning to a greenhouse for regrowth assays. The amount of regrowth is recorded and the number of surviving plants at each temperature is used to calculate the LT_{50} or the lethal temperature at which 50% mortality occurs (Tcacenco et al., 1989). These results can then be compared to field winter survival to assess the effectiveness of controlled freezing studies.

The most promising perennial flax species being evaluated as candidates for domestication are Asian flax (*L. austriacum* L.), Lewis flax (*L. lewisii* Pursh), and blue flax (*L. perenne* L.) (Tork et al., 2019) (Chapter 2). *Linum perenne* has been naturalized throughout North America, but is native to Europe and Asia (Ockendon, 1971; Ogle, 2002; Pendleton et al., 2008). Several sources report that *L. perenne* is native to USDA Z5-9, although ‘Appar’ is reportedly hardy to Z3 (Cornell University, 2006; Missouri

Botanical Garden, n.d.; USDA-NRCS, 2020). To the best of our knowledge, there are no reports for the hardiness of *L. austriacum* but, given that it has been grown in Canada and shares a similar native range to *L. perenne*, it may have comparable hardiness levels (USDA-NRCS, n.d.). *Linum lewisii* is native to North America, with a large north-south distribution that stretches from Mexico to Alaska across a range of elevations and hardiness zones. Thus, the species could possess greater variation in hardiness. *Linum lewisii* 'Maple Grove' is reportedly hardy to Z4, which can serve as a benchmark for the species (USDA-NRCS, n.d.).

The UMN has been breeding perennial flax for oilseed and ornamental uses for the past ~10 y. During that time, lines of USDA Z4 winter-hardy selections have been made. Perennial flax has been observed in field and greenhouse trials to produce shoots from underground portions of the stem and root system, known as non-emergent shoots (N. Anderson and D. Tork, unpublished data, 2019-20). In garden chrysanthemum (*Dendranthema* x*grandiflora* Tzvelv), the number of emergent and non-emergent rhizomes was correlated with winter survival (Anderson and Gesick, 2004). A similar mechanism could occur in flax since any underground structures are insulated from the air (Anderson and Gesick, 2004). The primary objective of this study was to screen the existing UMN perennial flax breeding populations for cold tolerance using a programmable freezer to select cold-hardy genotypes. Secondary objectives included (i) comparing the cold tolerance of breeding populations of unknown species composition to known accessions of *L. austriacum*, *L. lewisii*, and *L. perenne*, (ii) evaluating the correlation between field winter survival and controlled freezing tolerance, and (iii)

examining the relationship between cold tolerance and location of regrowth on the plants. Developing an effective controlled freezing protocol and understanding the perennial flax cold response will be critical for selecting winter hardy genotypes with consistent survival throughout USDA Z3-4.

Materials and Methods

Field sites

In 2005, a randomized common garden nursery was established in St. Paul, MN, which initially included *Linum altaicum* Ledeb. ex Juz., *L. austriacum*, *L. baicalense* Juz., *L. bienne* Mill., *L. campanulatum* L., *L. flavum* L., *L. hirsutum* L., *L. lewisii*, *L. perenne*, *L. sulcatum* Riddell, *L. tauricum* Willd., *L. tenuifolium* L., and *L. thracicum* Degen (N. Anderson and K. Betts, unpublished data)(Betts et al., 2008). However, it was noted at the time that *L. altaicum*, *L. campanulatum*, *L. sulcatum*, *L. tauricum*, *L. tenuifolium*, and *L. thracicum* lacked vigor and probably did not contribute seed to subsequent generations (K. Betts, personal communication, 2021). The open pollinated seed of the highest yielding plants from the 2005 common garden nursery was grown in 2006-7 to establish a second generation, i.e. Broad Based 1 "BB1" population. This procedure was repeated in 2008 to generate "BB2" as well as two additional populations, "KJ1" and "KJ2," which were selected for 'tuft' (upright, high branching) and 'bush' (spherical, low branching) habits, respectively. Since these early generations of seed were open pollinated, the species background of all early stage populations is unknown. In

wk. 17 (24 Apr. 2017), remnant seed from the 2005-2008 evaluations was sown at the Rosemount Research and Outreach Center, Rosemount, MN (44°42’58.2" N, -93°5’54.9” W) to restart the perennial flax breeding program. A single-row cone seeder was used to plant 2 x 5’ row plots with 1.5 g seed ea. (~8.5 lb/a) with 50.8 cm between rows, replicated 1-3x depending on the availability of seed and arranged using a completely randomized design. The plots were fertilized with urea (50 lb/a actual N), and chemical weed control was provided by pendimethalin (2 pt/a rate) [Prowl®, BASF Corporation, Ludwigshafen, DE]. Soil P and K levels were adequate based on soil tests (K. Betts, personal communication, 2021).

The most vigorous plants from each population in the restart nursery were harvested and planted in an adjacent field in wk. 41 (13 Oct. 2017) along with two check genotypes, ‘Maple Grove’ (Ames 27614; Table 4-1) and ‘Appar’ (PI 445972; not tested), to establish an “elite restart” nursery using the same planting design, fertilizer rates, and weed control as the original restart nursery (K. Betts, personal communication, 2021). In wk. 39 (2018), the two most vigorous plants per plot were flagged out of this population for seed harvest and freezing study tests.

In 2017, a common garden nursery was also established at the University of Minnesota, St. Paul Campus, St. Paul, MN (44°59′23″ N, 93°10′28″ W) to evaluate additional accessions of *L. austriacum*, *L. lewisii*, and *L. perenne* (Table 4-1). In wk. 5 (30 Jan 2017) seeds were stratified in petri dishes on damp blotter paper for 1 wk in darkness at 7.2 °C. From wk 6-9 (6 Feb. to 5 Mar. 2017) seeds were germinated in light at 18.3 °C, and then transplanted during wk 9-10 (27 Feb. to 12 Mar. 2017) to a

greenhouse set to 15.6 °C day/night with a 14 h photoperiod (0600–2000 h; long days) supplied by 400 w high pressure sodium high intensity discharge (HPS-HID) lamps, at a minimum of 150 μmol m^{-2} s^{-1} at plant level. In wk 16 (20 Apr. 2017), seedlings were transplanted to the field with a spacing of 60.96 cm O.C. within and between rows using a completely randomized design. The field was irrigated post-planting with 2.54 cm water, but after this no irrigation was used. The plots were fertilized with urea (50 lb/a actual N), and chemical weed control was provided by pendimethalin (2 pt/a rate) [Prowl®, BASF Corporation, Ludwigshafen, DE]. Weed control also consisted of mechanical tillage between rows and hand weeding within rows, as needed. In wk 39 (2018), the most vigorous plants in this population were flagged as selections for breeding.

Two commercial cultivars, *L. perenne* ‘Sapphire’ and ‘Blue Flax,’ were also grown at the West Central Research and Outreach Center, Morris, MN (45° 37' 41.520" N, 95° 53' 20.688" W). Plug trays (72s) of ‘Sapphire’ and ‘Blue Flax’ rooted liners and were obtained from The Nursery Stock Market, Inc. (Presswood, KY). Ten clones per cultivar were planted in spaced rows (45.2 cm O.C. within rows and 60.96 cm between rows) at each site.

Plant material

In wk 39 and 40 (2018), cuttings from 53 flax test genotypes were harvested for cold tolerance evaluation from UMN breeding populations (BB1, BB2, KJ1, KJ2),

species accessions (*L. austriacum, L. lewisii, L. perenne*), and commercial cultivars of *L. lewisii* 'Maple Grove' and *L. perenne* 'Blue Flax' and 'Sapphire.' Plants were located at field sites in MN [St. Paul, Rosemount (USDA Z4); Morris (USDA Z3/4)] (Table 4-1). Twenty stem tip cuttings per genotype (> 5 cm length) were harvested from the crown, labeled, sealed in bags [1.2 ml Get Reddi® Sandwich Bags, United States Plastic Corporation], and put into a cooler on ice for transport to St. Paul, MN, Plant Growth Facility, University of Minnesota (44°59'17.8" N, -93°10'51.6" W) before rooting. After removing the lower leaves, cuttings were trimmed to 5-7 cm length using a sterile razor [GEM Carbon Steel Extra Sharp Single Edge Blade, The Razor Blade Co., CA], and the cut stem base was dipped into 1000 ppm Indole-3-butyric Acid (IBA) in talc. Cuttings were then inserted into pre-moistened foam propagation strips [ROOTCUBES® PLUS WEDGE®, Oasis Grower Solutions, Kent, OH]. Cuttings were rooted for 5 wk in a glass mist house (21/21 °C, day/night, 16 h; 0600–2200 h lighting with high pressure sodium high intensity discharge lamps or HIDs at a minimum set point of 150 μmol m^{-2} s^{-1} at plant level). An intermittent mist system, at a mist frequency of 10 min intervals (mist nozzles, reverse osmosis water) during 0600-2200 h with a 7 s duration was used.

Cold acclimation conditions

After rooting, cuttings were transplanted in wk 45 and 46 into 10.12 cm square deep pots [SVD-355-DEEP-BK-40, T.O. Plastics, Clearwater, MN] filled with a soilless medium [Promix Mycorrhizae, Premier Horticulture Inc., Quakertown, PA] and grown in

a glass greenhouse at 16.7/15.5 °C day/night daily integral and a 10 h photoperiod (0600–1600 h; short days). Supplemental lighting was supplied during winter months and cloudy days by 400 w high pressure sodium high intensity discharge (HPS-HID) lamps, at a minimum of 150 μmol m^2 s^{-1} at plant level. Starting in wk 50 (11 Dec 2018) plants were acclimated by gradually decreasing temperatures by ~1.7/1.9 °C day/night every 2 weeks to a min. in wk 6 (5 Feb. 2019) of 10/7.8 °C day/night, at which point supplemental light was stopped. Plants remained under these conditions for one month before transfer to a walk-in cooler at 4/4 °C day/night in darkness, where they remained acclimated in cold storage for 1000-3000 h, based on the availability of the programmable freezer.

Cold tolerance assessment

After cold storage, plants were tested for cold tolerance using a randomized factorial design with two factors and three replicates. Factors included five test temperatures (0, -4, -8, -10, and -12 °C) and 53 genotypes, for a total of 795 experimental units. Temperature treatments were randomly assigned to experimental units. The freezer chamber accommodated 80 pots total, or five genotypes per run at 3 reps/temperature/genotype (n = 75 containers per run total tested, plus five empty pots containing buried temperature probes). A Tenney C-EVO Environmental Test Chamber [Thermal Products Solutions, Model: C30C2.0-A-S1.1-C] was set at 4 °C to commence each freezing run. Cold tolerance was assessed at soil temperatures of 0, -4, -8, -10,

and -12 °C, using a 2 h ramp time (decreasing temperature) and a 10 h soak time (constant temperature) (Figure 4-1). The ramp and soak times, and the air temperatures needed to reach the desired soil temperatures, were determined in pilot experiments using buried temperature probes (Digit-TL, LabJack) (Table 4-2). After freezing, plants were returned to a 4 °C cooler for 7 d until thawed. A graphical summary of the test shows the decreasing temperatures as the experiment was run (Figure 4-1). Buried temperature probes also revealed temperature variation of ~ ± 1 °C based on relative positioning within the freezer chamber (Figure 4-2). These temperature differences were mitigated by randomization of container locations within the freezer chamber.

Regrowth assay

After freezing, plants were moved back to the same cooler to thaw for 1 wk at 4 °C before moving to a greenhouse for regrowth and evaluation of cold damage (same conditions used at the onset of acclimation). After 4 wk, the total number of alive and dead shoots was recorded for all plants, which was then used to calculate the proportion of alive shoots (# alive shoots / (# alive + # dead shoots)) for comparison regardless of differences in initial plant size. Plants were also assigned a binary alive/dead score for the calculation of LT_{50}. For plants that survived, shoot counts were also recorded by plant sector location (above ground, crown, non-emergent (NE)). The above ground location on the plant was defined as > 3 cm above the soil line, the crown was defined as 0-3 cm above the soil line, and non-emergent (NE) shoots were defined as any shoot originating

below the soil surface (Figure 4-3, Figure 4-4; Tork, et al., 2019). From these shoot counts segmented by location, the proportion out of the total alive shoots was calculated (# shoots at location / total # alive shoots). This calculation determined, for the plants that survived, where the most growth occurred proportionally, and how those proportions differed across populations or genotypes.

The root mass was also scored for freezing damage based on a 1-5 visual Likert scale developed using the commercial *L. perenne* cultivar 'Blue Flax,' which was the first genotype evaluated in 2019. 'Blue Flax' exhibited a range of root damage across temperatures, from fully alive to fully dead, making it an effective reference (Figure 4-5). Representative samples from each genotype were also photographed during the data collection process.

Field % winter survival

For each genotype tested in controlled freezing studies, field winter survival (alive/dead) was recorded the following spring in wk. 17 (26 Apr. 2019). Number of replicates varied by location. St. Paul genotypes were single plant selections, therefore, only one replicate was available. The number of replicates in Rosemount varied from 2-6 plants, depending on if seed was available to replicate plots. Commercial varieties planted in Morris had 10 replicated clones per genotype. This data was used to determine the correlation between field percent winter survival and controlled freezing for this experiment.

Statistical analysis

The LT_{50}, or the determined lethal temperature at which 50% survival occurs, was calculated using probit analysis in R (Hulke et al., 2008; Tcacenco et al., 1989; Wiering et al., 2018). A generalized linear model for binomial data with a probit link function was fit to analyze the effects of temperature on whole-plant survival. The *dose.p* function in the "MASS" package was then used to calculate the median lethal temperature (LT_{50}) on a genotype and population basis.

Regrowth assay data was analyzed by two-way Analysis of Variance (ANOVA) and mean separations (5% Tukey's Honestly Significant Difference, HSD, $\alpha = 0.05$) using the Statistical Package for the Social Sciences (SPSS), v.25 for Windows (SPSS, Inc., Chicago, IL). For population comparison analysis, genotype data were pooled together for each population. For proportional shoot regrowth by location, means separations were not able to be calculated, as high mortality at the lower temperatures caused small and inconsistent sample sizes. Pearson correlations were calculated on a genotype mean basis using SPSS to compare LT_{50}, field percent winter survival, and regrowth data.

Results and Discussion

Determination of LT_{50}

At the species level, LT_{50}s ranged from LT_{50} = -3.51 °C (*L. lewisii*) to -10.73 °C (*L. austriacum;* Table 4-3) whereas the breeding populations ranged from -3.27 °C (KJI) to -5.99 °C (BB2; Table 4-3). Previous studies on herbaceous perennials have shown that genotypes with LT_{50}s ≤ -10 °C are predicted to be hardy to USDA Z4, while genotypes with LT_{50}s ≤ -12 °C are predicted to be hardy to USDA Z3 (Anderson et al., 2012; Du et al., 2013; Kim and Anderson, 2006); therefore, *L. austriacum* and *L. perenne* are likely USDA Z4 hardy (Table 4-3). This is consistent with known geographic distributions and hardiness levels for *L. perenne* (USDA-NRCS, n.d.). In contrast, *L. lewisii* is not predicted to be hardy in Minnesota (USDA Z3-4) based on these results. However, the native geographic distribution of *L. lewisii* is large, encompassing the western half of North America from Mexico to Alaska (USDA-NRCS, 2020), so the mean LT_{50} of -3.51 °C for *L. lewisii* is only relevant to 'Maple Grove' and accession Ames 31361 (Table 4-4).

Across all genotypes, LT_{50} values ranged from > 0 °C (0% survival; 4-9S) to < -12 °C (100% survival; 293-3, 293-6, 294-2) (Table 4-4). The relatively high temperature of LT_{50} values in the breeding populations, especially for BB1 and KJ1, demonstrate a substantial loss of cold tolerance despite field selection for winter hardiness in previous generations. Within BB1, genotype 6-19N had the lowest LT_{50} of -8.91 °C, and within BB2 the lowest LT_{50} of -8.75 °C was observed for genotype 1-20S (Table 4-4). Similarly, within KJ1, the genotype 4-20S was observed to have the lowest mean LT_{50} of -7.92 °C, and within KJ2 the lowest mean LT_{50} was -7.13 °C, observed for genotype 1-2N (Table 4). Interestingly, these breeding populations

consistently ranked lower than *L. austriacum* and *L. perenne*, and were instead more comparable to *L. lewisii* in terms of LT_{50}. The inconsistent relationship between LT_{50} and field percent winter survival may suggest that additional weather conditions, trait(s) or abiotic stressors may be involved, since recent winters have had atypical below zero air temperatures in December without adequate snow cover which could be lethal for acclimated crowns (Kunkel et al., 2013; Minnesota DNR, 2021; Wuebbles and Hayhoe, 2004).

Regrowth assay ANOVA

All main effects as well as their interactions were very highly significant for the proportion of alive shoots and root damage ratings (Table 4-5). This would be expected for these two traits when testing genotypes and populations varying in cold tolerance responses to soil temperatures. In contrast, the proportion of above ground shoots and the proportion of crown shoots were not significantly different among tested temperatures, populations and genotypes (Table 4-5). This demonstrates that these above ground traits in an herbaceous perennial are not predictive of winter hardiness. Rather, belowground structures are critical survival mechanisms since the proportion of non-emergent shoots were significantly different for temperature, genotypes, and their interaction (Table 4-5).

Additional factors, i.e. the number of alive shoots, number of dead shoots, number of above ground shoots, number of crown shoots, and number of non-emergent shoots were significantly different for all main effects (temperature, populations, genotypes) and

all of their interactions, although the level of significance varied (Table 4-6). However, for the proportion of alive shoots, the *F ratios* are much larger than number of alive or number of dead shoots alone, which is the first indication that the proportion calculations have greater discriminatory power to detect differences among temperatures, populations, and genotypes.

Proportional shoot regrowth by location

The hypothesis that winter hardiness is related to the ability to generate non-emergent shoots requires recording more than raw shoot counts, which only tell whether the quantity of regrowth changed based on the factors in the experiment. The question of whether patterns of regrowth differ in response to temperature requires examining the proportional contribution of new shoots from each segment of the plant, as defined (Figure 4-3). However, there are tradeoffs involved. Since proportional calculations require plant survival, the sample size is greatly reduced, especially at the lower temperatures, which prevented mean separations from being calculated for the factor of genotype. This creates an unbalanced analysis that must be interpreted with caution. For example, mean separations with temperature as a factor revealed that the -12 °C group had a proportion of non-emergent shoots significantly higher than the other temperatures (Table 4-7). However, if the low sample size of the -12 °C group (n = 10, due to mortality) and lack of separation among the other temperatures is taken into account, this association may not be real and, thus, will require further testing to confirm. This can be

further reinforced by examining the trait correlations (Table 4-8). There is no association between the proportion of non-emergent shoots and LT_{50}, indicating that genotypes with an increased proportion of non-emergent shoots did not tend to score better for LT_{50} (Table 4-8). The negative correlation between the proportion of non-emergent shoots and field percent winter survival may also require further testing since the winter survival measurements were taken on genetically identical clones.

Trait correlations

Trait correlations illustrate why the calculated proportion of alive shoots is superior to shoot counts alone for describing cold damage (Table 4-8). For comparisons involving LT_{50}, significant negative correlations approaching r = -1.0 are highly desirable since lower negative temperatures designate greater freezing tolerance. All shoot counts are significantly and negatively correlated with the proportion of alive shoots, as well as LT_{50}. However, none of these are more strongly correlated with LT_{50} than the proportion of alive shoots ($r = -0.918$, $p \leq .001$). Furthermore, root damage rating is even more highly correlated with LT_{50} ($r = -0.935$, $p \leq .001$), indicating that these two traits are effective at characterizing levels of cold damage in perennial flax. These correlations also confirm the negative associations found between LT_{50} and field percent winter survival (Table 4-3, Table 4-4); However, correlations with field percent winter survival should be interpreted with caution since winter severity differs from year-to-year. The St. Paul, MN site had the lowest field percent winter survival, but the genotypes tested at this site had

the lowest LT_{50}s in the programmable freezer (Table 4-4). This may be due to local environmental factors such as a lack of snow cover or wind exposure. The St. Paul field also has heavier soil (increased amount of clay) compared to Rosemount or Morris (Soil Survey Staff, n.d.). In contrast, the Rosemount site, which was the site of all other tested genotypes besides 'Blue Flax' and 'Sapphire', has a large windbreak, which may have provided protection from desiccation to the genotypes there. Thus, field percent winter survival is heavily dependent on unpredictable or landscape-based abiotic edaphic and environmental factors (Blum, 1988). While there were insufficient quantities of clones to test at multiple locations, future balanced studies at locations over years would demonstrate long-term stability.

Proportion of alive shoots

Since main effects are significantly different for the proportion of alive shoots (Table 4-5), mean separations show *L. austriacum* and *L. perenne* with significantly less cold damage than either the breeding populations or *L. lewisii* (Table 4-9). These findings are consistent with the LT_{50}s for these species (Table 4-3). The main advantage of using the proportion of alive shoots is that it accounts for differences in the initial size of the plants. For example, *L. lewisii* appears to have the worst performance based on the number of alive shoots alone (Table 4-10), but for the proportion of alive shoots, the performance of *L. lewisii* is statistically the same as all breeding populations (Table 4-9), indicating that raw shoot counts were biased by differences in initial plant size.

Furthermore, mean separations for the number of alive shoots (Table 4-10) provide significant, but less clear distinctions compared to the proportion of alive shoots (Table 4-9). *Linum austriacum* has a significantly greater number of alive shoots compared to other populations, whereas *L. perenne* overlaps with breeding populations BB1 and BB2 and *L. lewisii* is statistically similar with breeding populations KJ1 and KJ2 (Table 4-10). These data further support that cold damage in perennial flax is better described by the proportion of alive shoots (Table 4-9).

Temperature x population interactions for 0, -4, -8, -10, and -12 °C of the proportion of alive shoots demonstrate a distinct differentiation among *L. perenne* and *L. austriacum* from all other tested species and breeding lines (Figure 4-6). While *L. perenne* and *L. austriacum* overlap for the proportion of alive shoots at 0 and -4 °C, at -8 °C *L. perenne* drops below *L. austriacum* while still having higher mean values than the remaining populations and species (Figure 4-6). These data reinforce the significantly greater cold tolerance of *L. austriacum* and *L. perenne*, and also demonstrates a steep linear proportional decline as temperatures fall from 0 to -12 °C. Plotting the proportion of alive shoots by temperature pooled across populations confirms this strong linear relationship between the two variables ($R^2 = 0.9735$; Figure 4-7). This is further supported by phenotypic observations, which show how this proportion changes with temperature, regardless of initial plant size (Figure 4-8). Taken together, the proportion of alive shoots is an effective metric for describing cold damage in perennial flax that can supplement and reinforce LT_{50} findings.

Root damage rating

The root damage ratings showed significantly less root damage for *L. austriacum* (3.58) and *L. perenne* (3.19) relative to the other groups, although both species did display some damage (Table 4-9). The interaction of temperature x population for the root ratings showed a linear decline starting at 0 °C for all breeding populations and *L. lewisii*, whereas *L. perenne* decreased after -4 °C and *L. austriacum* after -8 °C (Figure 4-9). These results match those observed for the proportion of alive shoots (Figure 6). A linear relationship exists between temperature and mean root rating (Likert scale) for all populations (Figure 4-10), nearly identical to that of proportion of alive shoots (Figure 4-7).

Root damage rating is an effective metric for describing cold damage in perennial flax to supplement and reinforce LT_{50} values. Additionally, root damage rating is much faster to record than the shoot counts used to calculate proportion of alive shoots. Given the high correlation between these two measures ($r = 0.935$, $p \leq .001$; Table 4-8), root rating may be sufficient as an alternative to LT_{50} for characterizing cold damage in *Linum spp*, especially if the scale developed in this study is used (Figure 4-5). However, ratings are still more subjective than count data, so it is recommended that future studies of perennial flax cold tolerance use the proportion of alive shoots, root rating, and LT_{50} in concert, which will prove more informative than any single measurement.

Implications for breeding and selection

Since the factor of genotype was significant for proportion of alive shoots, root rating, and all shoot counts, means separations show statistical differences for these traits among genotypes that can be used along with LT_{50} for breeding and selection. For example, among the *L. austriacum* genotypes tested, 272-1 had the greatest proportion of alive shoots and root damage rating (Table 4-11), as well as the lowest LT_{50} of -12.06 °C (Table 4-4). The proportion of alive shoots observed for 272-1 was significantly greater than 33/34 breeding population genotypes; likewise, the root damage rating was significantly greater than 32/34 breeding population genotypes (Table 4-11). Therefore, integrating genotype 272-1 into future crosses and breeding populations could help to increase the overall cold tolerance of those populations.

A similar pattern is observed for *L. perenne* genotype 294-2, which had 100% survival at all temperatures (LT_{50} = < -12.00 °C; Table 4-4), the highest proportion of alive shoots, and the second highest root rating score among *L. perenne* genotypes (Table 4-11). Compared to breeding populations, genotype 294-2 significantly outperformed all genotypes for proportion of alive shoots, and 33/34 genotypes for root damage rating (Table 4-11). Thus, genotypes were identified among *L. austriacum* and *L. perenne* which can be crossed with breeding populations to recover cold tolerance lost through previous cycles of selection. In contrast, the two *L. lewisii* genotypes tested did not outperform breeding genotypes for these traits, so further evaluation is needed to determine if *L. lewisii* can contribute improved cold tolerance to existing breeding populations (Table 4-11).

The examples of *L. austriacum* 272-1 and *L. perenne* 294-2 further demonstrate why raw shoot counts are more difficult to interpret than their respective proportions. For example, 272-1 has a significantly greater number of alive shoots compared to all other genotypes studied, yet 294-2, which has a similar LT_{50} value (Table 4-4), significantly exceeds only one other genotype in terms of number of shoots, 4-9S from population KJ1 (Table 4-12). For the number of dead shoots, the trend is reversed: genotype 272-1 does not differ significantly from any genotype besides 5-8S from population KJ1, whereas 294-2 has significantly fewer dead shoots compared to 11/34 breeding population genotypes (Table 4-12). Only by considering the proportion of alive shoots (Table 4-11) does it become apparent that genotypes 272-1 and 294-2 exhibit similar levels of cold tolerance, as evidenced by LT_{50} (Table 4-4).

As discussed previously, the proportion of non-emergent shoots did not correlate with LT_{50} (Table 4-8), even though a significantly greater proportion of non-emergent shoots was observed at -12 °C relative to the other temperatures (Table 4-7); therefore, this trait cannot be used at present to select for greater cold tolerance. In contrast, desirable negative correlations were observed between LT_{50} and number of above ground ($r = -.676$), crown ($r = -.672$) and non-emergent ($r = -.526$) shoots (Table 4-8), but it is much more difficult to discern a clear pattern among genotypes (Table 4-12). For example, among breeding populations, the genotype with the greatest number of aboveground shoots, 5-8S (Table 4-12), had a relatively high LT_{50} of -2.01 °C (Table 4-4), a low proportion of alive shoots (0.14) and severe root damage (1.80) (Table 4-11). Likewise, the most cold-tolerant breeding genotype, 6-19N (LT_{50} = -8.91 °C; Table 4-4),

did not differ significantly from 5-8S for number of above ground or crown shoots (Table 4-12), but had significantly less root damage (Table 4-11). Altogether, for the populations studied, LT_{50}, proportion of alive shoots and root damage rating are the optimal traits to use for corollary selection for improved winter hardiness.

Conclusions

This research is the first known example of controlled freezing tests conducted on wild perennial flax germplasm. Significant differences were identified between populations for cold tolerance, with *L. austriacum* and *L. perenne* exhibiting improved response to cold stress compared to *L. lewisii* and the four breeding populations tested. The large number of genotypes tested in this experiment will serve as a useful reference for breeders of perennial flax in the future. Data collected for *L. perenne* 'Sapphire' and 'Blue Flax' and *L. lewisii* 'Maple Grove' will be useful to the ornamental landscape industry, which requires accurate information on cold hardiness for marketing purposes. Future perennial flax controlled freezing experiments should test different methods of acclimation along with the freeze test protocols developed herein to study the potential cause(s) for the significant inverse correlation among LT_{50} and field percent winter survival ($r = .494$). Future experiments should also consider increasing replications per genotype or the number of temperatures, which would produce even more accurate LT_{50} estimates, and potentially help to clarify the relationship between non-emergent shoot growth and cold tolerance. Once the controlled freezing protocol is sufficiently refined,

we plan to implement it as part of the regular perennial flax breeding cycle, which will enable the UMN perennial flax program to pre-screen genotypes which are well-suited for winter survival in USDA Z3-4.

Tables

Table 4-1. *Linum spp.* populations, genotypes, 2017-2019 field site location, and accession number with the geographic source of the collection in parentheses.

Population	Genotype	2017-19 site	PI Number (Source)
L. austriacum	262-1 (R1)	St Paul	PI 650295 (Poland)
	267-1	St Paul	Ames 29749 (Ukraine)
	272-1	St Paul	PI 650300 (Hungary)
	272-2	St Paul	PI 650300 (Hungary)
	272-3	St Paul	PI 650300 (Hungary)
	272-6	St Paul	PI 650300 (Hungary)
	274-2	St Paul	PI 650302 (Ukraine)
L. lewisii	104-4	St Paul	Ames 31361 (USA)
	'Maple Grove'	Rosemount	Ames 27614 (UT, USA)
L. perenne	292-1	St Paul	PI 650323 (Hungary)
	292-4	St Paul	PI 650323 (Hungary)
	293-1	St Paul	PI 650324 (Hungary)
	293-3	St Paul	PI 650324 (Hungary)
	293-6	St Paul	PI 650324 (Hungary)
	294-1	St Paul	PI 650325 (Hungary)
	294-2	St Paul	PI 650325 (Hungary)
	295-1	St Paul	PI 650326 (Hungary)
	'Blue Flax'	Morris	
	'Sapphire'	Morris	
BB1[a]	3-12N	Rosemount	
	3-16N	Rosemount	

Population	Genotype	2017-19 site	PI Number (Source)
	4-6N	Rosemount	
	5-14N	Rosemount	
	5-2N	Rosemount	
	5-8N	Rosemount	
	6-14N	Rosemount	
	6-19N	Rosemount	
	6-22N	Rosemount	
	7-18N	Rosemount	
	8-12N	Rosemount	
	8-17N	Rosemount	
	8-24N	Rosemount	
	9-2N	Rosemount	
BB2[b]	1-11S	Rosemount	
	1-20S	Rosemount	
	1-21S	Rosemount	
	1-7S	Rosemount	
KJ1[c]	3-12S	Rosemount	
	4-10S	Rosemount	
	4-11S	Rosemount	
	4-15S	Rosemount	
	4-16S	Rosemount	
	4-18S	Rosemount	
	4-19S	Rosemount	
	4-20S	Rosemount	

Population	Genotype	2017-19 site	PI Number (Source)
	4-9S	Rosemount	
	5-14S	Rosemount	
	5-6S	Rosemount	
	5-8S	Rosemount	
KJ2[c]	1-2N	Rosemount	
	2-11N	Rosemount	
	2-4N	Rosemount	
	2-5N	Rosemount	

[a] The BB1, or "broad based 1" population was established from the highest yielding plants from a 2005 common garden nursery containing thirteen randomly mated species
[b] BB2 is a breeding population established in 2009 from the highest yielding plants in BB1
[c] The KJ1 and KJ2 breeding populations were selected from BB1 in 2009 for plant habit

Table 4-2. Target soil temperature (°C) and corresponding air temperature (°C) setting determined in the programmable freezer using buried temperature probes

Target soil temperature	Air temperature
°C	
0	-3
-4	-6
-8	-9
-10	-12
-12	-14

Table 4-3. Mean ± S.E. LT_{50}s, sample size (n genotypes), and field % winter survival 2019 for all sites, USDA Z4, calculated in a population basis for the *Linum spp.* studied. Accessions are listed first, followed by breeding populations.

Population	LT_{50}	n	Field % winter survival 2019
L. austriacum	-10.73 ± 0.35	7	57.14%
L. lewisii	-3.51 ± 1.27	2	0.00%
L. perenne	-10.25 ± 0.45	10	24.40%
BB1	-4.32 ± 0.42	14	97.36%
BB2	-5.99 ± 0.70	4	87.50%
KJ1	-3.27 ± 0.48	12	95.83%
KJ2	-3.48 ± 0.92	4	100.00%

Table 4-4. Mean ± S.E. LT_{50}s determined via a programmable freezer for flax (*Linum spp.*) populations calculated on a genotype basis in comparison with field location-specific (Minnesota) % winter survival in the same year (2019).

Population	Genotype	LT_{50}	2019 location	Field % survival 2019
L. austriacum	262-1 (R1)	-10.13 ± 138.93*	St Paul	100
	267-1	-11.09 ± 0.93	St Paul	0
	272-1	-12.06 ± 109.28*	St Paul	0
	272-2	-6.00 ± 5225.25*	St Paul	0
	272-3	-6.00 ± 5225.25*	St Paul	100
	272-6	-10.09 ± 0.89	St Paul	100
	274-2	-6.00 ± 5225.25*	St Paul	100
L. lewisii	104-4	-6.00 ± 5225.25*	St Paul	0
	'Maple Grove'	-2.98 ± 1.68	Rosemount	0
L. perenne	292-1	-13.03 ± 3.36	St Paul	0
	292-4	-6.00 ± 5225.25*	St Paul	0
	293-1	-11.10 ± 0.89	St Paul	100
	293-3	< -12.00 b	St Paul	0
	293-6	< -12.00 b	St Paul	0
	294-1	-6.00 ± 5225.25*	St Paul	0
	294-2	< -12.00 b	St Paul	0
	295-1	-6.00 ± 5225.25*	St Paul	0
	'Blue Flax'	-8.17 ± 0.67	Morris	100
	'Sapphire'	-8.12 ± 144.55*	Morris	44
BB1	3-12N	-6.01 ± 2229.90*	Rosemount	83
	3-16N	-4.25 ± 252.95*	Rosemount	100
	4-6N	-2.01 ± 2697.74*	Rosemount	80
	5-14N	-0.25 ± 272.31*	Rosemount	100
	5-2N	-7.13 ± 1.17	Rosemount	100
	5-8N	-6.01 ± 2200.86*	Rosemount	100

Population	Genotype	LT_{50}	2019 location	Field % survival 2019
	6-14N	0.28 ± 381.75*	Rosemount	100
	6-19N	-8.91 ± 0.86	Rosemount	100
	6-22N	-4.25 ± 252.95*	Rosemount	100
	7-18N	-4.25 ± 252.95*	Rosemount	100
	8-12N	-2.98 ± 1.68	Rosemount	100
	8-17N	-0.25 ± 272.31*	Rosemount	100
	8-24N	-4.58 ± 1.49	Rosemount	100
	9-2N	-8.13 ± 115.90*	Rosemount	100
BB2	1-11S	-5.77 ± 1.30	Rosemount	67
	1-20S	-8.75 ± 1.16	Rosemount	100
	1-21S	-5.77 ± 1.30	Rosemount	83
	1-7S	-3.75 ± 237.24*	Rosemount	100
KJ1	3-12S	-7.86 ± 167.95*	Rosemount	100
	4-10S	-4.25 ± 252.95*	Rosemount	100
	4-11S	-4.25 ± 252.95*	Rosemount	100
	4-15S	-1.80 ± 1.71	Rosemount	100
	4-16S	-3.79 ± 237.24*	Rosemount	100
	4-18S	-6.01 ± 2200.86*	Rosemount	100
	4-19S	-0.76 ± 3.27	Rosemount	100
	4-20S	-7.92 ± 1.33	Rosemount	100
	4-9S	>0.00 [a]	Rosemount	75
	5-14S	-2.01 ± 2697.74*	Rosemount	100
	5-6S	-0.25 ± 272.31*	Rosemount	100
	5-8S	-2.01 ± 2697.74*	Rosemount	75
KJ2	1-2N	-7.13 ± 1.17	Rosemount	100
	2-11N	-2.40 ± 2.89	Rosemount	100
	2-4N	-3.75 ± 237.24*	Rosemount	100

Population	Genotype	LT_{50}	2019 location	Field % survival 2019
	2-5N	-0.25 ± 272.31*	Rosemount	100

[a] LT_{50} not calculated due to zero survival
[b] LT_{50} not calculated due to 100% survival
*Large S.E. values may partly be explained by abrupt drop in proportional survival between temperature treatments (see Figure B 1 for example)

Table 4-5. ANOVA (df, F ratio, Prob > F) for the effects of temperature, population, genotype and their interactions for the following traits: proportion of alive shoots (# alive shoots / total # shoots), root damage rating (1-5; 1 = dead, 5 = no damage), proportion of above ground shoots (# alive above ground shoots / total # alive shoots), proportion of crown shoots (# alive crown shoots / total # alive shoots), and proportion of non-emergent shoots (# alive non-emergent shoots / total # alive shoots). Non-emergent shoots are defined as those originating below the soil line, crown shoots as 0-3 cm above the soil line, and above ground shoots as > 3 cm above the soil line.

		Proportion of alive shoots		**Root damage rating**			**Proportion of above ground shoots**		**Proportion of crown shoots**		**Proportion of non-emergent shoots**	
Effect	**df**	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	**df**	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***
Temperature	4	151.28	≤.001	119.29	≤.001	4	2.18	.073	1.67	.158	2.03	.092
Population	6	57.39	≤.001	37.82	≤.001	6	0.24	.963	0.67	.672	1.88	.086
Temperature x population	24	4.01	≤.001	3.26	≤.001	14	0.58	.877	0.79	.675	1.27	.232
Temperature	4	348.99	≤.001	260.97	≤.001	4	2.39	.055	1.04	.392	6.20	≤.001
Genotype	52	14.55	≤.001	9.78	≤.001	44	1.13	.298	1.34	.106	8.10	≤.001
Temperature x genotype	178	2.73	≤.001	2.19	≤.001	43	0.97	.540	0.97	.527	2.03	≤.001

Table 4-6. ANOVA (F ratio, Prob > F, df) for the effects of temperature, population, and genotype and their interactions for the following traits: # alive shoots, # dead shoots, # above ground shoots, # crown shoots, and # non-emergent shoots. Non-emergent shoots are defined as those originating below the soil line, crown shoots as 0-3 cm above the soil line, and above ground shoots as > 3 cm above the soil line.

		# alive shoots		# dead shoots			# above ground shoots		# crown shoots		# non-emergent shoots	
Effect	**df**	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	**df**	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***	***F* ratio**	**Prob > *F***
Temperature	4	62.70	≤.001	14.69	≤.001	4	26.71	≤.001	19.81	≤.001	3.43	.009
Population	6	17.21	≤.001	15.85	≤.001	6	4.85	≤.001	8.05	≤.001	12.07	≤.001
Temperature x population	24	2.25	≤.001	1.73	.017	21	1.73	.023	2.54	≤.001	3.13	≤.001
Temperature	4	169.54	≤.001	1053.94	≤.001	4	56.34	≤.001	54.79	≤.001	6.12	≤.001
Genotype	52	8.19	≤.001	244.43	≤.001	45	2.94	≤.001	3.82	≤.001	10.30	≤.001
Temperature x genotype	178	2.59	≤.001	51.29	.003	151	1.61	≤.001	1.77	≤.001	2.59	≤.001

Table 4-7. Mean ± S.E. proportional shoot contributions by location and temperature treatment after four weeks of regrowth. The sample size (n) varied by temperature due to mortality. Mean separations based on Tukey's 5% HSD results for significant main effects.

Temperature (°C)	n	Proportion of above ground shoots	Proportion of crown shoots	Proportion of non-emergent shoots
0	123	0.44 ± 0.03	0.50 ± 0.03	0.06 ± 0.01 a
-4	56	0.44 ± 0.05	0.53 ± 0.05	0.03 ± 0.02 a
-8	12	0.35 ± 0.11	0.56 ± 0.11	0.09 ± 0.03 a
-10	13	0.28 ± 0.11	0.68 ± 0.11	0.04 ± 0.03 a
-12	10	0.11 ± 0.13	0.60 ± 0.13	0.28 ± 0.04 b

Table 4-8. Pearson correlation coefficients (r) for LT_{50}, field % winter survival (WS %), shoot counts, proportion of alive shoots, root rating (1-5 Likert scale), # of alive shoots, # dead shoots, proportion of alive shoots, proportion of dead shoots, proportion of non-emergent shoots, # of aboveground shoots, # of crown shoots, and number of non-emergent shoots. Note: r are calculated on a genotype mean basis to include LT_{50} and field % winter survival.

	LT_{50}	WS %	Prop alive shoots	Root dmg	# alive shoots	# dead shoots	Prop abv shoots	Prop crn shoots	Prop NE shoots	# abv shoots	# crn shoots	# NE shoots
LT_{50}	1											
WS %	.494 **	1										
Prop. alive shoots[a]	-.918 ***	-.607 ***	1									
Root dmg[a]	-.935 ***	-.537 ***	.935 ***	1								
# alive shoots[a]	-.758 ***	-.305 *	.698 ***	.746 ***	1							
# dead shoots	.532 ***	.562 ***	-.628 ***	-.459 **	-.063	1						
Prop. Abv. Shoots	-.054	-.101	.045	.097	-.010	-.068	1					
Prop. Crn. Shoots	.149	.363 *	-.230	-.175	.006	.345 *	-.690 ***	1				
Prop. NE shoots	-.035	-.418 **	.220	.092	-.040	-.373 *	-.249	-.492 **	1			
# abv shoots[a]	-.675 ***	-.268	.617 ***	.697 ***	.835 ***	-.047	.395 **	-.314 *	-.068	1		
# crn shoots[a]	-.772 ***	-.089	.522 ***	.600 ***	.877 ***	.147	-.286	.328 *	-.161	.493 **	1	
# NE shoots[a]	-.526 **	-.670 ***	.604 ***	.545 ***	.457 **	-.402 **	-.035	-.347 *	.541 ***	.476 **	.207	1

*, **, *** Significance at the 0.05, 0.01, and 0.001 probability levels, respectively (two-tailed)

[a] Note that because lower LT_{50} values indicate greater cold tolerance, a negative correlation is desired.

Table 4-9. Mean ± SE regrowth measurements by population of breeding lines and *Linum spp.* after four weeks post-freezing tests for traits: proportion of alive shoots, root damage rating (1-5 Likert scale; 1=dead, 5=no damage), proportion of aboveground shoots, proportion of crown shoots, proportion of non-emergent shoots. The sample numbers (n) varied for the traits examined. Mean separations based on Tukey's 5% HSD results for significant main effects. Accessions are listed first, followed by breeding populations.

Population	n	Proportion of alive shoots	Root damage rating (1-5)	n	Proportion of aboveground shoots	Proportion of crown shoots	Proportion of non-emergent shoots
L. austriacum	65	0.64 ± 0.03 b	3.58 ± 0.16 b	22	0.30 ± 0.11	0.57 ± 0.11	0.13 ± 0.04
L. lewisii	21	0.23 ± 0.05 a	1.77 ± 0.24 a	6	0.57 ± 0.17	0.43 ± 0.17	0.00 ± 0.07
L. perenne	137	0.54 ± 0.02 b	3.19 ± 0.09 b	26	0.32 ± 0.09	0.49 ± 0.09	0.19 ± 0.04
BB1	210	0.22 ± 0.02 a	1.97 ± 0.07 a	69	0.37 ± 0.08	0.58 ± 0.08	0.05 ± 0.03
BB2	60	0.27 ± 0.03 a	2.20 ± 0.14 a	25	0.33 ± 0.10	0.65 ± 0.10	0.02 ± 0.04
KJ1	180	0.18 ± 0.02 a	1.78 ± 0.08 a	49	0.27 ± 0.11	0.71 ± 0.11	0.02 ± 0.05
KJ2	60	0.18 ± 0.03 a	1.88 ± 0.14 a	17	0.23 ± 0.15	0.66 ± 0.15	0.11 ± 0.06

Table 4-10. Mean ± SE regrowth measurements by population of breeding lines and *Linum spp.* after four weeks post-freezing tests for traits: # alive shoots, # dead shoots, # above ground shoots, # crown shoots, and # non-emergent shoots. The sample numbers (n) varied for the traits examined. Mean separations based on Tukey's 5% HSD results for significant main effects. Accessions are listed first, followed by breeding populations.

Population	n	# alive shoots	# dead shoots	n	# above ground shoots	# crown shoots	# non-emergent shoots
L. austriacum	65	13.74 ± 1.01 c	6.12 ± 1.03 bc	36	7.23 ± 0.88 b	8.07 ± 0.88 b	1.70 ± 0.22 b
L. lewisii	21	3.47 ± 1.58 a	7.13 ± 1.60 bc	19	2.07 ± 1.15 a	1.43 ± .1.14 a	0.00 ± 0.28 a
L. perenne	137	8.83 ± 0.61 b	5.96 ± 0.62 c	34	3.94 ± 1.09 ab	6.80 ± .1.09 ab	1.58 ± 0.26 b
BB1	210	5.12 ± 0.47 ab	11.87 ± 0.48 a	210	2.23 ± 0.33 a	2.72 ± 0.33 a	0.05 ± 0.08 a
BB2	60	5.50 ± 0.88 ab	9.52 ± 0.90 ab	60	2.27 ± 0.63 a	3.25 ± 0.62 a	0.05 ± 0.15 a
KJ1	180	4.17 ± 0.51 a	11.87 ± 0.52 a	180	2.08 ± 0.36 a	2.03 ± 0.36 a	0.06 ± 0.09 a
KJ2	60	4.43 ± 0.88 a	12.25 ± 0.90 a	60	1.85 ± 0.63 a	2.45 ± 0.62 a	0.22 ± 0.15 a

Table 4-11. Mean ± S.E. trait values on a genotype basis for measures of regrowth (proportion of alive shoots, root damage rating [1-5 Likert scale; 1 = dead, 2 = 75% damaged, 3 = 50% damaged. 4 = 25% damage, 5 = no damage], proportion of aboveground shoots to total alive shoots, proportion of crown shoots to total alive shoots, proportion of non-emergent shoots to total alive shoots) four weeks after the freezing tests. The sample numbers (n) varied for the traits examined. Mean separations based on Tukey's 5% HSD results for significant main effects.

Population	Genotype	n	Proportion of alive shoots	Root damage rating (1-5)	n	Prop. above ground shoots	Prop. crown shoots	Prop. non-emergent shoots
L. austriacum	262-1 (R1)	9	0.45 ± 0.06 g-o	2.67 ± 0.29 c-i	5	0.45 ± 0.17	0.17 ± 0.21	0.00 ± 0.05
	267-1	14	0.59 ± 0.05 k-r	3.60 ± 0.24 h-j	0			
	272-1	9	0.70 ± 0.06 o-r	4.00 ± 0.29 ij	8	0.35 ± 0.13	0.51 ± 0.13	0.14 ± 0.04
	272-2	6	0.50 ± 0.07 j-q	2.83 ± 0.35 e-j	3	0.40 ± 0.21	0.10 ± 0.21	0.50 ± 0.06
	272-3	6	0.50 ± 0.07 j-q	2.50 ± 0.35 b-h	3	0.63 ± 0.21	0.29 ± 0.21	0.08 ± 0.06
	272-6	15	0.61 ± 0.05 l-r	3.53 ± 0.22 h-j	0			
	274-2	6	0.42 ± 0.074 f-o	2.83 ± 0.35 e-j	3	0.08 ± 0.21	0.92 ± 0.21	0.00 ± 0.06
L. lewisii	104-4	6	0.46 ± 0.07 i-p	2.33 ± 0.35 a-h	2	0.35 ± 0.26	0.65 ± 0.26	0.00 ± 0.07
	'Maple Grove'	15	0.19 ± 0.05 a-i	1.80 ± 0.22 a-f	4	0.69 ± 0.18	0.31 ± 0.18	0.00 ± 0.05
L. perenne	292-1	11	0.72 ± 0.06 a-j	3.58 ± 0.27 h-j	0			
	292-4	6	0.45 ± 0.07 h-p	2.67 ± 0.35 c-i	3	0.60 ± 0.21	0.26 ± 0.21	0.15 ± 0.06
	293-1	15	0.65 ± 0.07 m-r	3.20 ± 0.22 f-j	0			

Population	Genotype	n	Proportion of alive shoots	Root damage rating (1-5)	n	Prop. above ground shoots	Prop. crown shoots	Prop. non-emergent shoots
	293-3	12	0.77 ± 0.05 qr	4.17 ± 0.25 j	12	0.30 ± 0.11	0.53 ± 0.11	0.17 ± 0.03
	293-6	15	0.75 ± 0.05 p-r	4.20 ± 0.22 j	0			
	294-1	6	0.42 ± 0.07 e-o	2.67 ± 0.35 c-i	3	0.63 ± 0.21	0.11 ± 0.21	0.26 ± 0.06
	294-2	6	0.81 ± 0.07 r	4.17 ± 0.35 j	6	0.39 ± 0.15	0.41 ± 0.15	0.20 ± 0.04
	295-1	6	0.36 ± 0.07 c-m	1.83 ± 0.35 a-f	2	0.10 ± 0.26	0.00 ± 0.26	0.90 ± 0.07
	BF	30	0.40 ± 0.03 d-n	2.80 ± 0.16 d-j	0			
	SP	30	0.43 ± 0.03 f-o	2.70 ± 0.16 c-i	0			
BB1	3-12N	14	0.26 ± 0.05 a-j	2.33 ± 0.24 a-h	6	0.35 ± 0.15	0.65 ± 0.15	0.00 ± 0.4
	3-16N	16	0.43 ± 0.05 e-o	1.97 ± 0.22 a-g	6	0.21 ± 0.17	0.79 ± 0.17	0.00 ± 0.05
	4-6N	15	0.15 ± 0.05 a-g	1.33 ± 0.22 a-c	3	0.29 ± 0.21	0.71 ± 0.21	0.00 ± 0.06
	5-14N	15	0.11 ± 0.05 a-d	1.40 ± 0.22 a-d	2	0.00 ± 0.26	1.00 ± 0.26	0.00 ± 0.07
	5-2N	15	0.29 ± 0.05 a-k	2.40 ± 0.22 a-h	7	0.31 ± 0.16	0.69 ± 0.16	0.00 ± 0.04
	5-8N	15	0.31 ± 0.05 b-k	2.27 ± 0.22 a-h	6	0.43 ± 0.15	0.58 ± 0.15	0.00 ± 0.4
	6-14N	15	0.05 ± 0.05 ab	1.20 ± 0.22 ab	1	0.50 ± 0.37	0.50 ± 0.37	0.00 ± 0.10
	6-19N	15	0.34 ± 0.05 b-l	3.27 ± 0.22 g-j	9	0.46 ± 0.14	0.54 ± 0.14	0.00 ± 0.04
	6-22N	15	0.17 ± 0.05 a-i	1.87 ± 0.22 a-g	5	0.48 ± 0.17	0.52 ± 0.17	0.01 ± 0.05

Population	Genotype	n	Proportion of alive shoots	Root damage rating (1-5)	n	Prop. above ground shoots	Prop. crown shoots	Prop. non-emergent shoots
	7-18N	15	0.21 ± 0.05 a-j	2.00 ± 0.22 a-g	5	0.83 ± 0.17	0.16 ± 0.17	0.01 ± 0.05
	8-12N	15	0.16 ± 0.05 a-h	2.20 ± 0.22 a-g	4	0.81 ± 0.18	0.16 ± 0.18	0.03 ± 0.05
	8-17N	15	0.10 ± 0.05 a-c	1.33 ± 0.224 a-c	2	0.00 ± 0.26	1.00 ± 0.26	0.00 ± 0.07
	8-24N	15	0.26 ± 0.05 a-j	2.00 ± 0.22 a-g	5	0.39 ± 0.19	0.59 ± 0.19	0.03 ± 0.05
	9-2N	15	0.29 ± 0.05 a-j	2.20 ± 0.22 a-h	8	0.27 ± 0.13	0.56 ± 0.13	0.17 ± 0.04
BB2	1-11S	15	0.33 ± 0.05 b-l	2.73 ± 0.22 a-h	6	0.34 ± 0.17	0.66 ± 0.17	0.00 ± 0.05
	1-20S	15	0.33 ± 0.05 b-l	2.73 ± 0.22 c-i	9	0.36 ± 0.14	0.62 ± 0.14	0.02 ± 0.04
	1-21S	15	0.27 ± 0.05 a-j	2.00 ± 0.22 a-g	6	0.56 ± 0.17	0.44 ± 0.17	0.05 ± 0.05
	1-7S	15	0.16 ± 0.05 a-h	1.87 ± 0.22 a-g	4	0.31 ± 0.21	0.69 ± 0.21	0.00 ± 0.06
KJ1	3-12S	15	0.33 ± 0.05 b-l	2.13 ± 0.22 a-h	7	0.44 ± 0.16	0.55 ± 0.16	0.01 ± 0.04
	4-10S	15	0.25 ± 0.05 a-j	1.87 ± 0.22 a-g	5	0.53 ± 0.17	0.44 ± 0.17	0.03 ± 0.05
	4-11S	15	0.23 ± 0.05 a-j	1.87 ± 0.22 a-g	5	0.83 ± 0.17	0.18 ± 0.17	0.00 ± 0.05
	4-15S	15	0.08 ± 0.05 a-c	1.47 ± 0.22 a-e	3	0.00 ± 0.23	0.38 ± 0.23	0.63 ± 0.06
	4-16S	15	0.16 ± 0.05 a-h	1.67 ± 0.22 a-e	4	0.08 ± 0.21	0.90 ± 0.21	0.01 ± 0.06
	4-18S	15	0.26 ± 0.05 a-j	2.33 ± 0.22 a-h	6	0.51 ± 0.15	0.49 ± 0.15	0.00 ± 0.04
	4-19S	15	0.13 ± 0.05 a-f	1.53 ± 0.22 a-e	3	0.41 ± 0.23	0.59 ± 0.23	0.00 ± 0.06

Population	Genotype	n	Proportion of alive shoots	Root damage rating (1-5)	n	Prop. above ground shoots	Prop. crown shoots	Prop. non-emergent shoots
	4-20S	15	0.32 ± 0.05 b-l	2.53 ± 0.22 b-h	8	0.26 ± 0.15	0.74 ± 0.15	0.00 ± 0.04
	4-9S	15	0.00 ± 0.05 a	1.00 ± 0.24 a	0			
	5-14S	15	0.10 ± 0.05 a-c	1.67 ± 0.22 a-e	3	0.06 ± 0.21	0.94 ± 0.21	0.00 ± 0.06
	5-6S	15	0.12 ± 0.05 a-d	1.47 ± 0.22 a-e	2	0.35 ± 0.26	0.65 ± 0.26	0.00 ± 0.07
	5-8S	15	0.14 ± 0.05 a-f	1.80 ± 0.22 a-f	3	0.70 ± 0.21	0.29 ± 0.21	0.01 ± 0.06
KJ2	1-2N	15	0.25 ± 0.05 a-j	2.53 ± 0.22 b-h	7	0.26 ± 0.16	0.71 ± 0.16	0.03 ± 0.04
	2-11N	15	0.18 ± 0.05 a-i	1.53 ± 0.22 a-e	4	0.23 ± 0.21	0.59 ± 0.21	0.18 ± 0.06
	2-4N	15	0.20 ± 0.05 a-i	1.93 ± 0.22 a-g	4	0.45 ± 0.21	0.53 ± 0.21	0.02 ± 0.06
	2-5N	15	0.09 ± 0.05 a-c	1.53 ± 0.22 a-e	2	0.55 ± 0.26	0.41 ± 0.26	0.04 ± 0.07

Table 4-12. Mean ± SE trait values on a genotype basis for the number of alive shoots, number of dead shoots, number of aboveground shoots, number of crown shoots, and number of non-emergent shoots. The sample numbers (n) varied for the traits examined. Mean separations based on Tukey's 5% HSD results for significant main effects four weeks after the freezing tests, reported as mean.

Population	Genotype	n	Number of alive shoots	Number of dead shoots	n	Number of above ground shoots	Number of crown shoots	Number of non-emergent shoots
L. austriacum	262-1 (R1)	9	10.00 ± 1.78 c-h	7.67 ± 2.03 a-h	9	4.00 ± 1.45 a	6.00 ± 1.42 abc	0.00 ± .31 a
	267-1	14	14.97 ± 1.45 f-h	10.30 ± 1.65 a-i	0			
	272-1	9	26.56 ± 1.78 i	9.33 ± 2.03 a-i	9	13.11 ± 1.45 b	11.56 ± 1.42 cd	3.67 ± 0.31 d
	272-2	6	6.83 ± 2.18 a-f	2.83 ± 2.48 a-c	6	4.00 ± 1.77 a	1.00 ± 1.74 a	5.83 ± 0.37 e
	272-3	6	7.50 ± 2.18 a-g	4.33 ± 2.48 a-e	6	6.33 ± 1.77 ab	1.17 ± 1.74 a	0.67 ± 0.37 ab
	272-6	15	7.47 ± 1.38 a-g	3.13 ± 1.57 a-c	0			
	274-2	6	16.50 ± 2.18 h	14.33 ± 2.48 e-j	6	2.00 ± 1.77 a	14.50 ± 1.74 d	0.00 ± 0.37 a
L. lewisii	104-4	6	3.67 ± 2.18 a-d	2.33 ± 2.48 ab	4	1.75 ± 2.17 a	1.50 ± 2.13 a	0.00 ± 0.46 a
	'Maple Grove'	15	3.93 ± 1.38 a-d	7.53 ± 1.57 a-h	15	2.53 ± 1.12 a	1.40 ± 1.10 a	0.00 ± 0.24 a
L. perenne	292-1	11	8.04 ± 1.64 a-h	2.17 ± 1.86 ab	0			
	292-4	6	3.83 ± 2.18 a-d	1.67 ± 2.48 a	6	2.50 ± 1.77 a	1.17 ± 1.74 a	0.83 ± 0.37 ab
	293-1	15	13.47 ± 1.38 e-h	4.07 ± 1.57 a-d	0			
	293-3	12	15.67 ± 1.54 gh	5.00 ± 1.76 a-f	12	5.50 ± 1.25 a	9.33 ± 1.23 bcd	2.67 ± 0.27 cd

Population	Genotype	n	Number of alive shoots	Number of dead shoots	n	Number of above ground shoots	Number of crown shoots	Number of non-emergent shoots
	293-6	15	9.40 ± 1.38 b-h	3.07 ± 1.57 a-c	0			
	294-1	6	2.83 ± 2.18 a-d	2.83 ± 2.48 a-c	6	2.17 ± 1.77 a	0.33 ± 1.74 a	0.83 ± 0.37 ab
	294-2	6	8.83 ± 2.18 b-h	2.50 ± 2.48 ab	6	3.83 ± 1.77 a	4.17 ± 1.76 ab	1.83 ± 0.37 bc
	295-1	6	3.00 ± 2.18 a-d	3.17 ± 2.48 a-c	4	0.25 ± 2.17 a	0.00 ± 2.13 a	1.50 ± 0.46 abc
	BF	30	6.20 ± 0.98 a-f	8.77 ± 1.11 a-h	0			
	SP	30	9.30 ± 0.98 b-h	8.13 ± 1.11 a-h	0			
BB1	3-12N	14	3.73 ± 1.45 a-d	9.07 ± 1.65 a-h	14	1.47 ± 1.18 a	2.27 ± 1.10 ab	0.00 ± 0.25 a
	3-16N	16	6.62 ± 1.34 a-f	8.70 ± 1.53 a-h	16	1.48 ± 1.09 a	3.33 ± 1.10 ab	0.00 ± 0.23 a
	4-6N	15	2.67 ± 1.38 a-d	8.53 ± 1.57 a-h	15	0.87 ± 1.12 a	1.87 ± 1.10 a	0.00 ± 0.24 a
	5-14N	15	1.40 ± 1.38 a-c	13.27 ± 1.57 d-j	15	0.00 ± 1.12 a	1.40 ± 1.10 a	0.00 ± 0.24 a
	5-2N	15	8.47 ± 1.38 a-h	19.33 ± 1.57 ij	15	2.87 ± 1.12 a	5.53 ± 1.10 abc	0.00 ± 0.237 a
	5-8N	15	10.67 ± 1.38 d-h	10.87 ± 1.57 a-i	15	4.67 ± 1.12 a	5.87 ± 1.10 abc	0.00 ± 0.24 a
	6-14N	15	1.07 ± 1.38 ab	9.47 ± 1.57 a-i	15	0.53 ± 1.12 a	0.53 ± 1.10 a	0.00 ± 0.24 a
	6-19N	15	8.67 ± 1.38 a-h	11.20 ± 1.57 a-i	15	4.93 ± 1.12 a	3.73 ± 1.10 ab	0.00 ± 0.24 a
	6-22N	15	4.47 ± 1.38 a-d	15.73 ± 1.57 g-j	15	2.47 ± 1.12 a	1.93 ± 1.10 a	0.07 ± 0.24 a
	7-18N	15	4.07 ± 1.38 a-d	10.53 ± 1.57 a-i	15	3.20 ± 1.12 a	0.87 ± 1.10 a	0.07 ± 0.24 a

Population	Genotype	n	Number of alive shoots	Number of dead shoots	n	Number of above ground shoots	Number of crown shoots	Number of non-emergent shoots
	8-12N	15	4.67 ± 1.38 a-e	16.27 ± 1.57 g-j	15	4.00 ± 1.12 a	0.67 ± 1.10 a	0.13 ± 0.24 a
	8-17N	15	2.87 ± 1.38 a-d	15.87 ± 1.57 g-j	15	0.00 ± 1.12 a	2.87 ± 1.10 ab	0.00 ± 0.24 a
	8-24N	15	5.00 ± 1.38 a-e	6.33 ± 1.57 a-g	15	1.73 ± 1.12 a	3.27 ± 1.10 ab	0.07 ± 0.24 a
	9-2N	15	7.13 ± 1.38 a-g	10.93 ± 1.57 a-i	15	2.93 ± 1.12 a	3.87 ± 1.10 ab	0.40 ± 0.24 ab
BB2	1-11S	15	5.53 ± 1.38 a-e	8.00 ± 1.57 a-h	15	2.40 ± 1.12 a	3.13 ± 1.10 ab	0.00 ± 0.24 a
	1-20S	15	7.87 ± 1.38 a-h	11.60 ± 1.57 a-i	15	4.00 ± 1.12 a	3.93 ± 1.10 ab	0.13 ± 0.24 a
	1-21S	15	4.73 ± 1.38 a-e	8.33 ± 1.57 a-h	15	1.33 ± 1.12 a	3.40 ± 1.10 ab	0.07 ± 0.24 a
	1-7S	15	3.87 ± 1.38 a-d	10.13 ± 1.57 a-i	15	1.33 ± 1.12 a	2.53 ± 1.10 ab	0.00 ± 0.24 a
KJ1	3-12S	15	6.27 ± 1.38 a-f	7.80 ± 1.57 a-h	15	3.40 ± 1.12 a	2.87 ± 1.10 ab	0.07 ± 0.24 a
	4-10S	15	3.87 ± 1.38 a-d	6.53 ± 1.57 a-g	15	2.00 ± 1.12 a	1.73 ± 1.10 a	0.13 ± 0.24 a
	4-11S	15	5.53 ± 1.38 a-e	12.53 ± 1.57 c-j	15	4.27 ± 1.12 a	1.20 ± 1.10 a	0.00 ± 0.24 a
	4-15S	15	1.80 ± 1.38 a-c	11.87 ± 1.57 b-i	15	0.00 ± 1.12 a	1.47 ± 1.10 a	0.33 ± 0.24 ab
	4-16S	15	3.47 ± 1.38 a-d	10.53 ± 1.57 a-i	15	0.87 ± 1.12 a	2.47 ± 1.10 ab	0.13 ± 0.24 a
	4-18S	15	4.73 ± 1.38 a-e	8.67 ± 1.57 a-h	15	2.80 ± 1.12 a	1.93 ± 1.10 a	0.00 ± 0.24 a
	4-19S	15	3.00 ± 1.38 a-d	16.67 ± 1.57 h-j	15	1.13 ± 1.12 a	1.87 ± 1.10 a	0.00 ± 0.24 a
	4-20S	15	7.33 ± 1.38 a-g	12.27 ± 1.57 b-j	15	3.40 ± 1.12 a	3.93 ± 1.10 ab	0.00 ± 0.24 a

Population	Genotype	n	Number of alive shoots	Number of dead shoots	n	Number of above ground shoots	Number of crown shoots	Number of non-emergent shoots
	4-9S	15	0.00 ± 1.38 a	8.93 ± 1.57 a-h	15	0.00 ± 1.12 a	0.00 ± 1.10 a	0.00 ± 0.24 a
	5-14S	15	2.40 ± 1.38 a-d	11.13 ± 1.57 a-i	15	0.13 ± 1.12 a	2.27 ± 1.10 ab	0.00 ± 0.24 a
	5-6S	15	2.67 ± 1.38 a-d	13.47 ± 1.57 d-j	15	1.00 ± 1.12 a	1.67 ± 1.10 a	0.00 ± 0.24 a
	5-8S	15	9.00 ± 1.379 b-h	22.00 ± 1.57 j	15	5.93 ± 1.12 ab	3.00 ± 1.10 ab	0.07 ± 0.24 a
KJ2	1-2N	15	6.47 ± 1.38 a-f	15.00 ± 1.57 f-j	15	1.93 ± 1.12 a	4.27 ± 1.10 ab	0.33 ± 0.24 ab
	2-11N	15	3.53 ± 1.38 a-d	12.00 ± 1.57 b-j	15	0.80 ± 1.12 a	2.60 ± 1.10 ab	0.27 ± 0.24 a
	2-4N	15	4.80 ± 1.38 a-e	7.27 ± 1.57 a-h	15	2.93 ± 1.12 a	1.73 ± 1.10 a	0.13 ± 0.24 a
	2-5N	15	2.93 ± 1.38 a-d	14.73 ± 1.57 f-j	15	1.73 ± 1.12 a	1.20 ± 1.10 a	0.13 ± 0.24 a

Figures

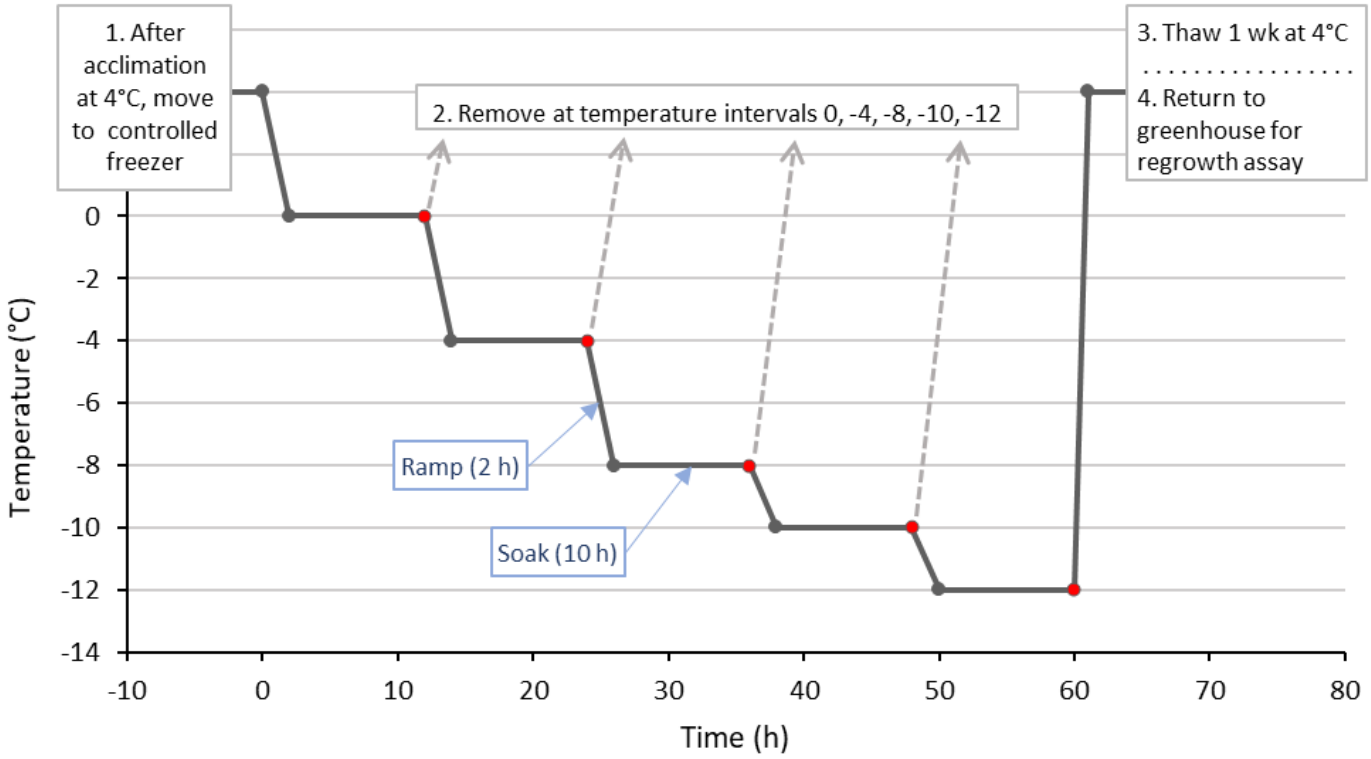


Figure 4-1. Graphical representation of the time (h) versus temperature (°C) programmed for each freezer run, including target soil temperatures, ramp and soak periods, point at which containers are removed, and the details regarding the initiation and conclusion of the experiment.

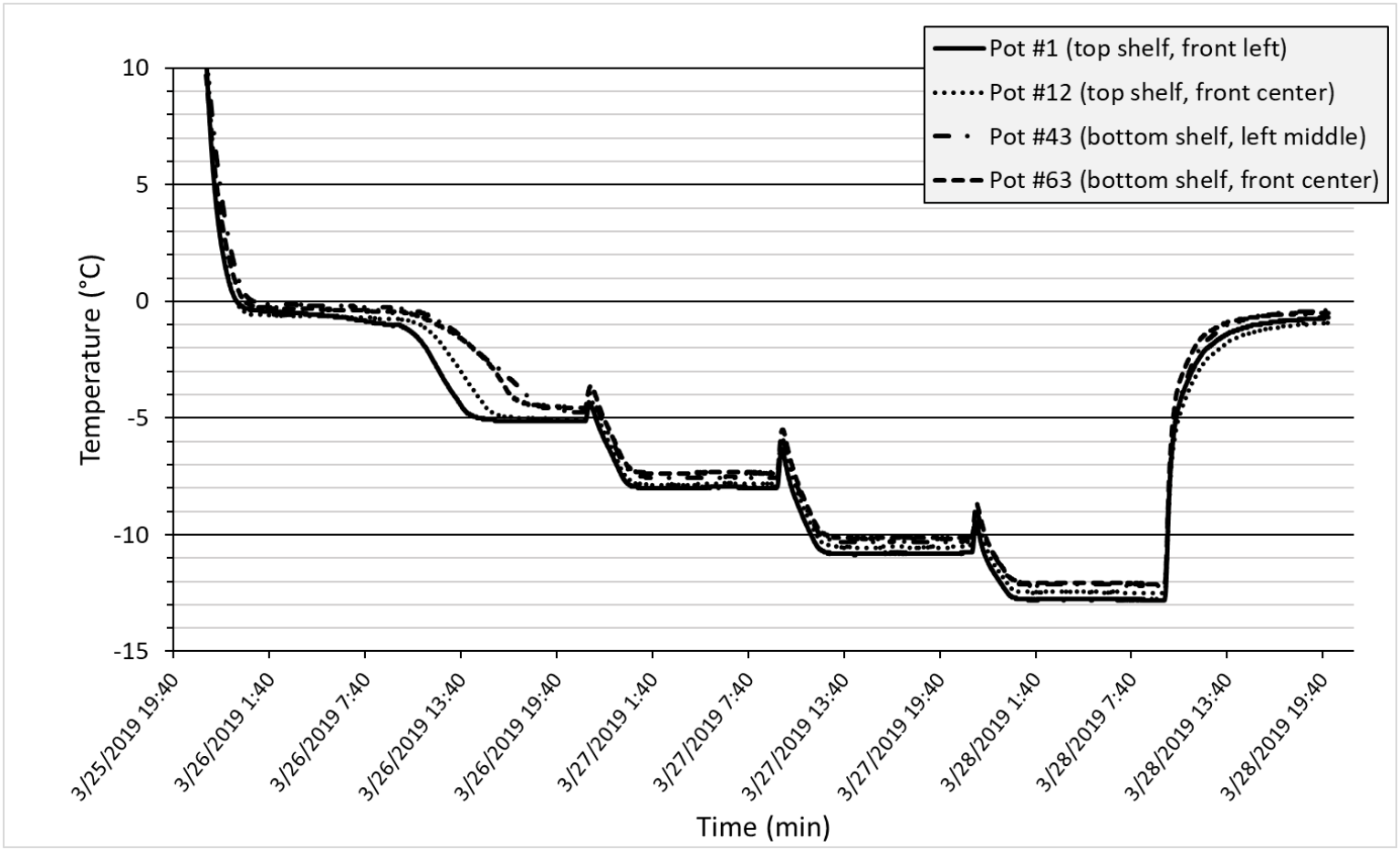


Figure 4-2. Actual soilless medium temperatures during an example experimental run across various locations within the freezer chamber. Temperature probes were buried within empty containers of soilless medium to monitor soil temperature during the experiment. The most extreme temperature variation occurs around 0 °C due to the release of latent heat during freezing.

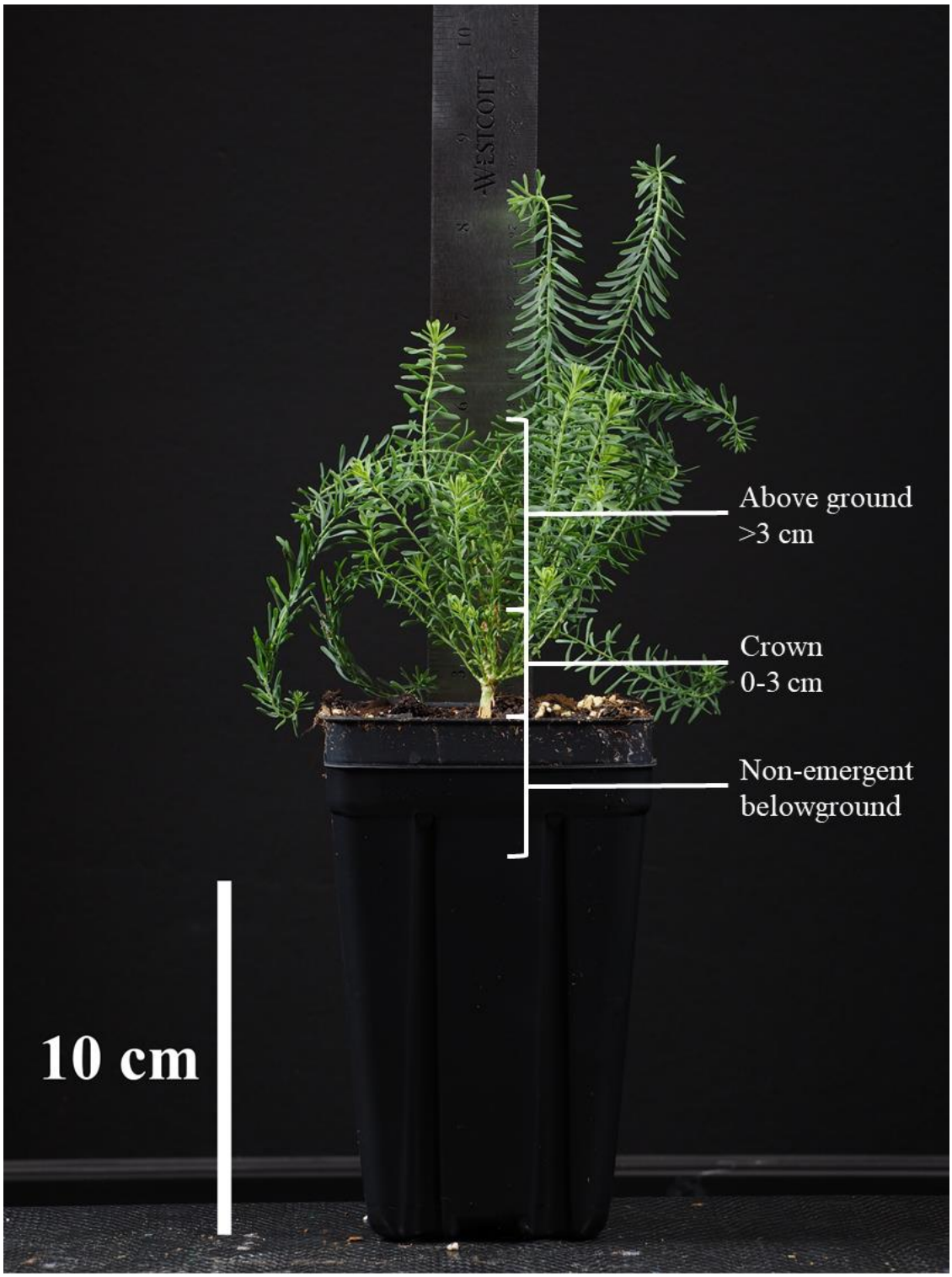
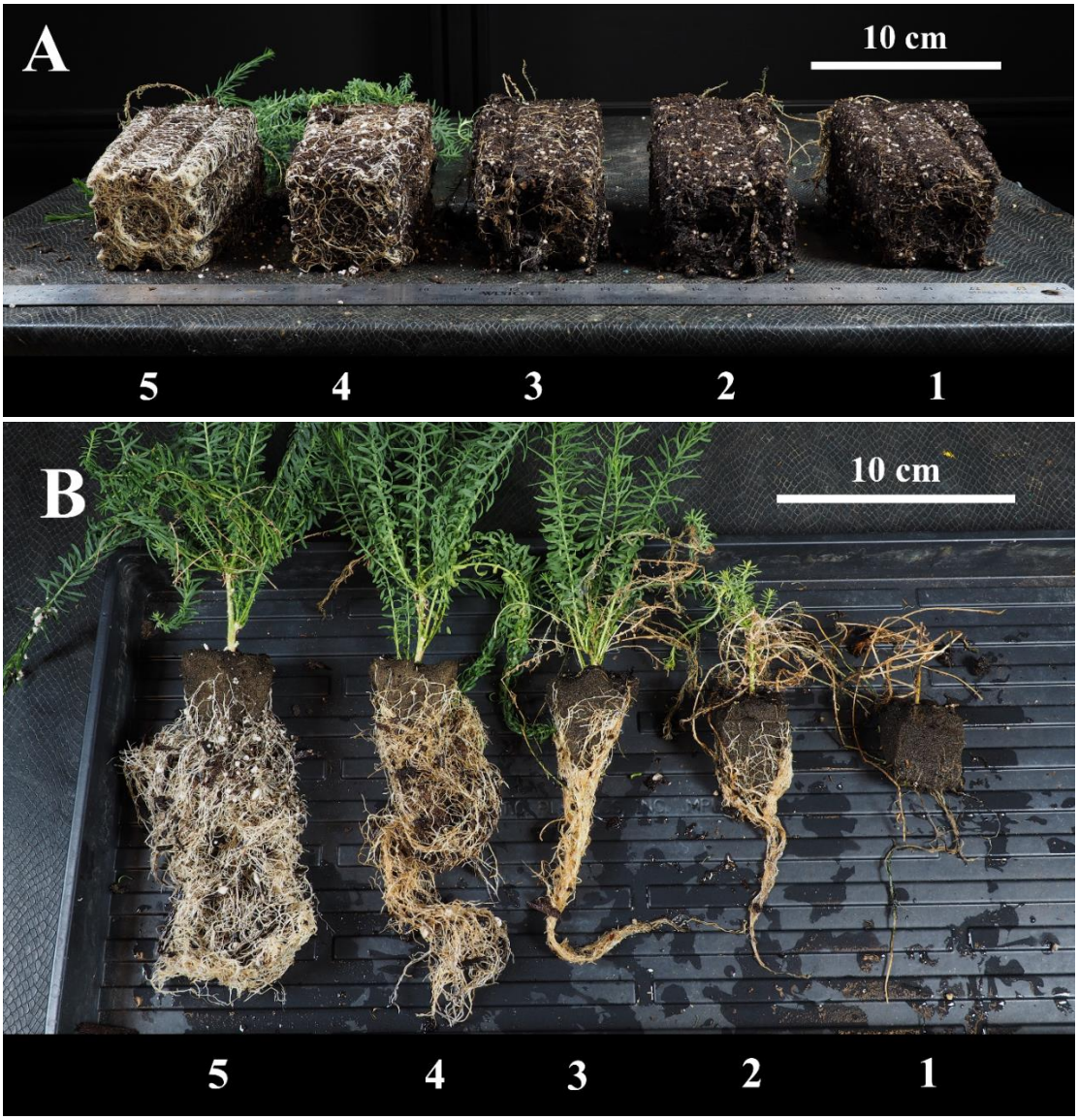


Figure 4-3. Diagram of above ground (> 3 cm), crown (0-3 cm) and non-emergent (belowground) shoot definitions.

Figure 4-4. Example of non-emergent shoots from beneath the soil line in *Linum spp.* sample with dead above ground and crown tissues.



Rating	% Root damage	Description
5	0	No visible damage, white healthy roots, vigorous regrowth
4	25	Mostly white roots, some discoloration visible, medium regrowth
3	50	Mix of white and discolored roots, some regrowth
2	75	Most roots discolored and/or rotten, little regrowth visible
1	100	All roots dead and discolored, no regrowth visible

Figure 4-5. Root damage rating visual scale for *Linum spp.*, including before (A) and after (B) washing. The table integrated above provides a detailed explanation of this scale based on percent root damage, and a textual description of each category.

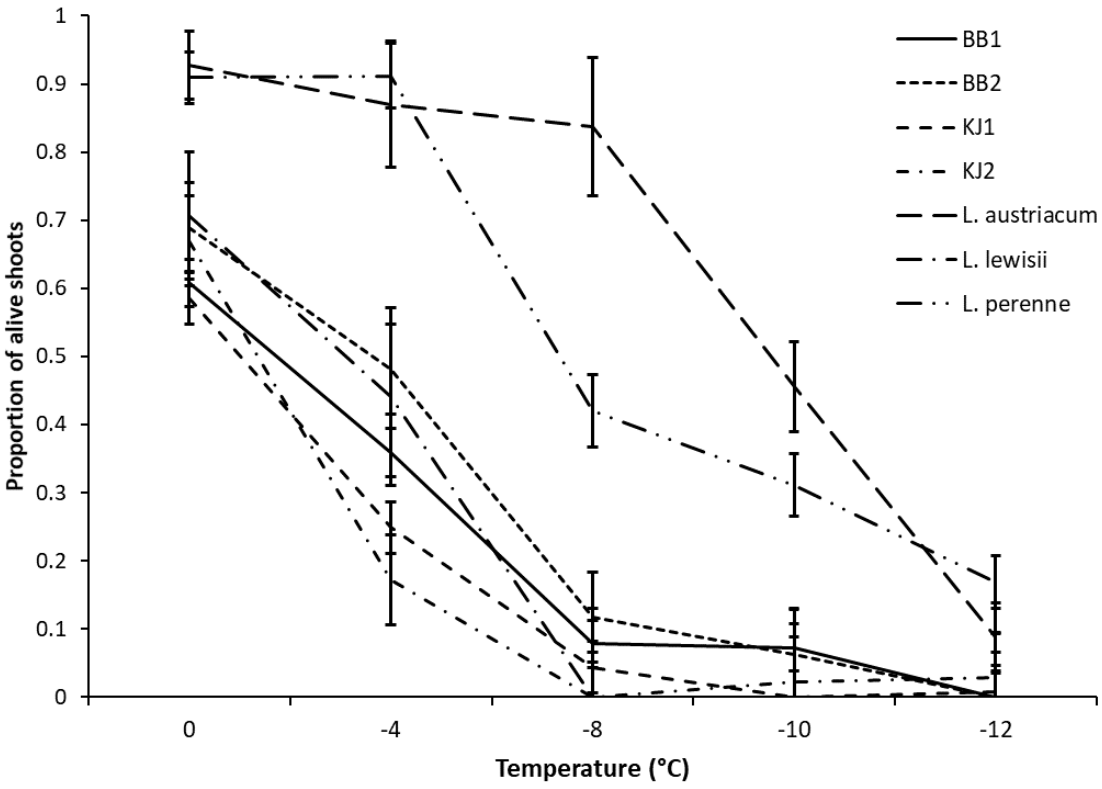


Figure 4-6. Interaction of freezing temperature and population on mean shoot regrowth in *Linum spp.* for the proportion of alive shoots (# alive shoots / total # shoots) to minimize the influence of initial plant size. Populations BB1, BB2, KJ1, and KJ2 are breeding populations of unknown species composition selected for yield or plant habit. Error bars are SE of the means.

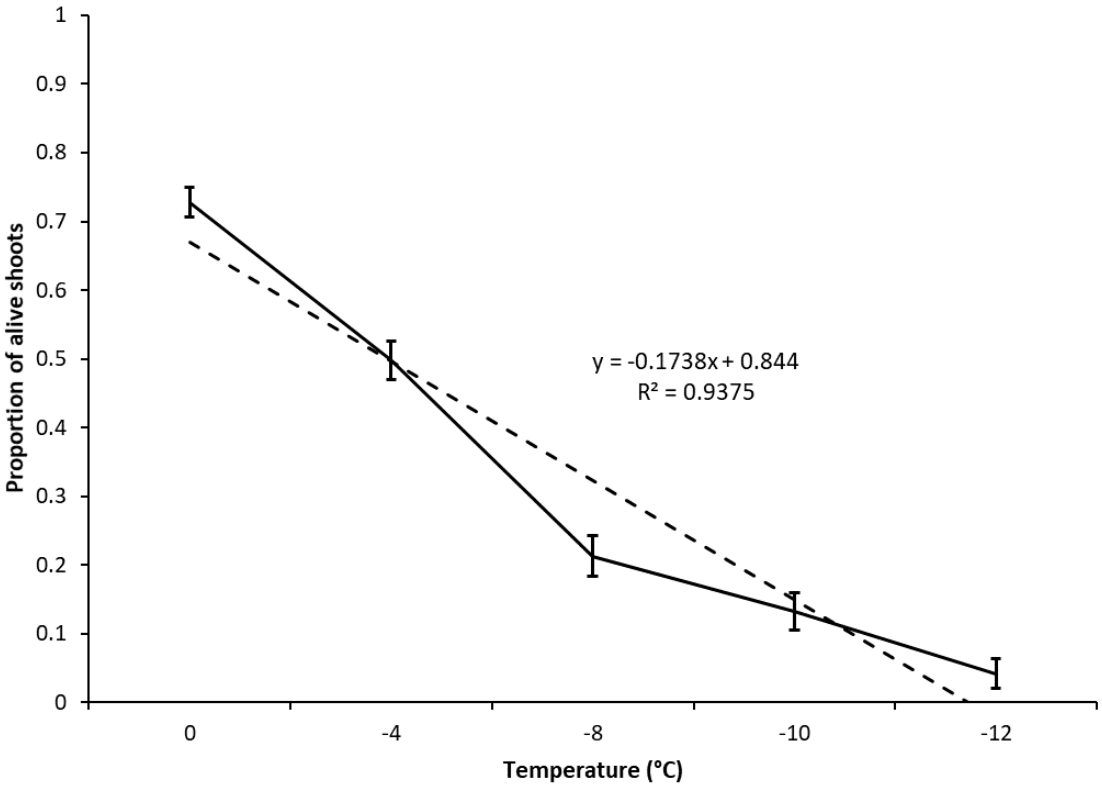


Figure 4-7. Effect of freezing temperature on mean shoot regrowth for all populations, presented as the proportion of alive shoots (# alive shoots / total # shoots) to minimize the influence of initial plant size. Error bars are SE of the means.

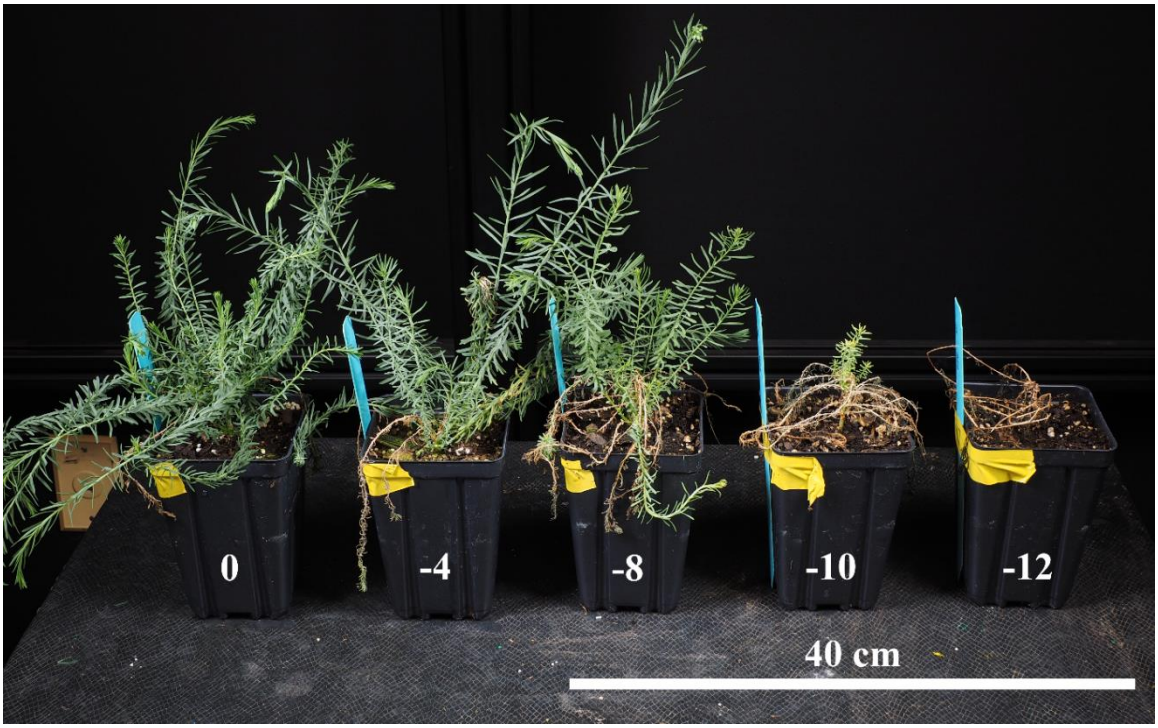


Figure 4-8. Proportion of alive shoots in flax genotype *L. perenne* ‘Blue Flax’ tested across freezing temperature treatments.

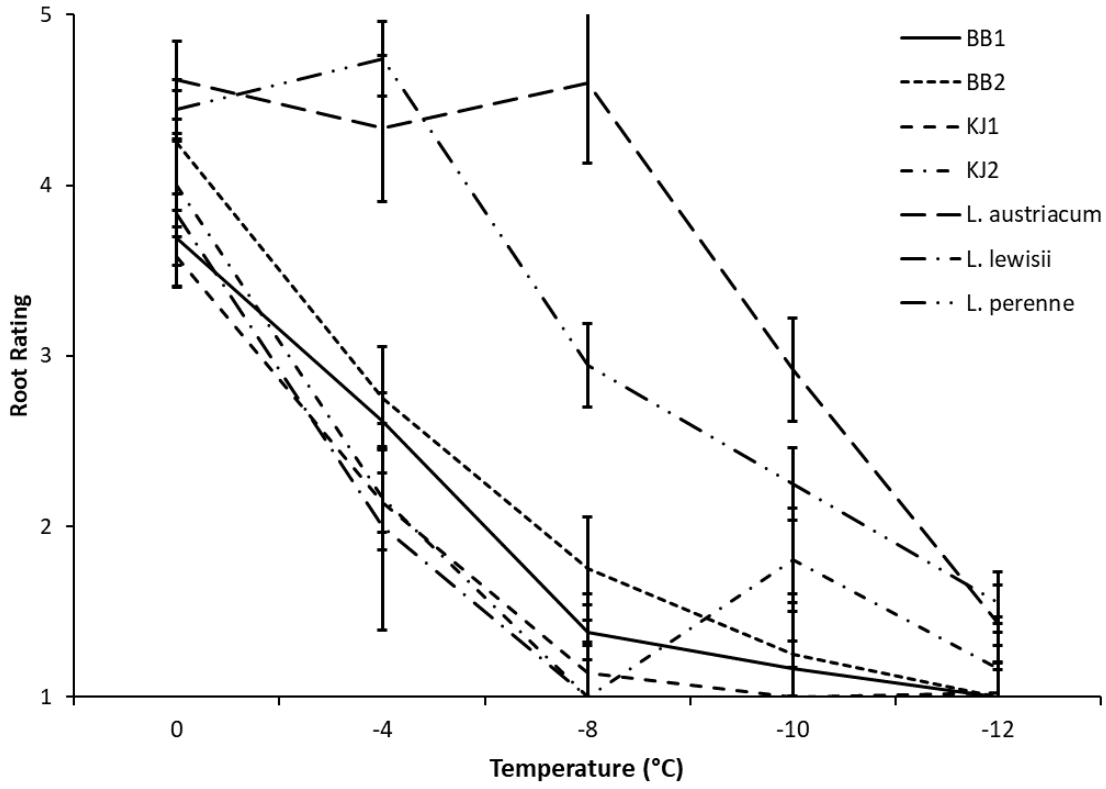


Figure 4-9. Interaction of freezing temperatures and populations on mean ± S.E. root ratings (Likert scale) for all populations of *Linum spp.* Root ratings: 1 (dead, 0% live roots), 2 (25% live roots), 3 (50% live roots), 4 (75% live roots), 5 (100% live roots).

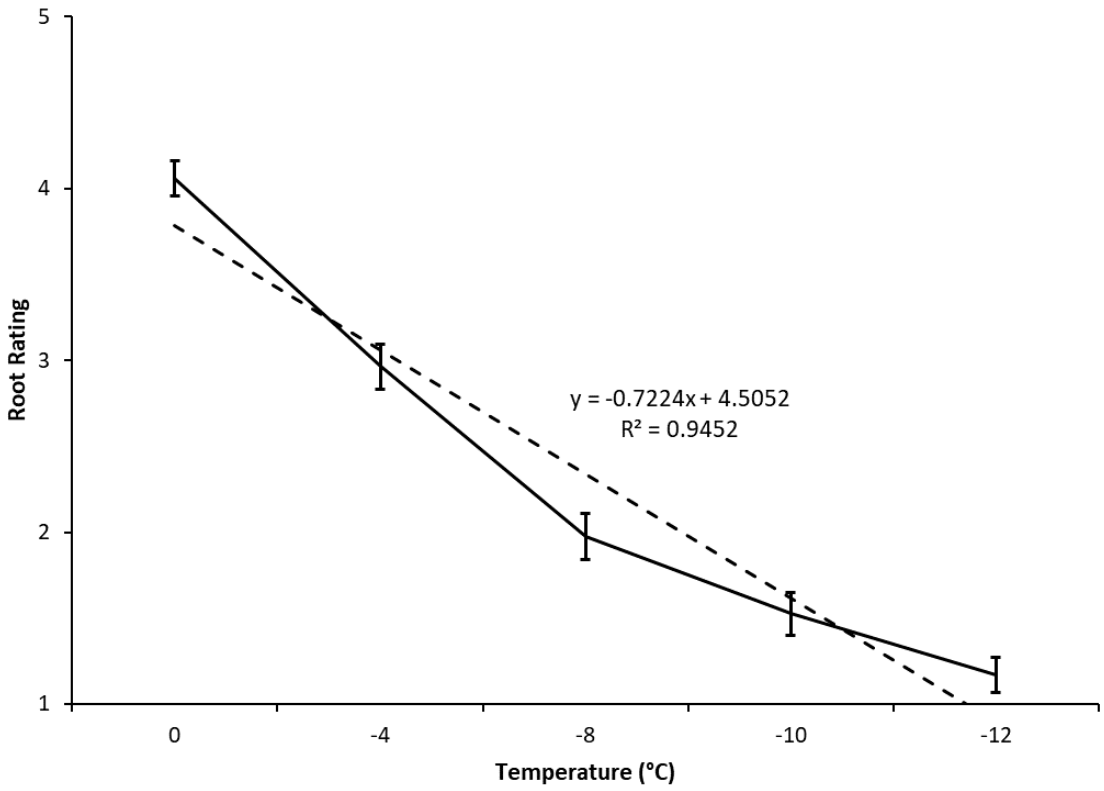


Figure 4-10. Effect of freezing temperature on mean root rating (Likert scale) for all populations of *Linum spp.* Root rating: 1 (dead, 0% live roots), 2 (25% live roots), 3 (50% live roots), 4 (75% live roots), 5 (100% live roots). Error bars are SE of the means.

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Appendix A

Chapter 2 supplemental tables

Table A 1. ANOVA (df, F ratio, Prob > F) for the effects of population (*Linum* species, selections) and genotype on boll rating (1-3; fully dehiscent to non-shattering), # underdeveloped seeds per capsule, length to the first branch > 5 cm (cm), proportion of stem unbranched, stature rating (1-3; prostrate to upright), and average width (cm).

	Species			Genotype		
Trait	**df**	***F* ratio**	**Prop > *F***	**df**	***F* ratio**	**Prop > *F***
Boll rating (1-3)	11	19.831	<.001	114	7.497	<.001
# underdeveloped seeds per capsule	11	4.629	<.001	114	3.222	<.001
Length to first branch (cm)	11	23.420	<.001	125	8.996	<.001
Proportion of stem unbranched	11	15.493	<.001	125	5.126	<.001
Stature Rating (1-3)	11	12.564	<.001	148	4.550	<.001
Avg. width (cm)	11	25.363	<.001	148	8.305	<.001

Table A 2. Percent of plants with horizontal laterals for flax species and cut flower (CF) and oilseed (OS) selections investigated for domestication potential. Horizontal laterals were recorded as a binary rating (y/n) for the presence/absence of branch angles roughly equal to 90 degrees.

Population	% of plants with horizontal laterals
L. altaicum	80.00
L. austriacum	70.33
L. baicalense	95.24
L. bienne	69.57
L. grandiflorum	13.04
L. hirsutum	78.57
L. lewisii	70.94
L. pallescens	83.33
L. perenne	56.38
L. usitatissimum	10.53
Selections - CF	61.54
Selections - OS	41.12

Chapter 2 supplemental figures

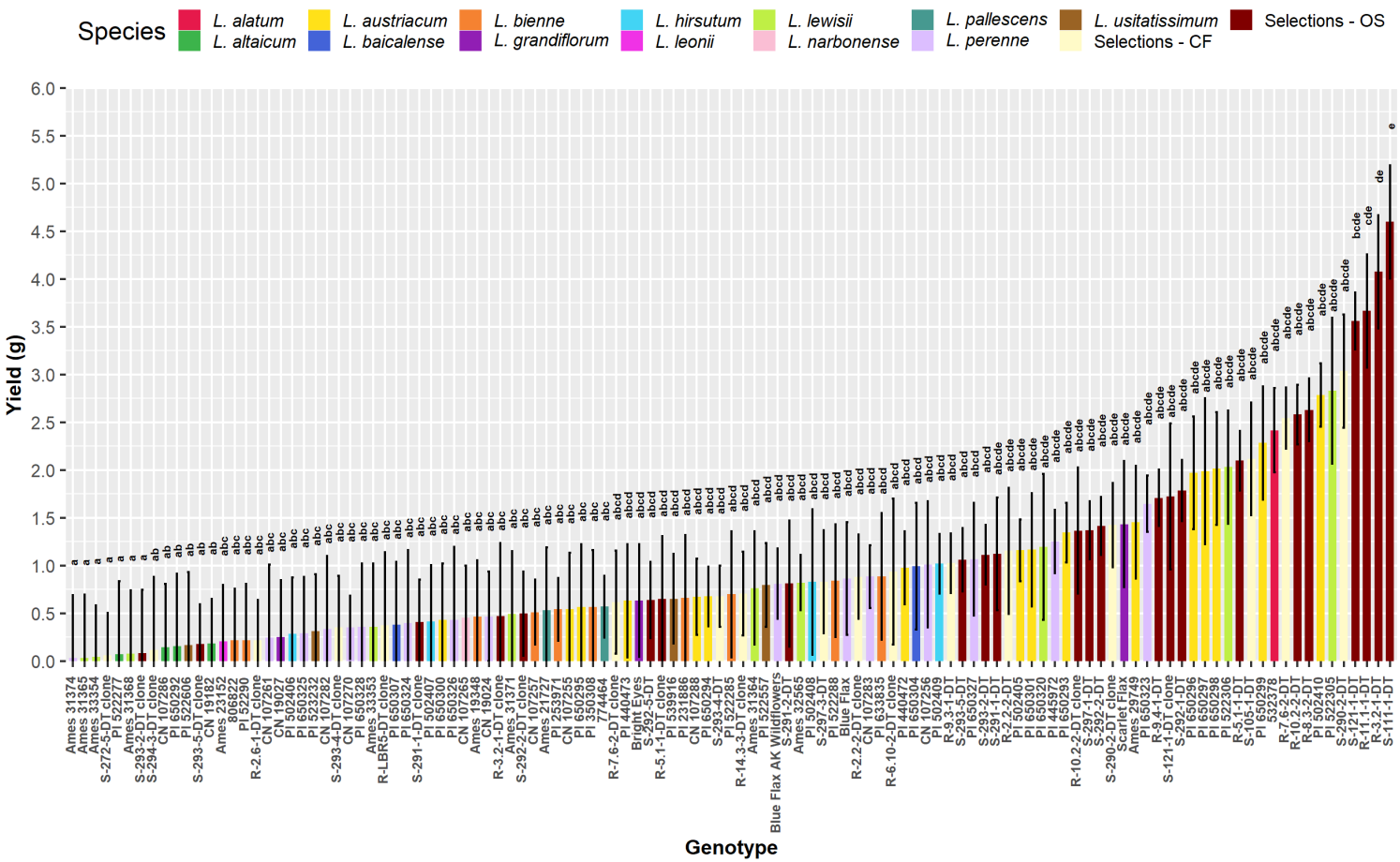

Figure A 1. Mean ± S.E. yield (g) values for all genotypes with n ≥ 3 observations. Means separations are displayed as letters above the columns denoting significance.

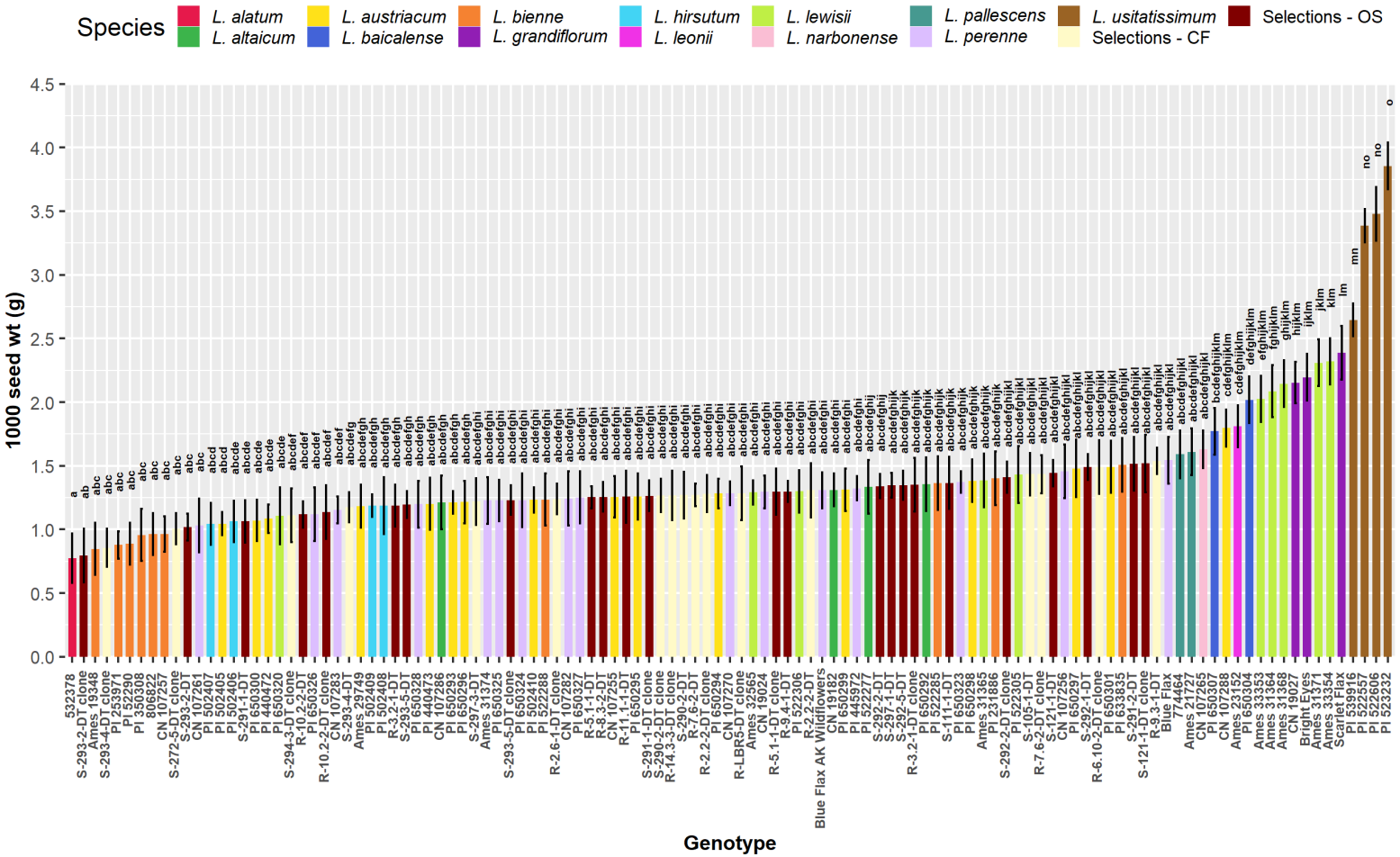

Figure A 2. Mean ± S.E. 1000 seed weight (g) values for all genotypes with n ≥ 3 observations. Means separations (5% HSD) are displayed as letters above the columns denoting significance.

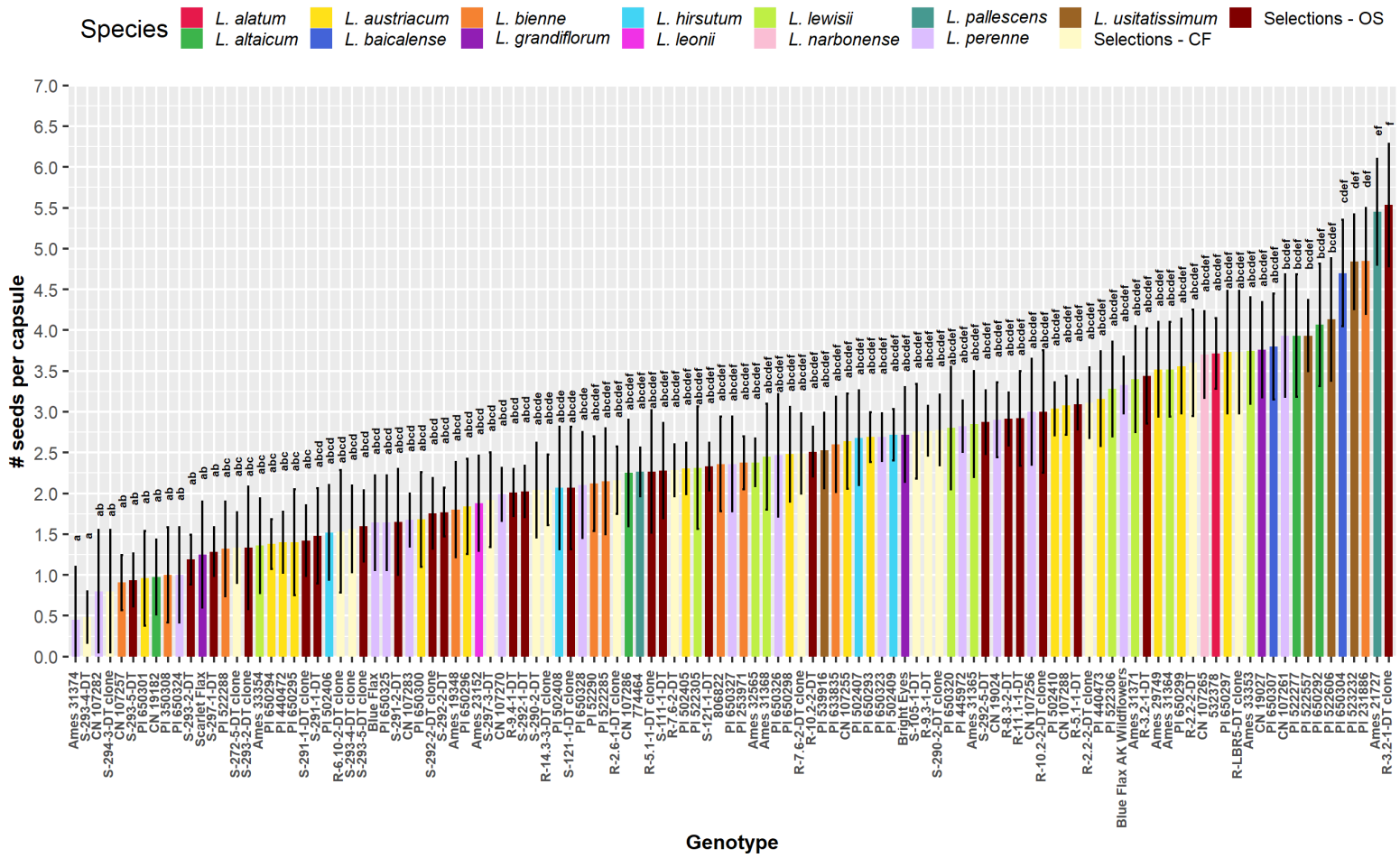

Figure A 3. Mean ± S.E. number of seeds per capsule values for all genotypes with n ≥ 3 observations. Means separations (5% HSD) are displayed as letters above the columns denoting significance.

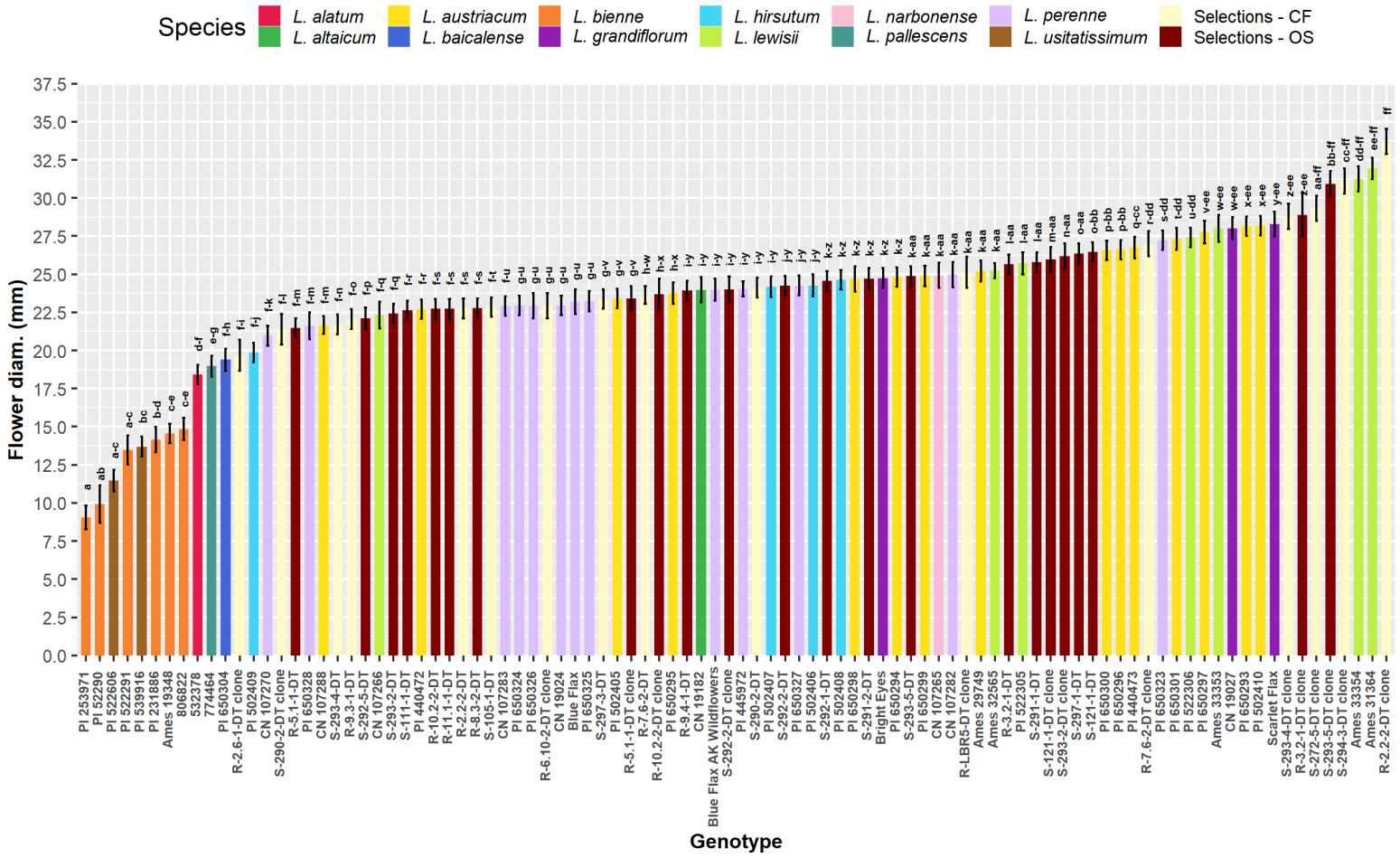

Figure A 4. Mean ± S.E. flower diameter (mm) for all genotypes with n ≥ 3 observations. Means separations (5% HSD) are displayed as letters above the columns denoting significance.

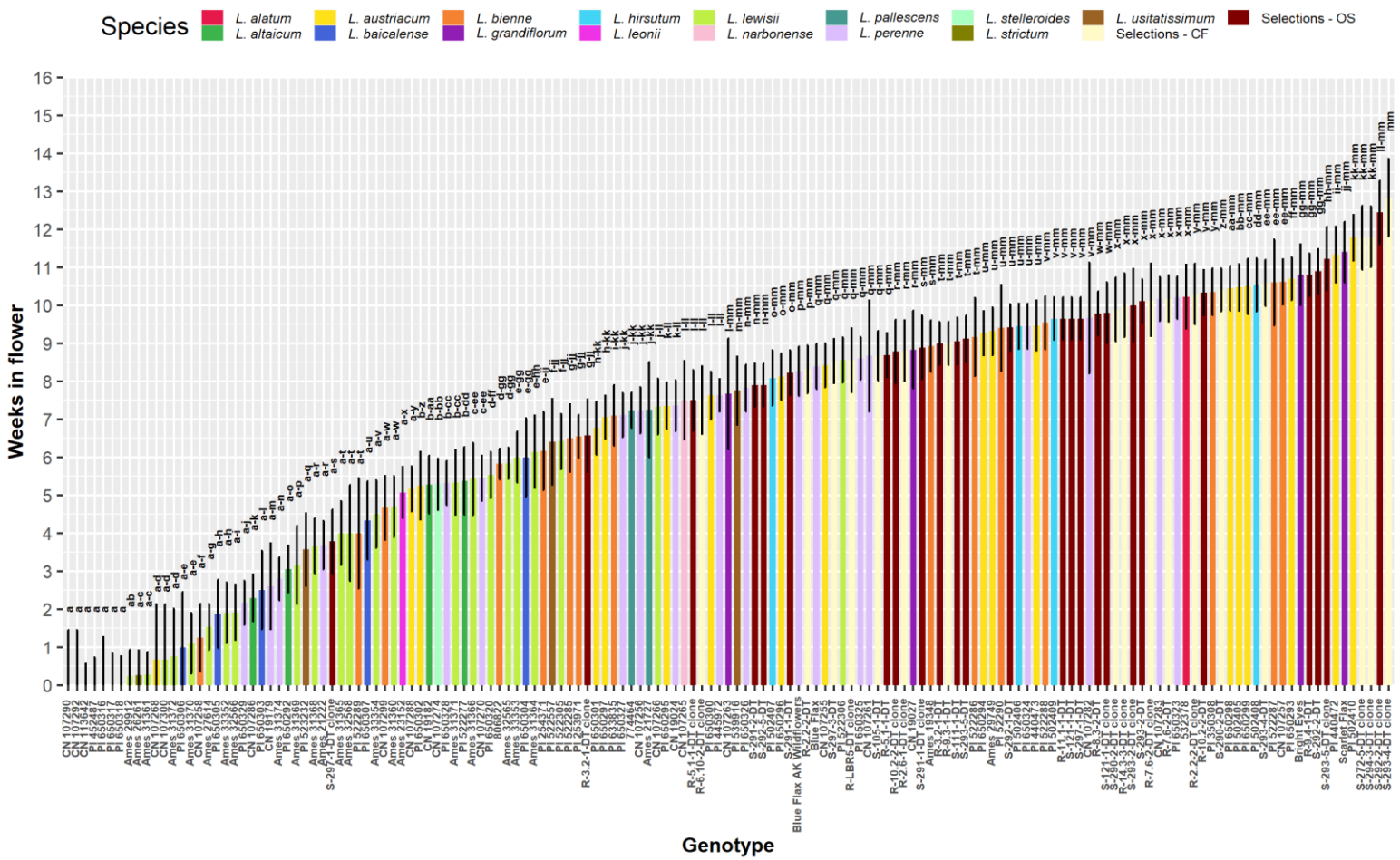

Figure A 5. Mean ± S.E. number of weeks in flower for all genotypes with n ≥ 3 observations. Means separations (5% HSD) are displayed as letters above the columns denoting significance.

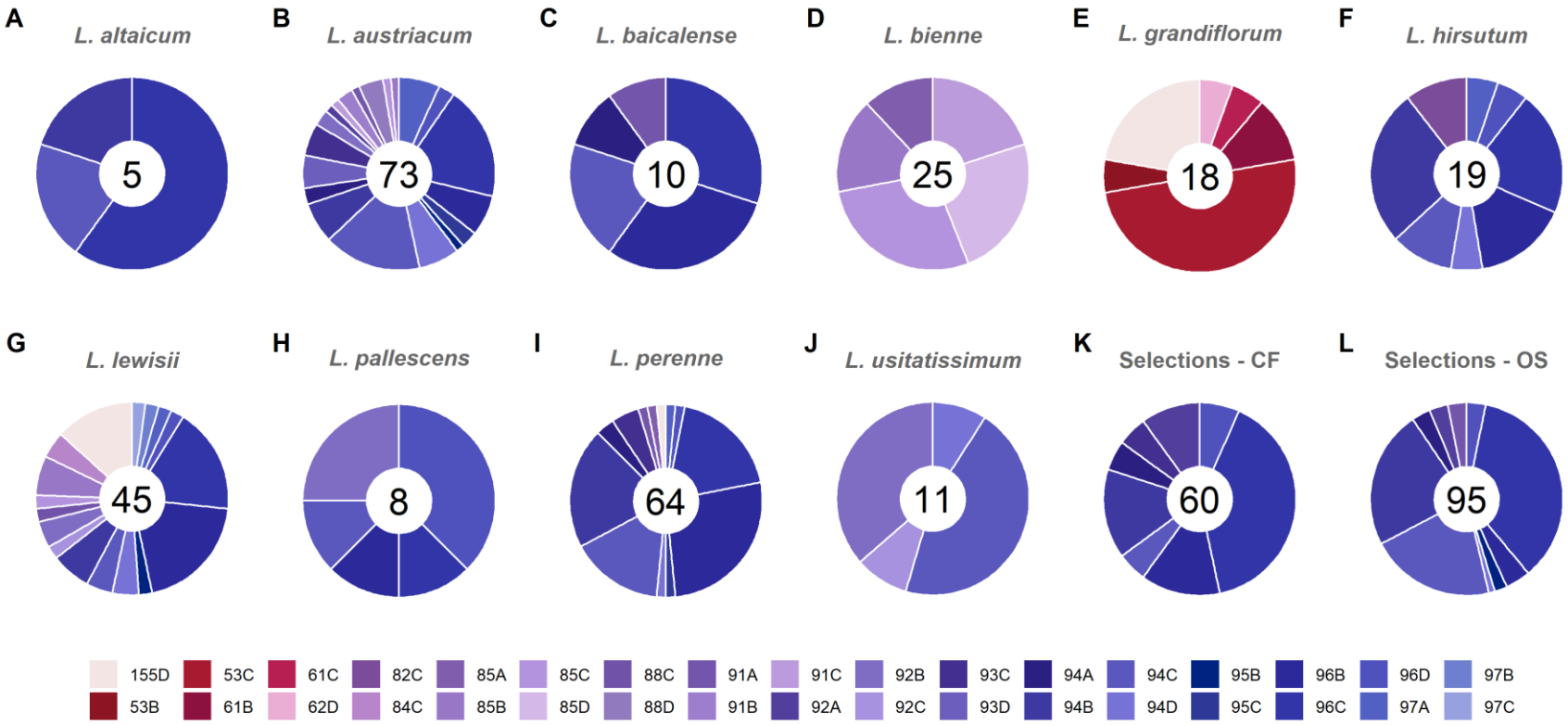

Figure A 6. Flower color (RHS code) proportion by species and population: (a) *L. altaicum*, (b) *L. austriacum*, (c) *L. baicalense*, (d) *L. bienne*, (e) *L. grandiflorum*, (f) *L. hirsutum*, (g) *L. lewisii*, (h) *L. pallescens*, (i) *L. perenne*, (j) *L. usitatissimum*, (k) Selections–CF, (l) Selections–OS. Number of observations are displayed in the center of each donut chart. RHS color codes converted to hexRGB values using the tool published by The Azalea Society of America website (https://www.azaleas.org/rhs-color-fan-1/).

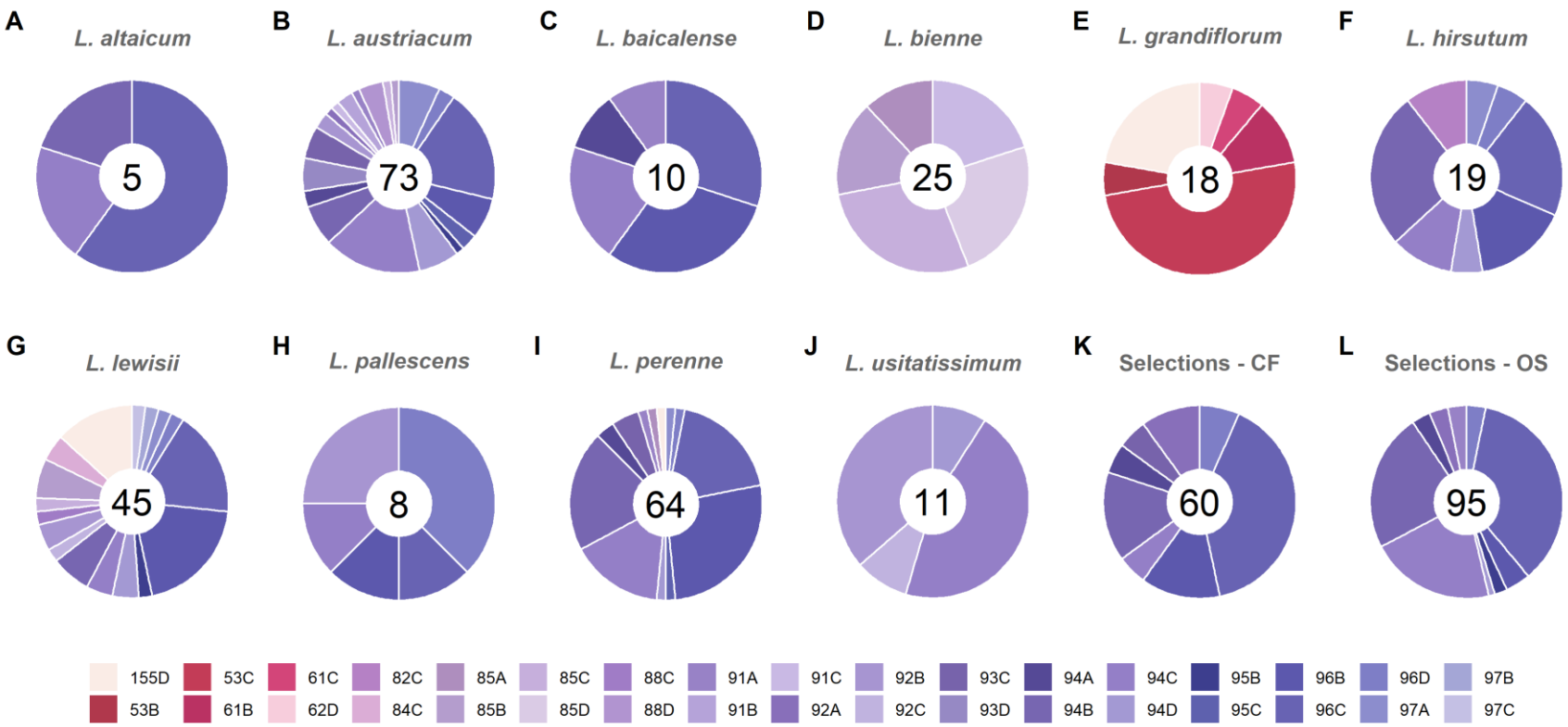

Figure A 7. Flower color (RHS code) proportion by species and population: (a) *L. altaicum*, (b) *L. austriacum*, (c) *L. baicalense*, (d) *L. bienne*, (e) *L. grandiflorum*, (f) *L. hirsutum*, (g) *L. lewisii*, (h) *L. pallescens*, (i) *L. perenne*, (j) *L. usitatissimum*, (k) Selections–CF, (l) Selections–OS. Number of observations are displayed in the center of each donut chart. RHS color codes converted to hexRGB values using unofficial colorimetric results published at: (http://rhscf.orgfree.com/).

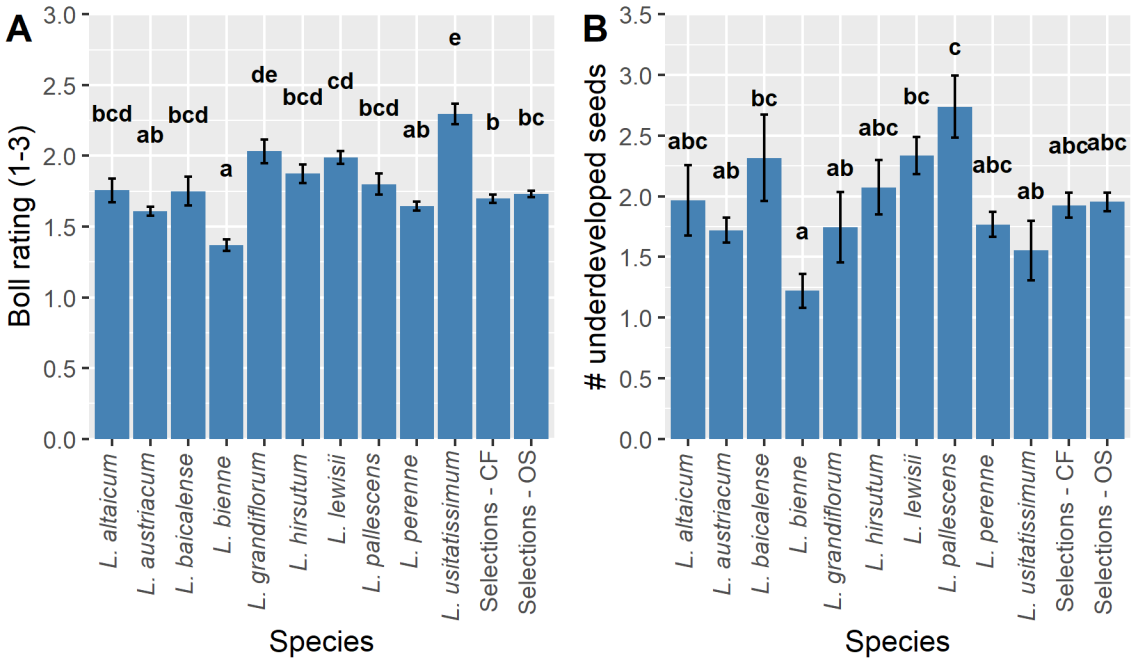

Figure A 8. Comparison of wild flax species with cut flower (CF) and oilseed (OS) selections for mean ± S.E. year one trait values related to oilseed ideotype: (a) boll rating (1-3; fully dehiscent to non-shattering) and (b) # underdeveloped seeds per capsule. Means separations (5% HSD): letters above columns.

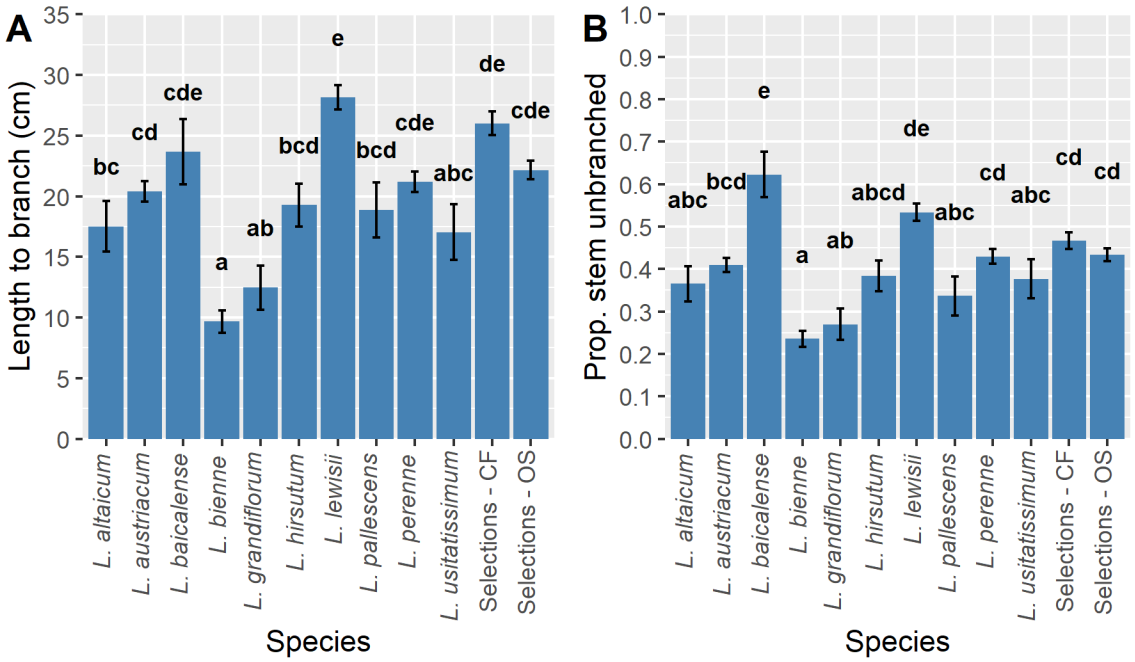

Figure A 9. Comparison of wild flax species with cut flower (CF) and oilseed (OS) selections for mean ± S.E. year one trait values related to the cut flower ideotype: (a) length to first branch > 5 cm (cm) and (b) proportion of stem unbranched. Means separations (letters above the bars) denote significance.

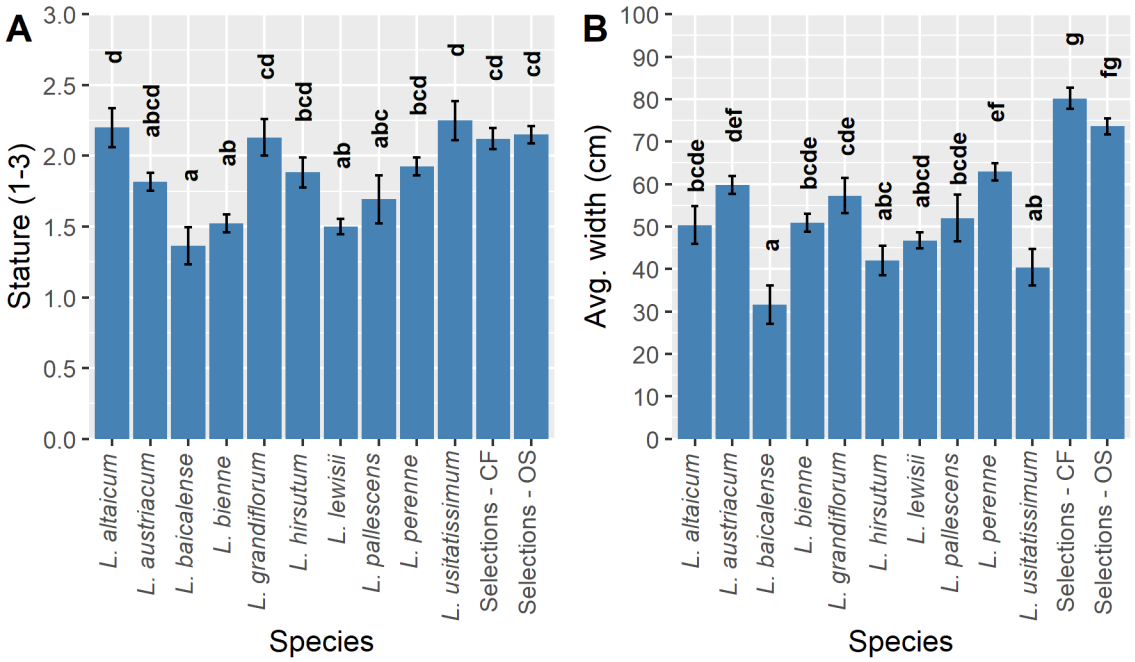

Figure A 10. Comparison of wild flax species with cut flower (CF) and oilseed (OS) selections for mean ± S.E. year one plant size and shape trait values: (a) stature (1-3; prostrate to upright) and (b) average width (cm). Mean separations are displayed as letters above the columns denoting significance.

Appendix B

Chapter 4 supplemental figures

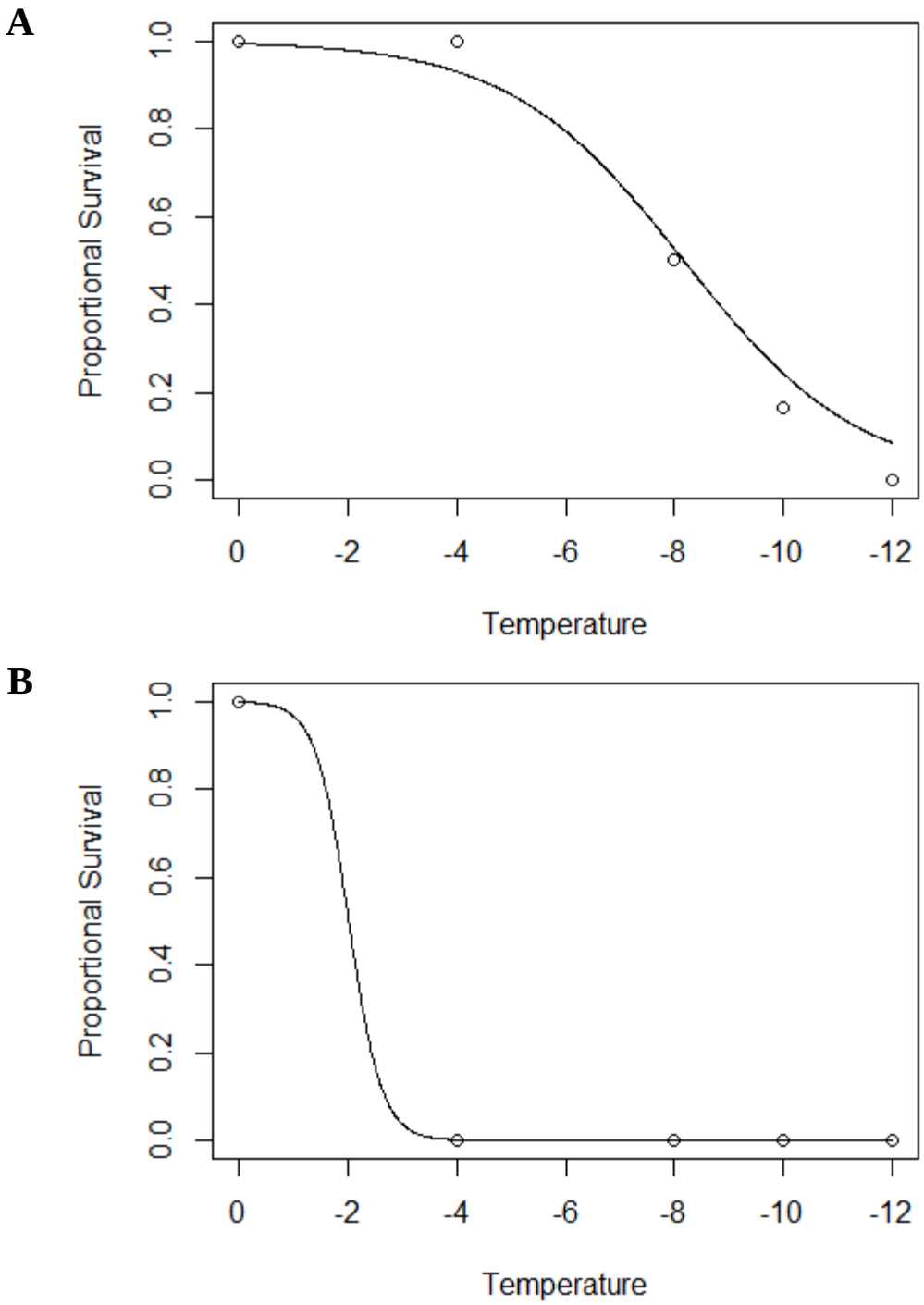

Figure B 1. Generalized linear model with a binomial distribution and a probit link function used to calculate LT_{50}s for all *Linum spp.* tested. (A) 'Blue Flax' showed gradually decreasing survival across temperature treatments and a relatively low SE. (B) Genotype 4-6N had 100% mortality in all temperatures < 0 °C. The lack of intermediate values helps to explain the high SE observed for genotype 4-6N and others when LT_{50} was calculated on a genotype basis.