FABA BEAN (*Vicia faba* L.) A PROMISING NEW PULSE CROP FOR SOUTHEASTERN WASHINGTON

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of ERIK J. LANDRY find it satisfactory and recommend that it be accepted.

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“There seems to be no limit to the variability available enabling plants to adapt to specific conditions of temperature, photoperiod, soil fertility, method of harvesting and the like.”

- Allard and Bradshaw, 1964
The successful adoption of faba bean (Vicia faba L.) in southeastern Washington is contingent upon the stability of supply, i.e., yield, and market demand. The lack of regional demand for faba bean has restricted research and development. Therefore, the objective of this research was to explore the adaptation of faba bean to southeastern Washington environmental conditions.

Winter-hardy northern European breeding lines and cultivars were acquired and screened for their ability to overwinter. Additional germplasm was sourced from the USDA-ARS Western Regional Plant Introduction Station (WRPIS) in Pullman, WA, to increase the genetic variation available for improvement of winter-hardiness via a bulk method of selection. Autumn and spring sowing conditions were trialed. Plot yield was most variable across location and year, rather than across populations. Percent survival confounded the differences in plot yield between entries; however, calculating the yield per plant helped to assess yield potential. The mean yield of the spring-sown trial (2.0 t·ha⁻¹) was considerably lower than when autumn-sown (2.8 t·ha⁻¹). The highest plot yield (>8.1 t·ha⁻¹) was obtained from an autumn sowing during the mild winter of 2011-12 at the Central Ferry Research Farm.

The increase in yield of autumn-sown faba bean is mainly attributed to a longer establishment phase and earlier development than when spring-sown. Flowering when daytime
high temperature exceeded 25°C was detrimental to pod set and growth. An early spring sowing in March would likely outperform later sowings given regional weather patterns. Earlier flowering and maturing genotypes are needed as the materials tested here exhibited delayed development when spring-sown.

A bulk method of selection improved winter-hardiness for most populations. Response to selection was estimated by comparing pre- and post-selection populations in 2013-14. WRPIS accessions showed the most gain in percent overwintering, ultimately rivaling the winter-hardiness of advanced northern European lines. Adaptive traits present within the germplasm include earlier flowering and maturity that would be important for dry-land agriculture where terminal drought limits the yield potential of northern European winter faba beans with late maturity.
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CHAPTER ONE

GENERAL INTRODUCTION

The rolling loessial hills of southeastern Washington and adjacent Idaho, regionally identified as the Palouse, are world-renowned for consistent high productivity and premium quality dryland soft white winter wheat (Papendick et al., 1985). The majority of Palouse cropland is in Whitman County, ~0.5 million hectares of the near ~0.8 million total crop hectares characterized as the Palouse, and has produced 120,000 ha of winter wheat annually since 1972 (2007, USDA AGCensus). The winter wheat yields in Whitman County have consistently increased since 1972. Washington’s average winter wheat yield (4.6 t ha$^{-1}$) exceeds the national average (3.2 t ha$^{-1}$) (NASS, 2012). Whitman County has been the leading wheat producing county in the country since 1978 (Washington Wheat Commission). Winter wheat in two or three year rotations, with spring sown wheat, barley, and/or a cool season forage legume or pulse (peas, chickpeas, and lentils) is currently common through the higher rainfall (38-66 cm) region, e.g. Whitman County, while summer fallow predominates throughout low rainfall regions. Cruciferous oil seed crops, such as rapeseed, canola, and camelina, will become even more important crops in the near future, if agricultural policy continues to shift support towards the domestic production of agricultural based fuels. Additional rainfall not only allows more crop diversification, but also improves yield and quality (Beus et al., 1990).

The history of cereal-based agriculture on the Palouse is a relatively short one compared to other parts of the world. From the early agricultural settlers of the 1880s who were primarily diversified animal, crop, and horticultural producers, through the early 20$^{th}$ century, where phases of consolidation and technological-specialization that led to the international export-
oriented, cereal-dominant, and input intensive green revolution period post 1950 (Goldstein, 1986).

Individual farmer livelihoods have improved since the 19th Century. For example, a Whitman County farmer earns, on average, a net annual income of $230,000 (USDA AGCensus, 2007). This prosperous agricultural development, while aiding the U.S. to be a net food exporter, has not come without a cost. Soil erosion has been a major environmental concern influencing public policy, leading to public sponsored conservation programs, as well as a general trend towards cultural management improvements (Young et al., 1994).

There are three main kinds of erosion: water, wind, and tillage. The Mediterranean precipitation pattern, in conjunction with fallow, or low vegetation-residue cover, on sloping hillsides, facilitates water erosion producing moderate, i.e., rills, to severe water erosion, i.e., gullies. The eroded topsoil settles within the valley flats or bottoms and can even eventually end up in the streams, rivers, and ultimately the Pacific Ocean, possibly contributing to the Pacific Northwest dead zone (Grantham et al., 2004).

The chocolate brown Palouse Falls attests to the persistent erosion issue. Young et al. (1994) estimated that 22 to 360 t ha$^{-1}$ yr$^{-1}$ of soil has been lost over a hundred years of cropping the Palouse. Reganold (1988) reported an annual average loss of 31.5 t ha$^{-1}$ due to water erosion as a result of winter fallow on the eastern Palouse. Wind erosion is of primary concern where precipitation is low and summer fallow is practiced in conjunction with rodweeding, which forms a dust mulch conserving water, but leaves the soil surface prone to wind erosion (NRCS, 2012).
Areas with severe tillage erosion can be identified as having lighter colored subsoil on ridgetops and mid-slope knobs (Reganold, 1988). Fencerows running parallel across the slope are often sites of stark soil height differences due to downslope plowing away from the dividing property line. The traditional cultural practice of moldboard plowing followed by disking, results in a fine seedbed suitable for germinating wheat, but also reduces clod size and stability leading to erosion. Disking has generally been eliminated by less intensive harrowing or other conservation tillage practices (Young et al., 1994).

The severity of erosion has, in general, declined (NRCS, 2012), especially where conventional uses of the moldboard plow have been replaced by no-till or conservation tillage-residue management systems. These, however, remain highly technological and input intensive curtailing broad adoption (Huggins and Reganold, 2008). Conservation reserve programs have also reduced the eroded or highly erodible crop land in production, but remain a stopgap measure for erosion mitigation (Young et al., 1994).

An alternative approach to the input intensive, production-oriented, conventional cereal model was highlighted by Reganold (1988). Two adjacent farms were compared; one following conventional and the other traditional practices. The traditional farm had never relied on synthetic chemical inputs and instead incorporated alfalfa and winter pea green manures for nitrogen, organic matter, and to break disease-pest cycles. These cultural practices were common prior to World War II when nitrogen fertilizer largely replaced legumes. Reganold found that the traditionally managed farm not only had a higher soil quality (higher organic matter, cation exchange capacity, nitrogen, potassium, water holding capacity, pH, polysaccharide content, enzyme activity, friability, and microbial biomass) but 16 cm more of it, than the conventional
operation. The loss of soil from the conventional farm was suspected to be due to erosion as a result of a conventional cropping system.

For many farm operations, the damage has been done and to restore the original virgin soil would be impossible. However, to not attempt some form of remediation or strive to slow the process of deterioration would be disastrous for regional agricultural sustainability. Busacca et al. (1985) estimated that all Palouse top soil could be removed within 100 years resulting in potential yield losses over 30%. To maintain winter wheat yields into the future, more inputs will be necessary to overcome the loss of soil tilth, organic matter, and water holding capacity. The results of higher production costs and degraded soils could include heightened environmental contamination, higher food costs, and increasing market volatility. To feed the coming 10 billion human inhabitants, soil fertility (Evans, 1998) and the organic matter that maintains it (Pieters, 1927) should remain primary concerns.

**ALTERNATIVE CROP SYSTEMS FOR COMBATING DECLINING SOIL QUALITY**

Two methods of curtailing soil loss and maintaining sustainable productivity on the Palouse are the traditional use of green manures and the modern use and continued development of no-till and/or conservation tillage systems. Animal manures would be an option in some locations, but overall the integrated animal-cereal cropping system of the early 20th century has been replaced by specialized cereal production for international export (Goldstein, 1986).

The value of green manure for maintaining soil health and productivity has been exploited since at least 1000 B.C. in China and the 3rd century B.C. in Greece and Rome (Pieters, 1927; Cubero, 2011). During the 1st century B.C., Virgil wrote about the treatment of the land in *Georgics*, “In alternate seasons you will also let your fields lie fallow after reaping, and the plain
idly stiffen with scurf or beneath another star, sow yellow corn in lands whence you have first carried off the pulse that rejoices in its quivering pods, or the fruits of the slender vetch, or the brittle stalks and rattling tangle of the bitter lupine”. From this, it appears Virgil is advocating for cereal rotations that either employ fallow or a pulse crop. Also during the 1st century B.C., Varro wrote in his books of agriculture that, “it is customary to plough under lupines as they begin to pod and sometimes field beans before the pods have formed so far that it is profitable to harvest the beans in place of dung, if the soil is rather thin”. Here Varro defines green manure; turning under vegetative plant materials in an effort to improve the soil for a succeeding crop. China’s historical use of green manures, complex rotations, and soil conditioners are detailed in the Ch’i Min Yao Shu (6th century A.D.), “If manure is unavailable, sow green Dolichos thickly in the 5th or 6th month. Plough the bean-plants in during the 7th or 8th month, just as if using manure-dressings. The effect is as good as ripened compost, and much labor is saved” (Sheng-Han, 1962).

Pieters in his seminal 1927 technical review on green manure proclaimed that organic matter was the most valuable constituent of the worked under plant material where the released nutrients are made available through decomposition. The beneficial effects of organic matter and humus on soil tilth and fertility were outlined by Pieters and probably known to most farmers who applied them, even if only through observation of crop productivity and soil characteristics. We may use more technical terms today, but the same principles apply (Dabney et al., 2001; Sainju, 2009). Benefits of organic matter include:
i. A crumbly or friable soil texture due to binding of soil particles by introduced organic matter, which decreases the tendency of the soil to blow and improves cultivation and water infiltration decreasing the potential for water erosion.

ii. Improvement of the soil’s aeration and water holding capacity enhances root growth, creating a positive feedback loop of organic matter deposition.

iii. Increase in the soil’s bacterial numbers, which help to solubilize minerals and further improves plant growth.

Historically, green manures were crucial where animal manures were scarce. However, with the introduction of readily available synthetic forms of nitrogen, i.e., fossilization, and other essential minerals during the early 20th century, green manures have been effectively replaced within the majority of modern cropping systems present in developed (and increasingly in undeveloped) countries (Jensen et al., 2010). Sarrantonio (1991) warned that even though there is a hidden or externalized cost of synthetic fertilizers, growers will generally not be receptive to green manure rotations that can otherwise grow cash crops. Intercropping (companion crop) and off-season production (catch crop) are offered by Sarrantonio as possible niches, but can also come along with considerable risk and technological and biological barriers. Some of these risks were highlighted by Murray and Swensen (1985), after researching mixtures of winter cereals and winter peas grown on the Palouse. The use of intercropping will, in the short-term, be restricted to organic growers of the developed and subsistence farmers of the developing world, where synthetic chemicals are restricted or unavailable (Jensen et al., 2010).

The most often limiting biological factor to the use of green manure throughout much of the Pacific Northwest is water. In semi-arid to arid regions, dryland farming conserves moisture
through a fallow period where a green manure could exhaust the stored water through its growth and decomposition (Pieters, 1927). Beus et al. (1990) highlighted a general reaction among conservation-minded farmers on the Palouse, where annual cropping dominates, that “unless the land is set-aside acreage, a green manure is too costly”. Goldstein (1986) proposed a low-input, purportedly ecologically sustainable alternative, to this prevailing notion. His plan’s economic viability was contingent upon high input costs, lower than normal wheat prices, and a less than supportive subsidy program. Today, current public policy and the perception of local farmers has likely not changed much since Goldstein’s economic analysis, especially with current >$6/bu wheat. High chickpea prices and fuel subsidies for oilseeds may allow a foothold for cereal alternatives in the near future, but the use of green manures, like that of polycultures, will largely be, at least into the near future, relegated to progressive conventional and organic producers and publicly funded researchers.

A compromise to the implementation of green manure that can maintain or at least slow the degradation of soil quality would be the use of pulses in a no-till or conservation tillage cereal rotation. The creation of Sheperd’s Grain Cooperative has been a concerted effort on the part of no-till farmers to capitalize on sustainable product premiums to finance the conservation of their land base. Some may argue that no-till is perhaps even more input and technologically intensive than conventional moldboard plow operations, but few would disagree that soil quality can be improved and erosion restricted substantially through crop residue conservation (Huggins and Reganold, 2008). What to do with the accumulation of cereal residues is still under debate, burning is costly and contributes to air pollution. Adoption of pulses into rotations could be one option for reducing stubble and crop residue, while incurring multiple secondary benefits similar to green manure.
Most of the benefits of pulses in rotation with cereals on the Palouse include a yield boost to subsequent cereals, cereal residue decomposition, more weed control options, reduced soil erosion and nitrogen requirement, disease cycle break, and greenhouse gas emission mitigation (Young, et al., 1994; Robertson, et al., 2000; Hauggaard-Nielsen, et al., 2011); nevertheless adoption is market driven. Pulses have traditionally been less competitive to higher value grain crops, although current demand for chickpea and its recent inclusion within the revenue based crop insurance program will decrease risk and likely increase grower adoption.

The main reason for the use of pulses in rotations currently is as a break (disease and weed control) crop for winter and spring wheat crops, although economic and production diversification stabilizes economic returns (Miller et al., 2002). According to the 2007 USDA NASS census, Whitman County had about the same land base for pulses (~40,500 ha) as that of barley, which was about ¼ of the total wheat in rotation. This would make sense, since a pulse is typically grown in one out of three to four years in a winter/spring wheat rotation. In 1997, for Washington State on the whole, chickpeas were grown on less than 2,000 ha, lentils on about 32,000 ha and peas on over 48,500 ha. Although USDA NASS does not provide data to the county level, for 1997, total pulse production was likely similar (about ½ of state production) since rotations have not drastically changed. Much of the change in rotations has been the choice of pulse, since production is aligned with market price. Chickpea production has consistently increased since 1997 to over 16,000 ha across the state in 2011, while lentils have decreased to about 24,000 ha as did peas to 28,000 ha.

Pulses are mainly spring planted on the Palouse with limited winter pea production for soil conservation or for animal feed. Autumn-sown varieties offer additional cropping options
and system diversification, while providing some distinct advantages over their spring counterparts. Winter-type pea, faba bean, and lupin have gained some popularity in Europe (Ney and Duc, 1997), and cultivars of lentil (Muehlbauer and McPhee, 2007) and pea (McPhee et al., 2007; McPhee and Muehlbauer, 2007) released by Fred Muehlbauer (Research Geneticist, USDA-ARS) were released during the early 1990s. More recently, winter faba bean was added as a research interest of the USDA’s ARS Plant Germplasm Introduction and Testing Station in Pullman, WA (Mwengi, 2011). Conservationists prefer autumn sowing over spring because an autumn-sown pulse would provide more winter cover to an otherwise fallow field, discouraging erosion and making more effective use of seasonally available moisture. Agronomists contend autumn-sown pulses would benefit the succeeding cereal and eliminate the variability of spring conditions on sowing, which would contribute to higher yields and an earlier harvest, improving crop rotation flexibility (Hebblethwaite, 1983). The diversification of pulse crops should also reduce soil-borne disease build up (Jensen et al., 2010).

Climate change and a general trend towards warmer, longer growing seasons, and erratic precipitation may favor winter over spring pulse production. Autumn-sown pulses would eliminate the compaction and poor seed establishment issues of wet cold soil, spreading out field operations, and increasing yield potential (Cutforth et al., 2007). The trade-off of synchronizing crop development with seasonal moisture is increased disease pressure, which will have to be met with breeding for disease resistance.

The development and adoption of autumn-sown pulse varieties has had its difficulties. Austrian winter pea, for example, has fluctuated from around 4,000-14,000 ha nationwide over the past 10 years (USDA NASS, 2012). Dr. Muehlbauer and his associates with the ARS Grain
Legume Genetics and Physiology Research Unit, Pullman, WA, hybridized the winter-hardy Austrian pea with locally adapted cultivars of spring dry pea in hopes to obtain a winter pea for human consumption. Although pea varieties like ‘Specter’ and ‘Windham’ have been released they are not as desirable for human consumption due to seed characteristics inherited from the Austrian parent. The red cotyledon winter-type lentil variety ‘Morton’ was released by the same group and could be used for human consumption but erosion and weed and disease pressure on the relatively small lentil plants carries significant cultural management risks. Other pertinent issues are stand establishment, due to drought-like conditions during sowing, which delays germination and reduces winter-hardiness, and market considerations. Autumn sowing into a no-till cereal stubble could help to alleviate the erosion and weed pressure issues, as well as improve winter-hardiness (Cutforth et al., 2007).

FABA BEAN

Faba, fava, horse, field, tic, or broad beans, are just a few of the common names given to *Vicia faba* L. (Kay, 1979). To reduce complications, faba bean, or winter bean, will be used here following Hawtin and Hebblethwaite (1983). Faba bean offers an additional route for obtaining an autumn-sown cool season pulse directed toward human-animal consumption or use as a green manure. Faba bean has been noted as one of the highest N$_2$ fixing and yield capacities of any locally adapted pulse (Herridge et al., 1994), but the extent of this capacity is relatively unknown on the Palouse. Like any other autumn-sown pulse, adoption of faba bean will be contingent upon winter-hardiness, yield, quality, and market viability. Market viability will only be briefly addressed; the emphasis here will be given to winter-hardiness.
Autumn sowing of faba bean is mainly practiced where winter temperatures remain above -5°C (Mediterranean types), but Northern European smaller seeded types (~0.5 g seed⁻¹) can tolerate winter temperatures below -10°C (Evans, 1992). With snow cover, survival down to -25°C has been observed (Picard et al., 1982), however, a tolerance of -12 to -15°C is more common for French and UK cultivars (Herzog, 1989). Sowing date depends upon autumn and winter climate, and ranges from early August to late October. If sown too early, hardiness can be reduced due to excessive branching and growth. If sown late, germination can be delayed, resulting in a poor stand, few productive branches, and reduced yield.

Autumn seeding rates are often lower than if spring planted, mainly due to the compensation of the higher branching capacity of winter-types. Where winter-kill reduces plant populations, seeding rates attempt to achieve an optimum spacing between 100-150 thousand plants ha⁻¹ (Li-juan et al., 1993). Temperatures in Pullman have been lower than -30°C and would be disastrous to a winter faba crop. Without snow cover, even winter wheat can be killed at this low a temperature (Young et al., 1994). Average seasonal lows are, however, above -10°C and should be tolerated by hardy winter-type faba bean.

HISTORY AND BACKGROUND

The faba bean has Middle Eastern origins and centers of diversity in the Far East and Europe; however, there are apparently no wild populations still in existence (Hebblethwaite, 1983). Faba bean was a common, possibly ideal, component of cereal rotations until synthetic fertilizers and alternative feed stocks lead to a general decline in production (Bond, 1976a). Domestication occurred during the late Neolithic period and spread through Europe during the Bronze Age (Renfrew, 1973; Tanno and Willcox, 2006). They reached China through the Silk
Trade possibly by 100 BC (Kogure 1979), although recent evidence suggests even earlier (Duc et al., 2010). Finally, they were introduced into the New World during the 16th century (Bond, 1976a). Interestingly, the faba bean was the ‘bean’ of the Old World replaced by the New World, common bean (*Phaseolus* spp.) (Kay, 1979). The historical use of pork and beans and the fairy tale Jack and the Beanstalk are thought to be based on faba bean rather than the common bean (Tannahill, 1973).

The earliest faba bean was likely short podded *V. faba* subsp. paucijuga and var. minor (100 seed weight <40 g or 800-1200 seeds lb⁻¹; >1.2 in long pods) and/or long podded var. equine (100 seed weight 40-80 g or 500-800 seeds lb⁻¹; ½-3/4 in long pods) (Evans, 1992; Duc, 1997). Larger vegetable type *V. faba* var. major (100 seed weight >80 g or 200-500 seeds lb⁻¹; >3/4 in long pods) became common after 500 AD (Lawes et al., 1983). Hawtin and Hebblethwaite (1983) consider the distinction between size classes or subgroups as botanical varieties, rather than subspecies, mainly because there are no reproductive barriers. Further, Lawes et al. (1983) noted that the distinctions between botanical varieties are not hard and fast. An interesting study of hybrids between the subspecies is found in Erith (1930).

**ECOLOGICAL SERVICES**

Köpke and Nemecek (2010) addressed in detail the ecological services of faba bean, specifically. Biological nitrogen fixation (BNF) was highlighted as the principal agronomic and economic advantage for including faba bean in a cereal rotation. There is quite a range in BNF between genotype and environment, but overall grain yield shows a high correlation with nitrogen fixed. For example, a grain yield of 4 t ha⁻¹ with a nitrogen content of 4.5% would yield about 180 kg N ha⁻¹ of BNF. However, if the grain is removed and not recycled through animal
manures, then residual nitrogen typically shows little increase or can even decrease. An interesting point made by the authors, however, involves the energy efficiency of BNF compared to producing synthetic nitrogen. The use of annual and perennial legume green manures will likely become more advantageous, as energy consumption and nitrous oxide-carbon dioxide gas emissions become cost prohibitive and carbon farming is supported (http://www.carboncoalition.com.au).

Even if faba bean is not used as a green manure and the grain is removed off-farm, there can still be rotational benefits, however slight, for the subsequent cereal (McEwen et al., 1990; Dyke and Prew, 1983; Jensen et al., 2010; Thomas et al., 2010). It appears that even though residual nitrogen is rather recalcitrant (<20% taken up by the following crop), nitrogen fertilization of a cereal can be reduced up to 30-50 kg N ha\(^{-1}\) without yield loss, compared to a cereal-cereal rotation (Prew and Dyke, 1979; Köpke and Nemecek, 2010).

Other ecological services mentioned by Köpke and Nemecek (2010) are directed to diversification of the cropping system providing stability and soil improvements. Diversification in space, or intercropping, is a technique that mimics natural systems and aims to decrease weed, disease, and crop competition by using components that have synergistic cultural requirements. While intercropping provides some insurance against abiotic and biotic stress factors and can stabilize yield, it will not likely be synergistic with the intensive farming systems on the Palouse. As mentioned before, intercropping will more likely be efficacious for regional low-input organic producers or when/if input costs outstrip returns.

Benefits to the soil when replacing a cereal with a faba bean crop include increased microbial diversity, soil sanitation, soil structure, and less water use (Jensen et al., 2010). The
adaptability of faba, like that of the other pulses and oilseed mustards, to conservation and no-till is also quite promising (Hebblethwaite, 1983). The use of a faba bean crop to alleviate soil compaction has been demonstrated by Rochester et al. (2001) and could help to increase soil porosity and stabilize aggregates of a no-till or low-till system. In some locations on the Palouse, where periodic waterlogging is an issue, faba bean would be an alternative to other common pulses, since it is one of the most tolerant cool season pulses to waterlogging (Solaiman et al., 2007).

The ecological services reviewed by Köpke and Nemecek (2010) were specifically oriented towards spring planted faba in Europe. Winter faba grown on the Palouse may be a more sustainable choice for growers. Other than higher yields and a lower seed cost (Link et al., 2010), earlier maturity enables winter faba to take advantage of seasonally available moisture avoiding summer drought (Duc, 1997), weevil (Sitona lineatus), and aphid (Aphis fabae) infestation (Link et al., 2010), reducing virus infection (Hebblethwaite, 1983). Further, winter faba has a greater capacity to root and fix nitrogen than spring types (Herzog and Geisler, 1991).

Mediterranean and oceanic climates with consistent winter rainfall, while providing the potential for a high yielding environment, are also ideal for specific diseases. In England and Australia, chocolate spot (Botrytis fabae), seed-borne ascochyta blight (Ascochyta fabae), alternaria leaf spot (Alternaria alternate), Mycosphaerella pinodes, and cercospora leaf spot (Cercospora zonata) have to be managed for autumn-sown crops (Hebblethwaite, 1983; Paull et al., 2011). Soil fungi, mainly Fusarium spp., black root rot (Thielaviopsis basicola), and stem rot (Sclerotinia trifoliorum), are more difficult to control apart from long-term non-host rotations (Biddle, 2007). Bean leafroll virus and rust (Uromyces viciae-fabae) epidemics are more of a
concern where mild winters are coupled with ample summer rains or high humidity. Seed-borne broad bean stain virus, aphid-transmitted bean yellow mosaic virus, as well as pea enation mosaic virus, are common viruses to faba bean and pea (Biddle, 2007). A list of the most important pests and viruses associated with faba bean was provided by van Emden et al. (1988) and Bos et al. (1988), respectively. Other potentially destructive pests include the stem eelworm (*Ditylenchus dipsuci*) and seed-borne nematodes, which like the fungal pathogens, can build up in continuous long-term legume rotations (Dyke and Prew, 1983; Robertson and Saxena, 1993). Stoddard et al. (2010) reviewed the common pests and integrated management strategies for their control.

Considering only the ecological services of faba bean, without addressing the economics, will not lead to wide-scale producer adoption. The faba bean is relatively unknown to the typical domestic consumer and internationally has lower value when compared to other cool season pulses. Thomas et al. (2010) examined different crop rotations that would improve the sustainability of dryland grain production in northeast Australia and found that even though faba bean provided the ecological services outlined above, gross margins were too low to compete with the higher yields and economic return of chickpea.

**WINTER-TYPES**

Faba bean is grown overwinter in the warmer regions of the Mediterranean, Middle East, China, and Australia. These regions are exposed to minimal frost events and hence most of the genetic material, while potentially frost tolerant, is not winterhardy per se. True winter-types (winterhardy varieties from Northern Europe) are small seeded (var. equine and minor) and are more tolerant to freezing, showing some vernalization requirement for early-node flowering (i.e.
late flowering), as compared to overwintering broad bean from warm climates (Saxena, 1982; Ney and Duc, 1997; Link and Bond, 2011). Winter faba bean has been a mainstay of English agriculture since 1825 (Bond and Crofton, 1999), reportedly introduced as Russian beans (minor type) (Lawes et al., 1983). Côte d’Or, a French landrace, was cultivated since at least 1812 and was considered to be the hardiest, tolerating temperatures as low as -18°C (Bond et al., 1994; Link and Bond, 2011).

According to Hebblethwaite (1983), the English were the primary innovators when it came to winter faba bean the developing the first winter equine types between 1925 and 1945 (Link and Bond, 2011). Farmers traditionally ploughed the broadcasted seed from September to early-November, as it was thought that deep sowing increased winter-hardiness. According to Murray et al. (1988), deep and high density sowing of winter pulses at a soil temp <10°C, or air temp between 5-8°C, increased winter survival. Broadcast sowing has been replaced by various drilling technologies; a five to over 10 cm sowing depth is recommended between mid-October and mid-November in England (Hebblethwite, 1983). While the exact sowing window is expected to change depending on location, the resulting plants going into winter should have two pairs of leaves and a strong root system (Murray et al., 1988). Nitrogen fixation will likely begin before winter but activity stops when soil temperature is below 5°C at a10 cm depth (Roughley et al., 1983).

Optimal spacing has a wide distribution between 30 x 40 cm and 8 x 40 cm (15-30 plants m⁻²), because plants have a strong ability/adaptability/plasticity to branch at varying densities (Link et al., 2010). The targeted density of spring plantings is typically higher than 30 plants m⁻², because spring types branch less than winter types (Key, 1979). Poulain (1984) achieved
optimal yield at 25 plants m$^{-1}$ for a winter sowing in France, but original sowing density, taking losses overwinter into account, was 30-60 plants m$^{-1}$ depending on population. Experimental yield in England can be upwards of 9 t ha$^{-1}$ (Roughley et al., 1983), but in production, will rarely yield over 7 t ha$^{-1}$ (Stelling et al., 1994). Seed size is limited to 0.4-0.6 g seed$^{-1}$ due to marketing and grain equipment capacity (Lawes et al., 1983). However, there is also a genetic association between hardiness, small seed size, and yield (Ney and Duc, 1997), therefore, 0.2 g seed$^{-1}$ is the target. Further husbandry practices of faba bean (e.g. fertilizing, sowing, herbicides, harvest, and storage) can be found within Hebblethwite (1983).

Older winter cultivars (Banner: British, Côte d’Or: French, Webo- registered 1979 and Hiverna- registered 1986: German) were assessed for freezing tolerance by Herzog (1988) and were determined to withstand temperatures as low as -14°C after four weeks of 8/2°C cold acclimation. Herzog (1987b) identified a simple leaf conductivity assay for determination of seedling freezing tolerance. More recently, Arbaoui et al. (2008) concluded Hiverna/2 (inbred of Hiverna), Hiverna, Karl, Bulldog/1 (inbred of Bulldog), and Gö-Wibo-Pop to be the most consistently winterhardy across 12 European environments as compared to other cultivars. Mwengi (2011) found Gö-Wibo-pop to be the most winterhardy and Hiverna/2, W6 12024, and W6 12026 to be reasonably hardy for the Palouse as compared to other USDA accession or European cultivars. Gö-Wibo-pop is short for Göttingen Winter Bean population, a composite of 11 European cultivars (Webo, Wibo/1, Hiverna, Banner, Bourdon, Bulldog, Côte d’Or, Arrissot, and other breeding lines) subjected to natural selection for winter survival and was found to be more winterhardy than either Striker (registered 1993), or Clipper (registered 1996), two common UK winter faba bean synthetic cultivars (Gasim et al., 2002).
It would appear that a rhizosphere temperature between -7°C (Mwengi, 2011) and -9°C (Saxena, 1982) determines survival of even the most hardy genotypes, rather than ambient temperature alone, since temperatures < -20°C do not result in complete kill if snow is present. Link and Bond (2011) have observed this as well, but have not directly measured the rhizosphere temperature. The most cold-tolerant commercially available cultivar in the Pacific Northwest is ‘Banner’ but winter-hardiness is inconsistent at best (Sattell et al., 1998). So far all registered winter faba bean cultivars are either minor, or equine, or are composites of both.

Frost tolerance is a major component of winter-hardiness ($r = 0.74$), but some genotypes will be frost tolerant and have low survival, as well as the opposite (Arbaoui et al., 2008). Frost tolerance is a complex trait involving water status, chemical composition of plant tissues, photoperiodism, rosette morphology, and damage repair mechanisms (Ney and Duc, 1997). Herzog (1988), using artificial freezing tests, found most of the frost tolerance variation could be explained by low leaf water content, slow plant development, short plant height, and reduced leaf area. Link and Bond (2011) found that the accumulation of proline and the desaturation of membrane-bound fatty acids were involved in the hardening process and frost tolerance. Heritability for frost tolerance was found to be high ($h^2 = 0.89$) (Arbaoui et al., 2008), but heterosis for winter-hardiness could confound applicable estimates of open pollinated genotypes (Bond, 1966; 1989; Duc and Petitjean, 1995). QTLs for frost tolerance have been identified, but they only explain <10% of the phenotypic variation (Link et al. 2010).

Laboratory screening for freezing tolerance can be effective at increasing cold tolerance, as shown in pea (Cousin et al., 1993). However, field survival can also be influenced by the extent of vernalization and dehardening, waterlogging, desiccation tolerance, resistance to biotic
stresses, and capacity to tiller (Murray et al., 1988; Bond et al., 1994; Link et al., 2010). While the importance of field-testing cannot be understated, Arbaoui et al. (2008) did find a significant relationship ($r = 0.41$) between frost tolerance and overwintering.

The two primary field survival selection events occur at the beginning and end of winter. The first selection coincides with early frosts and depends directly on the frost tolerance and extent of cold acclimation. Herzog (1987a) showed the importance of establishment in that frost tolerance increased between the first and second leaf of the faba bean seedling during cold acclimation. The second selection, through the end of winter, is due to wind injury, freeze-drying (inability to absorb water from the frozen soil) and frost tolerance, due to premature dehardening (Herzog, 1988) and growth (Annicchiarico and Iannucci, 2007). Tolerance to additional abiotic and biotic stresses is likely important depending on prevailing conditions. Link et al. (2010) highlighted some of the possible physiological mechanisms, which promote winter-hardiness:

- Sufficient vernalization requirement: photoperiod and temperature effects the transition from vegetative to reproductive phase, i.e., late flowering (Ney and Duc, 1997; Patrick and Stoddard, 2010)

- Adequate hardening and dehardening behavior: associated with lipid unsaturation of the cell membrane and cell water status

- Frost tolerance pre- and post-hardening: Osmoprotectant sugars and proline

- Tolerance to continuous snow-cover: related to plant architecture and fungal resistance

- Tolerance to frost-drought (desiccation from wind and solar radiation under frost)
• Tolerance to high soil moisture through winter.

• Endurance to frost heaving: root morphology and tensile strength.

• General resistance biological pathogens.

Compared to historical advances in winter cereals, a fraction of the global resources have been allocated to improving winter faba bean, and in the U.S., the crop is practically unknown. European publicly funded breeding programs have largely been transitioned to three privately held companies: Wherry & Sons in the UK, Agri-Obtentions in France, and NPZ Lembke in Germany. Wolfgang Link with the University of Göttingen in Germany and some recent interest in Serbia (Mikic et al., 2010) are among the few publicly active groups interested in developing winterhardy faba bean. Given faba bean has been reported to be the most winterhardy of the annual pulses (Lakhanow, 1984), one would expect there to be reference to research here in the Pacific Northwest. Unfortunately, only a limited number of research projects (Bezdicek, personal communication) and growers (Slinkard and Blain, 1988) have considered spring planting of faba bean and only recently has there been interest in autumn-sown types (Mwengi, 2011).

NUTRITION

Part of any alternative crop adoption program should identify the potential markets and the end products of production. Faba bean is quite dynamic in that the foliage and seed, in both the immature (fresh) and mature (dry) stages, can be consumed. Further, high starch and protein content provides raw materials for a number of industrial production applications (Olsen, 1980). Human consumption of fresh and dry seed predominates in China, the Middle East, and the Mediterranean. According to the FAO, 2010 production estimates indicated China (882,000 ha)
as the world leader, with Ethiopia (512,000 ha) and Australia (160,000 ha) responsible for the remaining bulk of world production. Overall, world production has decreased from 4.7 million ha in 1970 to about 2.5 million ha in 2010 and can be attributed to a decline in China’s production from three million ha in 1970 (FAO, 2012). This decrease in China’s production area was the result of an overall change in farming systems where faba bean was historically grown. Li-juan et al. (1993) described the replacement of higher value barley, rapeseed, and rice over the use of this legume. While there are industrial processing facilities using faba bean for bean noodles in China, it would appear the decline in faba bean production could be traced back to either the increase in use-availability of synthetic nitrogen or demand for meat over vegetable based protein (Hebblethwaite, 1983).

The U.S. does not produce recognizable amounts of faba bean. Vegetable types are produced on a small scale by backyard-market gardeners and green manure types are available through some seed catalogues. Evans (1992) was a relatively recent proponent of faba beans in the U.S. and offered some interesting recipes for falafel and hummus, which he claims were originally made with faba. Traditional recipes, and renditions thereof, were compiled by the International Center for Agricultural Research in the Dry Areas and can be found through their website (www.icarda.org).

Any wide-scale adoption of faba bean into the agricultural cropping systems on the Palouse will inevitably be destined, like the majority of other agricultural products, for international markets (Slinkard and Blain, 1988). The ramifications of this cannot be adequately conveyed here but the current globalized food system is questionably sustainable. According to Pimentel and Pimentel (2003) it is not a question of if, but when, the meat-based diet of
Americans will have to transition to a more resource use efficient lacto-ovo vegetarian diet. When this time comes, pulses will be a major source of protein, as they are for over half of the world’s population. In the meantime, pulses serve as a healthy alternative to meat, and promoting their use should be a concern of national health, since they are a key component in combating type II diabetes, obesity, colon cancer, and cardiovascular disease (Champ, 2001).

The nutritional value of faba bean, like that of other pulses, lies in their protein quality and quantity (~25-37%) (Duc et al., 1999). Faba bean is not only higher in total amino acids and vitamins than wheat, corn, and rice, but complements a cereal-based diet limited in lysine. Cereal, dairy, or eggs provide methionine, which faba bean is deficient in (Jonas, 1980; Li-juan et al., 1993). This may be why traditional consumption of faba bean is usually accompanied by a cereal, dairy, or egg product. In general, of the faba bean producing/consuming countries, minor and equine types are used as animal feed and major types as a fresh vegetable for human consumption in the developed world. The less developed countries consume the equine or major types in their dried or fresh form and graze livestock on the stubble (Hebblethwaite, 1983).

Recent efforts to promote sustainable cropping systems in developed countries have advocated the use of cereal-legume, faba bean included, intercrops for animal forage and feed (Strydhorst et al., 2008; Jensen et al., 2010).

Antinutritional factors are commonly associated with pulses (Norton et al., 1985), and for faba bean, the major elements of concern are oligosaccharides, tannins, and vicine-convicine. Oligosaccharides contribute to flatulence, tannins impart a bitter flavor to the seed, and vicine-convicine aglycone derivatives inflict the rare genetic disorder favism (Crepon et al., 2010).
Further, oligosaccharides are mainly concentrated within mature seeds, tannins are confined to the seed coat, and vicine-convicine predominates in the cotyledons of the developing seed.

It has been reported that two independent-complementary recessive genes, \( zt1 \) (\textit{Vicia faba} var. major L. origin) and \( zt2 \) (\textit{Vicia faba} var. minor L. origin), control the absence of seed coat tannins and block the synthesis of anthocyanin or anthocyanin precursors producing white flowered plants (Crofts et al., 1980; Metz et al., 1992; Duc et al., 1995). Crofton et al. (2000) identified Rowlands and Corner (1962) to be the first to identify complementation between different tannin-free genes, producing a wild type flowered plant in the \( F_1 \) of a \( zt1 \) x \( zt2 \) cross. Furthermore, upon selfing, the hybrid segregate in a 9:7 ratio (colored:white) (Fig. 3). Crofton et al. (2000) also ascertained at least two independent sources of \( zt2 \) and four for \( zt1 \). The majority of tannin-free commercial cultivars have the \( zt1 \) allele originating from ‘Threelfold White’ and \( zt2 \) from Ch170 (Crofton, et al., 2001). Sequence characterized amplified region (SCAR) molecular markers sequenced from digested random amplified polymorphic DNA (RAPDs) are now available for identification of \( zt1 \) plants at 9.7 cM (Gutierrez et al., 2007). The 20 different color variants controlled by the \( dp \) locus Chapman (1983) are silenced (pleiotropy) by either \( zt \) allele.

The reduction in the tannin content of dry seed associated with the white flower phenotype can be reduced below 0.1 g kg\(^{-1}\) dry matter (DM) from 5-10 g kg\(^{-1}\) DM for wild type flowered plants (Bond, 1976b; Duc et al., 1999). Tannin-free seed is preferred for direct consumption, as it is suggested to have a higher digestibility than tannin containing seed (Bond, 1976b) and resists oxidation when canned (Crofton et al., 2000). The \( zt2 \) gene is preferred over \( zt1 \) because it is associated with improved digestibility and slightly higher seed protein.
concentration (Cubero and Duc, 1995; Crofton et al., 2000). Dehulled, heat-treated, or NaOH (4%) soaked seed are also effective at reducing tannin concentrations (Garrido et al., 1989; Marquardt, 1989).

White flowered lines eliminate the need for dehulling or other seed treatments to reduce tannin concentration, but can also be low yielding, slow to emerge, and susceptible to insects and diseases (Bond and Smith, 1989). Cabrera et al. (1989) suggested low tannin lines could be selected with wild type flowers using a red testa color as a marker for low tannin. Another research group was able to show that soil-borne disease resistance was a factor of genetic background, rather than tannin concentration, and that poor emergence could be eliminated with a fungicide seed treatment (van Loon et al., 1989). Furthermore, Cubero and Duc (1995) confirmed that there were no obvious linkages between disease and absence of antinutritional factors, but rather, the genetic background determined resistance or susceptibility.

Vicine-convicine concentrations of seed across genotypes exhibit a continuous variation from 0.1% to over 1.5% depending on author and methodology (Jamalian, 1978; Gardiner et al., 1982; Bjerg et al., 1984, Frauen et al., 1984; Duc et al., 1989). Low vicine-convicine genotypes have been identified (Hussein et al., 1986). Reductions of both vicine and convicine by 10-20 fold in dry seed and 20-30 fold in fresh seed, was achieved through the discovery of the monogenic allele \(vc^-\); the expression is additive and maternally highly heritable (Duc et al., 1989). Duc et al. also mentioned that the allele contributes no negative effects (disease, yield, protein, and seed size) on plant growth. Hilum color has been used as a phenotypic marker, but there are two independent genes controlling these traits, 10.1 cM apart (Crepon et al., 2010). According to Gasim et al. (2004), the wild type black hilum is dominant to the light gray or clear
hilum associated with low vicine-convicine and is controlled by the genotype of the maternal parent. An earlier review by Crofton (1998) reported incomplete dominance of the black over the clear hilum where crosses segregated with a 1:2:1 ratio of black:gray:clear. Cleavage amplified polymorphism markers have been identified that can help identify the vc- allele, however, it appears hilum color had a tighter linkage (7.7 cM) than the closest identified molecular marker (14.8cM) (Gutierrez et al., 2006).

Zero-vicine-convicine varieties are preferred for fresh seed consumption, since over 90% of favism cases are the result of undercooking fresh seed (Arese and De Flora, 1990; Crepon et al., 2010). The antinutritional side effects of vicine-convicine on livestock are rather negligible when compared to tannins, but negative effects on the size of chicken eggs are a concern (Pitz et al., 1981; Crepon et al., 2010). Therefore, the recommended inclusion of high vicine-convicine was 7% for a hen’s diet, while low vicine-convicine could be added up to 20%. Cold (Lacassagne, 1988) or steam pelleted (Farrell, et al., 1999) compound feeds with faba bean is one option to further increase digestibility.

Faba bean has been shown not to alter the growth performance of growing or finishing pigs up to 10% of a compound feed and with supplemental amino acids, inclusion rates may be even higher (Crepon et al., 2010). The excretion of phosphorus in the form of limited available phytate is a potential environmental concern, due to phosphorous deposition (Hussein et al., 1983; Ray and Georges, 2010). Replacement of soybean meal with ground faba bean for lactating cows up to 30% was shown to not result in a comparative loss in production, and in some cases, with the addition of rapeseed meal, could even eliminate the need for soybean (Crepon et al., 2010). The double zero (low vicine-convicine and tannins) cultivars registered
with the Fevita trademark from the French company Agri-Obtentions, are intended for poultry feed, while the older cultivar ‘Maris Bead’ is a specialty pigeon feed component (Metayer, 2004).

L-DOPA is a glucoside with a molecular structure similar to vicine-convicine, but does not reduce the biological value of protein to the same extent (Bjerg et al., 1984). Molecular pharming of L-DOPA has been proposed as an alternative to the production of the synthetic form for the treatment of Parkinson’s disease (Ray and Georges, 2010). Levels range from 0.25 g to 2.29 g 100 g⁻¹ of seed DM (Arese and De Flora, 1990) and is highest (>6%) in developing pods-seeds with a moisture content of ~80% (Burbano et al., 1995). Rivoira (1979) reported a white flowered cultivar ‘triple white’ that did not contain L-DOPA. Bjerg et al. (1984) recommended the quantitative analysis of individual glucosides (vicine, convicine, L-DOPA), through the use of high performance liquid chromatography. Hu et al., (2013) found higher concentrations in flower buds than young leaves in six accessions of different flower colors and that two of the accessions with white flower color had different concentrations of L-DOPA.

Apart from direct human or animal consumption and the medicinal value of L-DOPA, there exists a number of different industrial applications for the air classified seed starch (10 µ) and protein (1 µ), or the alkaline-extracted protein-isolate (Olsen, 1980). Possible uses include: livestock-fish feed additive, meat extender, fortification of cereal products, gluten-free pasta, sweetener, starch-bean noodles, soy alternative, and tempeh (Olsen, 1980; David and Verma, 1981; Andrew et al., 1984; Chillo et al., 2008; Maskus, 2010).

BREEDING AND SELECTION

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*Vicia faba* is taxonomically isolated from other species of *Vicia*, having fewer chromosomes (n=6) and the greatest amount of DNA in the leguminosae (~13 pg or ~13000 Mb) (Lawes et al., 1983). From over 6000 years of anthropomorphic evolution, faba bean is still considered to be incompletely domesticated, hampering its acceptance by producers accustomed to modern cereals (Bond, 1987). For any breeding to be successful, the species must contain or be able to obtain, through some treatment, heritable genetic variation that upon selection results in the ideotype set forth at the onset. Although a wild progenitor has yet to be identified, there remains considerable variation available within the genus for many traits (Schaefer et al., 2012). However, since much of the interest lies in yield, which is of low heritability, breeding gains have been limited (Bond, 1987).

The reproductive biology of faba bean follows a mix of self- and cross-fertilization, i.e., partial allogamy. Whether a pollination event results in a self- or cross-fertilization is by chance, since a membrane (papillae) covers the stigmatic tip preventing self-pollination until the flower is tripped. Primarily, pollinating bees facilitate flower tripping, depositing pollen as they forage for nectar. In working with faba bean in 1876, Darwin found that if the flowers were not disturbed and bees are excluded, plants produced 30% the seed of similar plants that were tripped (Drayner, 1959). Rupture of the stigmatic membrane releases protein exudates promoting pollen germination of both self and foreign pollen. This results in an equal chance of fertilization, since there is presumed to be no host incompatibility (Toynbee-Clarke 1971). Outcrossing ranges from 4%-84% and is influenced by environment and genotype (Hebblethwaite, 1983; Hu et al., 2011). An outcrossing rate of ~50% is a general rule of thumb (Poulsen, 1981). Intercrossing can be significantly curtailed by a 20 m isolation distance (Pope and Bond, 1975) to as close as 3 m (Li-juan et al., 1993) depending on bee activity and biological barriers (Poulsen, 1981). A practical
isolation of 3 m for a breeding nursery and a certified seed isolation of 200 m were recommended by Fehr and Hadley (1980).

Patrick and Stoddard (2010) estimated “if every ovule of a faba bean crop produced a seed, the yield would be 38 t ha\(^{-1}\) and at a harvest index of 50% that would presuppose dry matter production of 76 t ha\(^{-1}\), which is five to 10 times more than achieved dry matter levels and greatly exceeds potential solar energy capture”. Suffice to say, only a fraction of flowers develop into harvestable pods. Along with extra-floral nectarines, excess flower production is a common ecological strategy to compete for pollinating insects (Schoonhoven et al., 2005).

Breeders have attempted to reduce dependence on pollinators through selecting for autofertility. Autofertility is the ability to set seeds without tripping and is dependent upon the spontaneous release of stigmatic exudates (Paul et al., 1978). However, autofertility may not favor self-pollination over cross-pollination, since spontaneous stigmatic exudation usually occurs after a typical pollination event (Link, 1990), resulting in equal cross- and self-pollinations (Monti and Frusciante, 1984). Inbreeding has been observed to increase autosterility, promoting outcrossing, possibly due to less pollen production as compared to a hybrid or preferential embryo viability (Drayner, 1959; Toybbee-Clarke, 1971; Kambal et al., 1976). Yield depression, when pollinators are rare, has been attributed to the effect of multiple generations of inbreeding and subsequent autosterility (Monti and Frusciante, 1984). The selection of autofertile inbred lines with improved yield stability is an attempt to adapt the species to autogamous breeding schemes (Cubero and Moreno, 1984).

Drayner (1959) showed when bees are excluded, higher seed set will occur on hybrid plants than extensively inbred ones, due to autofertility and autosterility, respectively. Heterotic
autofertility is generally the result of increased pollen grain production rather than a spontaneous stigmatic exudation (Kambal et al., 1976). Of the 11 European spring and winter genotypes tested by Stoddard (1986) the only autosterile cultivar was ‘Polar’ a white flowered winter type. The impact of autofertility and autosterility on an open pollinated population is that extensively inbred plants experience more outcrossing, producing less seed when bees are rare, compared to hybrids (Free and Williams, 1976). When bees are adequate, von Kittlitz (1981), using a simple autofertility index (# of pods/# of flowers-for three flowers/node), observed no appreciable yield difference between parents with contrasting autofertility.

Autofertility and autosterility or autogamy and allogamy vary according to genotype (von Kittlitz, 1981; Hawtin, 1982; Stoddard, 1986; Metz, et al., 1993; Link, 1994a; Robertson and El-Sherbeeny, 1995; Gasim et al., 2004). Absolute autofertility was discovered by Cubero and Moreno (1984), and while heritable, there was still noticeable heterosis for yield. Obligate autogamy has not been identified to my knowledge, other than in V. faba subsp. paucijuga (Drayner, 1959), a recent ancestor, or cousin to the larger seeded faba bean. Increased self-pollination through the use of closed flowered mutants (cleistogamous flowers) was not found to associate with increased levels of autofertility per se, but limit bee visitation and potential cross pollination, resulting in effective autogamy (Stoddard, 1986). Low seed set associated with the closed flower mutant led Robertson and Filippetti (1991) to suggest that introgression of autofertility may improve induced autogamy. Hayes and Hanna (1968) (see Stoddard, 1986) proposed that a single dominant allele controlled autofertility, but pod fill followed an additive model, while Metz et al. (1993) observed limited response to selection, concluding that outcrossing was polygenic and influenced by epistatic and/or dominance effects. The objective to treat faba bean as a fully autogamous crop, while enticing, may in fact prove elusive.
Determining the reproductive biology of a crop is prerequisite to breeding as the genetic structure and breeding method will be influenced by whether or not the crop is self- or cross-pollinated. Partial allogamy is somewhat unique to faba bean as a crop plant; many of the major crops are treated as autogamous, (pea and wheat), or allogamous (brassicas and corn). Without stable autofertility, or stable male sterile genes, hybrid cultivars via inbred line development are of limited commercial value (Picard et al., 1982). For the breeder, a balance has to be sought between uniformity of particular traits and exploitation of heterosis. Rye, like faba bean, is partially allogamous with varying degrees of self-fertility/sterility and would be a model for novel breeding techniques (Hayes et al., 1955).

The reproductive biology is as important to the curator, researcher, or seed grower, as is it to the breeder. Intercrossing of two unique genotypes-populations increases the risk of unique allele(s) being lost, which will depend on the frequency of the allele in question, the degree of outcrossing, and isolation distance between accessions (Witcombe, 1982). Changing allele frequencies may be the objective of the quantitative geneticist, but in situations where characterization, strict purity, or stability of the phenotype is the objective, isolation should be followed. Where strict inbreeding is followed care should be taken to limit drift of low frequency alleles. Witcombe (1982) recommended that where isolation is not followed, predominating alleles (high allele frequency within a population) will have a lower risk of loss compared to rare alleles, and therefore, a conservative 10% outcrossing could be tolerated without losing accession identity. If isolation by physical means is not practical, large plot size (> 16 m²), discarding the border rows and surrounding plots with Brassica spp. interplanted with a cereal were suggested by Witcombe (1982) as means to reduce outcrossing (<10%). The distinction between conservation and improvement through breeding is important, however, both are similar
in the sense that they are restricted by availability of time and resources. Choice of population management methodology is, in other words, restricted in space and time.

The simplest improvement or breeding method of faba bean, closest to its natural reproductive mode, is bulk selection, where little to no artificial selection is made and natural selection is the driving force, i.e., negative selection (Hawtin, 1982). This method is most effective for traits with high heritability. Mass selection is a derivative of bulk selection in that selected individuals are bulked and sown without any pollination restriction. This method was popular with Egyptian faba bean breeders creating composites for release (Nassib and Khalil, 1982). A breeder may establish an index of selection because a valuable trait like yield has a low heritability, as is the case for faba bean (Bond et al., 1985). Selection for discontinuous quality traits or phenotypes with additive genetic variation helps with maintaining a high frequency of desirable alleles within the population. Since heterosis can confound artificial selection, because of its low heritability (Gasim et al., 2004), progeny testing and selection enables the breeder to distinguish apparent and fixable variation (Lawes et al. 1983).

The natural selective pressure of overwintering has been shown to increase winter-hardiness of autumn-sown faba bean (Gasim et al., 2004). From the work of Dr. Link and colleagues from the University of Gottingen, the Göttingen Winter Bean Population or Gö-Wibo-Pop was formed from a composite of diverse winter genotypes. It appears as long as the selection pressure is not too severe, resulting in a genetic bottleneck (drift) and inbreeding depression, mass selection is a suitable method to improve favorable allele frequencies with additive gene effects and optimizes the maintenance of heterogeneity over generations (Gasim et al., 2004).
Mass selection and bulk population improvement were the two main techniques adopted by farmers since the dawn of agriculture (Jensen, 1988; Harlan, 1992). Over time, locally adapted, yet diverse populations or landraces, were developed and serve as the source material of modern-day breeders (Hawtin, 1982). Single plant selection and subsequent progeny testing was the first major breakthrough in crop improvement since mass selection of bulk populations (Allard, 1960). For self-pollinated species, a pedigree selection procedure can be carried through till homozygosity and then homogeneous lines released. For crops like faba bean, however, inbreeding depression can be an impediment if using strict pedigree selection. Bulk pedigree selection has been implemented for faba bean as a compromise of both selection methods (Bond, 1985), although Hawtin (1982) advised against this approach since superior plants would have a high likelihood of exhibiting hybrid vigor.

Recurrent selection is one alternative to the limitations of the pedigree method, allowing alternating generations of selfing (exposing recessive alleles) and outcrossing (breaking linkage groups) to concentrate genes of poor heritability (Bond, 1987; Muehlbauer et al., 1988). In conjunction with progeny testing, cycles of recurrent selection can produce desirable individuals, but can also narrow the allelic diversity, over time, depending on selection intensity and the allelic frequency of desirable alleles in the population. The key to recurrent selection is obtaining a high outcrossing rate, which improves recombination and recombination within linkage blocks (Lawes et al. 1983). Le Guen and Berthelem (1986) introgressed a male sterility gene into their faba bean breeding populations to improve outcrossing and maintain heterosis throughout various recurrent schemes. Later this male sterility trait was found to be unstable due to its association with an RNA virus transmitted with the cytoplasm (Robertson and Saxena, 1993).
Inbreeding programs are useful in the context of conservation (Witcombe, 1982; Robertson, 1988), characterization of recessive alleles (Hawtin, 1982), selection (Bond, 1966; Arbaoui et al., 2008; Link et al. 2011), estimating general combining ability, specific combining ability, and heritability (Poulsen, 1977), but commercial cultivars of inbred faba bean are not economical. The fact that Poulsen (1981) empirically observed no further inbreeding depression after the 4-5th selfing generation and that one generation of open-pollination restored lost yield suggests that inbreeding could be a valuable tool for breeding faba bean. Inbred lines are often used in the creation of synthetics, but combining ability is rarely estimated (Lawes et al. 1983).

The incorporation of multiple recurrently selected inbred lines or populations into an open-pollinated composite, i.e., synthetic, is one way to limit the negative effects of inbreeding while taking advantage of quality, heterosis, and yield stability (Hayes and Garber, 1919; Bond, 1967; 1982; 1987; Lawes et al. 1983; Bond et al., 1994). Most faba bean cultivars are synthetics, the first released winter faba bean synthetic (1960) was ‘Throws MS’, created and maintained through the recurrent selection of four component populations (Lawes et al., 1983). The synthetic was released simply by bulking equal proportions of the four populations. Recurrent selection was also used at ICARDA to develop synthetic populations with high levels of residual heterozygosity (Hawtin, 1982). Their system employed mass selection of the F2 for biotic resistance, plant architecture, and maturity; then for the F3, single plants are selfed and bulked according to yield characteristics; followed by progeny row selection during the F4. A portion of plants from the F4 through F7 are bagged or open pollinated and tested for autofertility or yield, respectively.
Phenotypic heterosis, or genotypic overdominance in faba bean was identified as a yield increase (>20%) over the inbred parents due to additional reproductive stems (Bond, 1966) or pods plant$^{-1}$ (Link, 1994a). Apart from differences of maturity dates, quality traits, abiotic and biotic resistance, and yield, there should be a high number of elite lines that are as distinct as possible to sustain heterosis though multiple generations of above average cross-fertilization (Wright, 1977; Bond, 1982; Link 1990, Link et al., 1994a) and to provide stability across environments (Stelling et al., 1994). General combining ability of selected lines is more often determined using performance of the synthetic variety itself rather than the general-specific combining ability of its components, as these effects are limited to hybridization events (Bond, 1982; Hebblethwaite, 1983). Inbred lines with highly heritable traits, such as pod number, seed number, and seed weight should be tested for general combing ability, since these are additive components of yield (Bond, 1966).

The frequency of outcrossing between the genotypes originally composing the synthetic will ultimately determine the rate of heterotic decline (Sprague and Tatum, 1942; Wright, 1977). In addition, the higher the component’s outcrossing rate, the higher the yield of the synthetic over the mean yield of its inbred parents will be, but the decline towards equilibrium will be sooner than if the components were preferentially autofertile (Bond, 1967; Wright, 1977; Link, 1990, Link et al., 1994a). Components can be greater than ten but more often between four and five for efficiency and necessity to conform to seed registration requirements of uniformity and stability (Bond, 1982).

A classic concept in genetics is the theoretical advantage in yield stability of a mixture over any one of its components in monoculture (Marshall and Brown, 1973). Heterozygous
genotypes and heterogeneous populations can have greater buffering capacity over a broader range of environments than homozygous-homogeneous populations (Lewontin, 1957; Allard, 1961; Bond, 1982; Schnell, 1982; Stelling et al., 1994). Flexibility of phenotypic traits, i.e., cooperation of genotypes (Allard, 1961), could ultimately contribute to yield stability (Westerman and Law, 1970), which follows a dynamic response to the prevailing environment (Becker and Léon, 1988). For other traits like winter-hardiness a static concept of phenotypic stability would be preferred. In other words, equally winterhardy in all environments of interest. Ultimately, stability may be the result of multiple adaption strategies that buffer a population against environmental perturbation. The greater use of winter faba bean synthetics than with spring faba bean (Bond, 1982) may capitalize on this heterozygosity for stability of winter-hardiness.

The regression approach implemented by Finlay and Wilkinson (1963) and Eberhart and Russell (1966) characterized the use of regression coefficients (b_i) and deviations from regression (s^2), respectively. These two metrics provided the breeder with additional information about the genotype x environment interaction. Estimates of stability are calculated by plotting the phenotypic trait of interest (typically yield) onto an environmental mean or index for the trait across all genotypes in that environment. A regression line and deviations from this line can then be estimated across all trail locations. Another stability parameter, similar to s^2, is ecovalence (W_i=∑i(X_ij-X_i.-X._j+X..)^2 (Becker and Léon, 1988). By transforming the ecovalence (100*((√ecoval.)/(n-1))/X_i) into a variation coefficient the phenotypic stability can be compared as weighted estimates according to yield level, i.e., low yielding varieties are given more weight than high yielding (Schnell and Becker, 1986).
The application of stability has often been met with mixed interpretations (Becker and Léon, 1988; Cleveland, 2001). Allard and Bradshaw (1964) classified a universal variety as one with high and consistent performance, while a specialized variety was one capable of high, yet inconsistent performance across differing locations. In other words, broad or wide adaptation for a trait such as yield is one that is closest to a $b_i$ of one, i.e., dynamic stability, whereas for a trait like winter-hardiness a $b_i$ of zero would be ideal. In both cases, no deviation from regression would be ideal, indicating little to no instability, i.e., phenotypic stability (Becker and Léon, 1988). It is important to remember that stability is not monolithic but provides additional tools to characterize varieties. Estimates of geographical adaptation are contingent upon the breadth of testing sites, as well as growing seasons, i.e., environmental adaptation. A genotype or variety may have high yield stability, but be relatively low yielding. Furthermore, varieties may have narrow geographical adaptation to marginal environments, but have high yield stability from year to year (Ceccarelli, 1994; Cleveland, 2001). Ultimately, the relevance of yield stability is limited to the varieties and the selection or testing environments screened (Hill et al., 1998).

Stelling et al. (1994), working with a diverse set of faba bean breeding material with contrasting population structures, showed that stability was improved by increasing heterozygosity and heterogeneity. Further, heterozygous variety structures were more responsive ($b_i>1$) to high yielding sites. It would be important to validate if under conditions that deviate from the optimum, the advantages of the heterozygotes increases proportionately (Phillips and Wolfe, 2005), as shown in lima bean, another partially allogamous species (Allard and Workman, 1963). Moderate levels of heterozygosity-heterogeneity have been exploited through the use of synthetic cultivars, which make up the majority of commercial cultivars (Bond, 1982). However, due to a polymorphic population structure, selection based on stability would be
biased by the level of heterozygosity present, which is why creating stable inbred lines with optimal general combining ability through trialing across >14 environments for multiple years is suggested (Link et al., 1994b). Other than genetic architecture, certain physiological modifications (indeterminate growth, autofertility, and early maturity), as well as biotic and abiotic resistance, or tolerance, can improve broad adaptation (Dantuma et al., 1983; Bond, 1987). Allard and Bradshaw (1964) coined the terms individual buffering and population buffering to describe these two methods of stabilization. Individual buffering or phenotypic plasticity (Phillips and Wolfe, 2005) can be derived from heterozygosity or a property of the specific genotype, where as population buffering relies on synergistic interactions among different coexisting genotypes.

The alternative to broad geographical adaptation, typically applied to favorable environments, i.e., high input agriculture, is specific or narrow seasonal adaptation within marginal environments (Ceccarelli, 1994; Phillips and Wolfe, 2005), or where a large variety x location interaction indicates special environments (Allard and Bradshaw, 1964). Marginal environments are characterized as low input, i.e., no irrigation, synthetic fertilizer, or chemical pesticides. Marginal environments are typically low yielding and are at risk of crop failure, due to variable abiotic and biotic stresses. For these reasons, for the subsistence farmer, yield stability is minimization of crop failure, rather than yield potential. This is where environmental sensitivity or plasticity of landrace varieties with time tested adaptive complexes may contribute to yield stability in marginal environments, but not necessarily when under intensive production (Allard, 1962).
Genetic gain in yield over the history of modern agriculture through breeding has been possible mainly because of synergistic advancements in agronomy and breeding (Rowntree et al., 2013). Where Green Revolution technologies have proven unsustainable, crop yields have stagnated, and sustainable intensification is the most recent term for creating awareness around improving yield in marginal environments (Garnett et al., 2013). Sustainable intensification and other holistic agendas aim to develop the capacities of target populations so they may achieve adequate food security and cope with climate change. A significant part of this coping strategy is through breeding cultivars or modern landraces adapted to the specific environment instead of altering the environment to fit the cultivar (Simmonds, 1993).

Where a genotype by environment interaction exists, direct selection on farm may be more effective and empowering than indirect selection at an experimental station (Ceccarelli, 1994). Furthermore, breeding for specific adaptation can improve yield over broadly adapted cultivars. This is where participatory and evolutionary breeding strategies could compliment sustainable agricultural development policies in achieving food security.

The evolutionary plant breeding method was first coined by Suneson (1956), as an effective, but long-term strategy that relies on natural selection of bulk hybrid populations derived from diverse seed stocks. The major disadvantage of this is the number of generations (>12) suggested to achieve production gains equivalent to pure line selection. However, adaptation (stability) via natural selection and maintenance of genetic diversity are corollary advantages. The method is an extension to the bulk method of breeding from Florell (1929) and natural selection experiments utilizing composite hybrid mixtures from Harlan and Martini (1938).
Pure line varieties offer uniformity at the cost of possibly exploiting genetic resiliency. Suneson (1956), provided the example of yellow dwarf virus changing the relative frequencies of resistant types in an F$_{25}$ bulk population. Residual diversity allows for further change (resilience) as biotic and abiotic stresses fluctuate over time (Phillips and Wolfe, 2005). Evolutionary breeding is most often applied to self-pollinated species; however, utilization of a partially allogamous species would allow both recombination and segregation to occur without much human intervention. The creation of a long-term bulk faba bean composite that is subjected to natural selection over contrasting environments (see dynamic management of Enjalbert et al., 1999) would be a cost effective way to improve adaptation, while maintaining a reservoir of genetic diversity for other traits of interest.

**QUANTITATIVE TRAITS AND RESPONSE TO SELECTION**

Given the rather narrow genetic background of winter-hardy genotypes, Côte d’Or being one of the only reliable sources, selection gains have been minor (Link et al., 2011). Genotypes with improved frost tolerance have been sought as one way of complementing genes and selecting for transgressive segregates within inbred lines (Arbaoui et al., 2008). Link et al. (2011), using a frost-tolerant genotype from China, were able to select for inbred lines with comparable frost tolerance as Côte d’Or, but winter-hardiness results have not yet been published.

To increase winter-hardiness in autumn-sown faba bean it would seem important to identify unrelated genotypes with additive genetic variance for this quantitative trait. The Western Regional Plant Introduction Station (WRPIS) of the National Plant Germplasm System
(NPGS) is one readily available source of diverse germplasm holding over 400 available accessions of *Vicia faba*.

Mwengi (2011) compared commercial with identified NPGS sources of winter-hardiness, but did not determine if any possessed novel winter-hardiness alleles. Since the heritability of frost tolerance is high and correlated with winter-hardiness (Link et al., 2010), it would be expected that mass selection would be an effective method to select for winterhardy genotypes from a heterogeneous accession or population (Srd et al., 1957; Gasim et al., 2004). Rather intense selection over winter should result in a rapid response to selection over the first few generations and then slow, as major genes are fixed and variance declines, if inheritance is additive (Srd et al., 1957; Allard, 1960; Murphy et al., 2008). The number of generations it takes for the allele frequency to shift the population mean towards higher survival rates would primarily depend on: heritability, population size, outcrossing, initial gene frequency-diversity, selection intensity, linkage, epistasis, assortative mating, environmental variance, maternal effects, and heterozygosity-dominance (Srd et al., 1957; Allard, 1960; Muehlbauer et al., 1988; Falconer and Mackay, 1996).

Quantifying the response to selection for improving winterhardy faba bean would be complicated by the presence of heterozygous individuals in the population. Link et al. (2010) described both maternal and overdominant effects present in the F₁ generation for frost tolerance of faba bean. The selection differential after positive winter-hardiness selection will therefore, likely depend on the allelic diversity, outcrossing rate and heterozygosity of individuals. Mather in Allard (1960) described this effect on selection as free and potential variability. Free variability was defined as the homozygous alleles that can be selected upon, while the potential
variability is bound in the heterozygotes, and if selected, does not lead to progress, predicted by simple additive inheritance. Since faba bean F₁ hybrìds are more apt to inbreed and inbred individuals apt to outcross (Free and Williams, 1976), a large heterogenous random mating population should be relatively stable for heterosis (Sprague and Tatum, 1942; Wright, 1977). Random mating under the directional selection pressure of winter would, after multiple generations of recombination and segregation, free the potential variability up for selection allowing for incremental improvement, but would likely decrease the population variance, limiting progress proportionately (Allard, 1960). Inbred offspring from heterozygotes or heterozygotes themselves, depending on their inbreeding coefficient, may also regress to lower levels of hardiness, resulting in the overestimation of additive variance (Srd et al., 1957).

Without conducting an intricate genetics study to estimate broad-sense heritability ($h^2_B = \frac{V_G}{V_T}$, where $V_G$ is the mean sums of squares of the genetic component of variation and $V_T$ is the mean sums of squares of the total variation in the population), realized heritability ($h^2 = \frac{R}{S}$, where $R$ is the response to selection and $S$ is the selection differential) is much more practical for determining the efficiency of mass selection from a bulk population (Hayes et al., 1955; Falconer and Mackay, 1996). Response to selection ($R$) is the difference between offspring and parental means. The selection differential ($S$) is the selection intensity [$i = z/p$, where $z$ is the z score corresponding to the proportion of population selected ($p$)] multiplied by the parent population variance ($\sigma_P$).

**PROJECT GOALS AND OUTCOMES**

When implementing breeding research and identifying objectives the crop’s reproductive system, agronomic requirements, germplasm, target environments, and market should be taken
into consideration (Mayo, 1980). Locally relevant considerations have been briefly outlined above in the context of *Vicia faba* with an emphasis on autumn sowing winter-types. Since faba bean is a minor crop and there are few breeders or companies with programs globally, it would be initially important to cater to a stakeholder with limited available resources. Providing novel alleles and/or genotypes that would support the development of synthetic populations, with favorable quality traits, would stabilize genotype x environment interactions and help to facilitate adoption, respectively. Therefore, this research evaluates the winter-hardiness and characterizes the inheritance of faba bean quality traits in accordance with the ARS Plant Genetic Resources, Genomics, and Genetic Improvement National Program (301). The continued evaluation and selection of winter-hardy faba bean follows the recent advances of Mwengi (2011) and satisfies the mandate to capitalize on untapped genetic diversity by incorporating novel diversity into adapted breeding pools. The second research component focuses on the characterization of low molecular weight sugars and the inheritance of phenotypic traits; e.g., height and leaf, flower, hilum, and stem color. This objective falls under the ARS mandate to identify genes and alleles that control important traits. The underlying purpose of this research was to broaden the genetic diversity available to breeders so they can develop cultivars that help to improve the sustainability of agriculture on the Palouse and beyond.
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CHAPTER TWO

The effect of autumn planting date on winter-hardiness, plant development, and yield of faba bean (*Vicia faba* L.) in southeastern Washington

(To be submitted to Agronomy Journal)

ABSTRACT

Autumn-sown faba bean (*Vicia faba* L.) could improve yield stability over spring sowing in terminal drought environments like southeastern Washington. Timing of sowing and genotype are suspected to influence winter-hardiness and yield. Therefore, a variety trial testing the effects of two sowing dates was conducted for two seasons (2011-12 and 2012-13) at three locations [Central Ferry Research Farm (CF), Pomeroy, WA; Spillman Agronomy Farm (SF), Pullman, WA; Whitlow Farm (WF), Pullman, WA] in southeastern Washington using 20 northern European breeding lines, or cultivars, and USDA sourced germplasm with predetermined winter-hardiness. Overwintering survival and yield were influenced by autumn sowing date and winter low temperatures. Survival was greatest at CF where the low air temperature was -6°C, yet multiple entries tolerated an extreme low of -14°C in Pullman. In both years, the second sowing at the CF location improved survival, whereas the first sowing was only slightly improved at the Pullman locations under certain environmental conditions. Providing a longer establishment and cold acclimation period was expected to optimize hardiness and yield potential. However, achieving 2-3 nodes prior to the first hard frost was difficult at dryland managed SF and WF locations where soil moisture was limiting. Contrastingly, at CF, irrigation aided stand
establishment, but showed reduced overwintering of the first sowing. Few of the entries tested across six site years stood out for winter-hardiness or yield; the majority exceeded a mean survival of 65% and yield of 2800 kg ha\(^{-1}\). Breeding adapted cultivars and optimizing stand establishment will be critical prior to commercial adoption.

Faba bean is grown as a winter annual across the Mediterranean and in parts of Australia and China, where winter temperatures typically remain above -5°C. True winter faba bean or winter field beans (Bond and Crofton, 1999) from northern Europe are small seeded (var. equine and minor) and can tolerate winter temperatures from -10°C (Evans, 1992) to -25°C (Picard et al., 1982). Generally, cultivars from France and the United Kingdom can tolerate temperatures between -12 and -15°C without risk of crop failure (Herzog, 1989). Annual minimum temperatures in southeastern Washington are generally above -20°C, but temperatures below -25°C do occur posing a significant risk for autumn-sown faba bean. Therefore, our objective was to evaluate European cultivars and breeding lines, while characterizing NPGS accessions with adequate winter-hardiness for this region.

The diversification of cereal-dominated rotations with pulse crops reduces the build-up of soil-borne disease and need for synthetic nitrogen inputs (Jensen et al., 2010). Spring sown faba bean has a high capacity to fix nitrogen and can also outyield many other cool-season pulses (Herridge et al., 1994). In southeastern Washington, the mean yield of spring-sown faba bean compared favorably with regional mean chickpea yield (Chapter 4). Winter faba bean can have a higher yield potential and capacity to root and fix nitrogen than when spring sown (Herzog and Geisler, 1991). A winter crop can also show earlier development than a spring sown crop, making efficient use of seasonal moisture (Herzog, 1989) and avoiding thermal stress.
Experimental yields of autumn-sown faba bean in England have been reported as high as 9 t ha\(^{-1}\) (Roughley et al., 1983), but in production, they rarely yield over 7 t ha\(^{-1}\) (Stelling et al., 1994). Improving the yield potential and stability of faba bean would help support the regional adoption of this pulse crop.

Pertinent issues facing adoption are weed pressure and erosion control over the winter. High density sowing is one method to reduce erosion, weed pressure, and crop failure due to winter-kill (Murray et al., 1988). However, wide spacing (before winter: 30-60 plants m\(^2\) and after winter: 15-30 plants m\(^2\)) is recommended for grain production, as plants have a strong ability to produce reproductive branches and show less lodging (Poulain, 1984; Link et al., 2010). Sowing into no-till cereal stubble may be a useful approach to conserve moisture and alleviate erosion and weed pressure issues (Gnanasambandam et al., 2012), as well as possibly improving winter-hardiness (Cutforth et al., 2007).

In southeastern Washington winter faba bean would likely be sown into relatively dry soil in October, after harvesting spring wheat or barley. Late August to early October is the recommended sowing time for autumn-sown pea in this region, as sowing much earlier results in slow germination, due to a lack of soil moisture, and late sowing results in a decline in winter survival (R. McGee, per. comm.). Low soil moisture can also affect the stand establishment of winter wheat, resulting in a shorter cold acclimation phase and heightened risk of winter-kill (Young et al. 1994). According to Murray et al. (1988), winter faba bean seedlings should have two to three pairs of leaves and a strong root system going into winter. Mwengi (2011) suggested sowing date had an effect on winter survival with an earlier sowing being more successful. Yet it
remains unknown how early is necessary to optimize winter-hardiness and yield. Further evaluation of sowing dates would be beneficial for estimating the optimal sowing window for winter-type faba bean.

This study aimed to further evaluate winter-type faba bean germplasm previously identified as winterhardy in southeastern Washington (Mwengi, 2011). Due to uncertain soil moisture conditions during early autumn, two sowing dates at each of three locations helped to identify a sowing window. This research sought to identify winterhardy genotypes and agronomic practices adapted for producing winter-type faba bean in southeastern Washington.

METHODS

An autumn-sown variety trial with twenty European breeding lines and cultivars and NPGS accessions and bulks (Table 2.1) was conducted for two seasons (2011-12 & 2012-13) across three locations [Central Ferry (CF) Research Farm in Central Ferry, WA, 46°39’5.1”N; 117°45’45.4”W; and Washington State University’s Whitlow Farm (WF), 46°44’3.2”N; 117°7’25.8”W, and Spillman Agronomy Farm (SF), 46°41’45.1”N; 117°9’2.7’’W, near Pullman, WA] following a split-plot experimental design with three replications. The main plot was planting date (Table 2.2) and subplot was entry.

Field sites represented different elevation and climatic zones of southeastern Washington, as well as field management. The CF location has a Chard silt loam soil and is characterized as low elevation (198 m) and was irrigated (subsurface drip irrigation at 10 min d⁻¹) during the active growing season, whereas SF and WF are higher in elevation (770 and 790m, respectively), dryland managed, receiving on average 53 cm of rainfall annually, and have heavier Palouse silt
loam and Palouse-Thatuna silt loam soils, respectively. Treflan (a,a,a-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; Dow Chemical), to control monocot weeds and Thio-Sul ((NH₄)₂S₂O₃; Texas Sulfur Co.), to reduce bird damage to seedlings were applied post-plant pre-emergent at all locations. Warrior ([1a(S*),3a(Z)]-cyano(3-phenoxypHENYL)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; Syngenta) was used to control pea leaf weevil (Sitona lineatus L.) as necessary.

Plot dimension varied by location due to irrigation constraints at CF, but area (2.7 m²) was constant. Plots at WF and SF had four rows, while CF had two. Rows were spaced 35 cm apart at each location. Plot dimensions were 1.5 x 1.8 m at WF and SF and 0.75 x 3.6 m at CF, with a 40 cm space separating subplots. A Hege 120 planter was set for 48 seeds per plot, equivalent to 80-120 kg of seed ha⁻¹ depending on seed size. Sowing density was toward the lower end of what is commercially recommended for autumn sowing (170-200 kg of seed ha⁻¹) (Soper, 1956; Hebblethwaite et al., 1983; Link et al., 2010) to facilitate individual plant measurements. Planting stock was sampled from the previous year harvest at WF annually. Complete isolation was not practiced in an effort to allow gene flow between entries and increase winter-hardiness, following a bulk method of selection (Chapter 3). Therefore, entries were maternally/cytoplasmically distinct, but open to paternal gene flow. Heterogeneous NPGS bulks were derived from 466 USDA germplasm accessions, bulked, and selected for winter-hardiness at four locations across southeastern Washington (Chapter 3).

Percent survival was determined from the ratio of seed sown to spring stand count. Mid-winter and spring stand counts were taken to estimate frost tolerance during early and late winter, but were not presented due to inconsistent stand establishment and delayed emergence. Soil
temperature, electrical conductivity, and moisture were collected at each of the three sites at three soil depths (2.5, 7.5, and 15 cm) using data loggers (EM-50, Decagon Devices, Pullman, WA) equipped with 5TE probes.

Physiological development was partitioned to characterize vegetative and generative stages (Knott, 1990). Early branching and height measurements were taken when plants started to bloom (203), and late branching and height just prior to harvest and leaf shedding (310 and 410) (Table 2). Number of branches and height measurements were based on 20 representative plants from each plot. Where less than 20 plants survived winter, all remaining plants were scored. Total plot yield was extrapolated to kg ha\(^{-1}\). Plant yield (g plant\(^{-1}\)), pods plant\(^{-1}\), and mature branches plant\(^{-1}\) were based on five representative plants at harvest. Yield adaptability (b\(_i\)) based on plot yield was estimated according to Finlay and Wilkinson (1963) as the regression coefficient of each individual entry’s site year yield across the mean yield of all entries at each site year. Ecovalence (W\(_i^2\)) (Becker and Léon, 1988) was transformed into a variation coefficient (VCW\(_i^2\)) according to Stelling et al. (1994) to weight variation according to yield. Calculation of reliability indices was based on mean yield and b\(_i\) of each entry.

Survival, phenological characteristics, and yield least squares means (LS-means) and Pearson correlation coefficients were compiled and analyzed using ANOVA with PROC MIXED and PROC CORR (SAS Institute, 2008), respectively. Entry, location, sowing date, and year were treated as fixed effects and block and block x sowing date (main plot) were set as random effects within the model following a split-plot experimental design. Treatment mean comparisons were assessed based on Fisher’s Least Significant Differences (LSD) test at \(P = 0.05\).
RESULTS

Daily low air (Fig. 2.1) and minimum monthly temperatures over winter (Table 2.3) at WF and SF were similar to one another and colder than at CF. Pullman locations having a colder climate than CF is typified by 30 year average low and record setting temperatures. This difference in winter severity was reflected in percent survival, with a higher rate of overwintering at CF than at either of the Pullman locations. Both 2011-12 and 2012-13 winters were above normal for temperature. Percent survival was also slightly higher across entry, location, and sowing date for the 2011-12 (65.5%) compared to the 2012-13 (63.7%) season. The 2011-12 winter was slightly warmer and shorter than for 2012-13.

The lowest temperature throughout this trial was just below -14°C on 13 Jan. 2013 in Pullman. The lowest soil temperature at sowing depth (7.5cm) was -3.1°C at SF during this same cold period, which was slightly colder than WF (-2.2°C). The active growing season in 2012 was very similar to the 30 yr average, but 2013 temperatures were warmer than normal.

Percent survival not only responded to year and location effects, but sowing date also influenced overwintering across entry (Table 2.4). The second sowing at CF was more advantageous than the first, mainly in 2012-13. This can largely be explained by the incidence of Pea Enation Mosaic and Bean Leaf Roll viruses that were likely vectored by late season aphid migration. The first sowing at CF emerged before the first hard frost and viral symptoms were observed across the majority of the seedlings, while the second sowing emerged after the first hard frost and viral symptoms were less pronounced.
The first sowing at WF showed a slight improvement in overwintering as compared to the second for both seasons; however, at SF there was no clear trend. The sowing date at SF was a week later than at WF for both seasons (Table 2.1). As a result, seedling emergence for both sowing dates was delayed considerably. For 2011-12, the first and second sowing dates emerged by 1 Nov. at WF. However, at SF, germination scoring of the first sowing was delayed until 5 Dec. and the second sowing could not be rated fully until late March. Limited soil moisture at SF during the autumn of 2012 likely impacted germination of the first sowing, whereas the second sowing showed greater germination rates across entry and survival overwinter.

No single entry stood out across all site years or in any one location across years for percent survival overwinter, likely due in part to the variation in response to environment (Table 2.1). Many of the entries exceeded 70% survival compared to the unselected check, which averaged 30% across all treatments. The W6 accessions were comparable to proven European lines and bulk populations were only statistically inferior to the hardiest of entries. The WF bulk population was on average comparable to elite northern European lines, suggesting that winterhardy genotypes are present in the NPGS collection and may serve as a source for improving winter-hardiness.

Percent survival was moderately correlated with plot yield \( r = 0.45; P < 0.0001 \), however, it was not complete. For example, 13 Karl/2-3 was among the hardiest of entries with a mean survival of 70.3 %, yet was among the lowest for mean yield per plant at 23.2 g across site years. Plot yield, like percent survival, was dependent on environmental conditions and ranged from over 8 t ha\(^{-1}\) to less than 100 kg ha\(^{-1}\).
For both years, the first sowing at CF, on average, yielded less than the second sowing (Table 2.4). This was primarily the result of a higher survival rate for the second sowing. In addition, at CF in 2012-13 there appeared to be more plants that failed to set or only set a few seeds per plant from the first sowing. This was likely related to viral infection the previous autumn, exacerbated by above normal temperatures (>30°C) during flowering.

The yield at the Pullman locations did not respond to different sowing dates, apart from the first sowing at WF in 2011-12, which was likely the result of higher percent survival, rather than more productive plants. The relatively high percent survival at SF in 2012-13 did not improve yield, because plant productivity was extremely low and losses from shattering were appreciable. In general, yield was higher in 2011-12 (4297.1 kg ha\(^{-1}\)) than in 2012-13 (1403.4 kg ha\(^{-1}\)), mainly as a result of higher plant yield, i.e., pod number, and greater percent survival.

Number of branches, height, and pods per plant (Table 2.4) contribute to per plant yield. Taller plants with more branches tended to set more pods than shorter plants with fewer branches. Plants shorter than 50 cm were low yielding and likely stressed due to biotic and/or abiotic factors. Increased branching at CF can be attributed to a longer period of autumn development allowing more auxiliary meristems to develop with less injury to dormant nodes over winter.

Plot yield of individual entries varied across year and location, but not sowing date; however, all 12 sowing date x location mean plot yields were included in estimating yield stability (Fig. 2.2). Four contrasting entries were chosen based on their differential reliability indices. Scout had the highest mean yield across site years (3390.9 kg ha\(^{-1}\)), an average stability
(b_i=1.02), and low VCW_i^2 (682.7), whereas the NPGS Dayton Bulk had the lowest mean yield (2299.1 kg ha^{-1}), apart from the original bulk, with average stability (b_i = 1.05), and high VCW_i^2 (1478.4). Alternatively, 13 Karl/2-3 was the most stable (b_i = 0.75), apart from the original bulk, but had a low mean yield (2484.9 kg ha^{-1}) with a high VCW_i^2 (1315.9). The F_{3:5} population was the most responsive (b_i = 1.33) and had a high mean yield (3146.0 kg ha^{-1}) and VCW_i^2 (1896.9).

Not all entries with above average stability had low yields. Striker, for example, had a mean yield of 3002.3 kg ha^{-1} and was very stable (b_i = 0.85). The majority of lines with a mean yield >3000 kg ha^{-1} were also responsive (b_i >1.0). Wibo/1 and Hiverna/2 had the lowest VCW_i^2 of any entry at 484.8 and 480.2, respectively, showing their yield was highly predictable.

The F_{3:5} population was among the entries with the highest mean yield under favorable conditions, 8070.4 kg ha^{-1} at CF and 4826.7 kg ha^{-1} at WF in 2011-12, but among the lowest at SF, 1252.3 kg ha^{-1} in 2011-12 and 436.7 kg ha^{-1} in 2012-13. This response may be partly explained by the shorter than average height (mean 57.8 cm) of this population, whereas, the European materials all exceeded a mean of 70 cm at maturity. Other contributing factors may have been earlier flowering, pod set, and maturity observed for this population, as compared to European entries. Early maturity may have been advantageous at CF and WF where plants were threshed by hand, but limiting at SF, where a late harvest using a plot combine likely resulted in higher losses than with later and taller materials. Differences in height can be traced back to its pedigree; ‘Extra Precoce Violetto’ has a short height and is an early flowering Mediterranean large seeded type when grown under dryland conditions.

Contrasting phenology may explain differences in yield stability. The responsive F_{3:5} population was consistently early to flower and resumed growth in the spring with some of the
tallest (31.5 cm) and most branched (2.4) plants of any entry at flowering, whereas, the relatively stable 13 Karl/2-3 was among the shortest (24.9 cm) and had fewer branches (2.0) across site years. At maturity, 13 Karl/2-3 was taller (73.2 vs. 57.8 cm), had a higher mean branch number (3.7 vs. 3.1) and pod number (32.0 vs. 22.9) than the F3:5 population.

In addition to general differences in phenology and reproductive traits, yield differences, at least between the F3:5 population and 13 Karl/2-3, can be partly explained by seed size. The F3:5 population had the highest mean seed size of 88.2 g 100 seeds⁻¹, while 13 Karl/2-3 was among the lowest at 56.6 g 100 seeds⁻¹.

NPGS Dayton and Spillman bulks also had above average seed sizes of 75.8 and 73.2 g 100 seeds⁻¹, respectively. Like the F3:5 population, both NPGS bulks, while having relatively high yields at CF and WF, were among the lowest yielding at SF. Yield was generally low across populations and locations in 2012-13, however for these three populations in particular stunted plants, early dehiscence, and below average survival overwinter at SF reduced yield more so than for the other populations including the NPGS CF and WF bulks.

At CF in 2011-12, where survival of the bulk-selected populations all exceeded 80%, seed size was also the largest, except for the WF bulk (58 g 100 seeds⁻¹), which did not vary significantly across site years. Interestingly, the other bulk-selected populations showed a reduction in seed size approaching or exceeding the WF selected bulk when survival was below 70%.

**DISCUSSION**
Winter faba beans have been a mainstay of English agriculture since 1825 (Bond and Crofton, 1999), reportedly introduced as a small seeded Russian bean (Lawes et al., 1983). The crop is relatively unknown in the United States and has received little attention in southeastern Washington, which is a major pea, lentil, and chickpea growing region (Slinkard and Blain, 1988).

‘Côte d’Or’, a French landrace, was cultivated since at least 1812 and was considered to be the hardy to at least -18°C (Bond et al., 1994; Link and Bond, 2011). Older winter cultivars (Banner, Côte d’Or, Webo, and Hiverna) were assessed for freezing tolerance by Herzog (1988) to be -14°C. More recently, Arbaoui et al. (2008) concluded cvs. Hiverna/2, Hiverna, Karl, Bulldog/1, and Gö-Wibo-Pop to be the most consistently winterhardy across 12 European environments. Mwengi (2011) found ‘Gö-Wibo-pop’ to be the hardiest and cvs. Hiverna/2, W6 12024, and W6 12026 to be reasonably hardy for the Palouse region. It would appear that a rhizosphere temperature between -7°C (Mwengi, 2011) and -9°C (Saxena, 1982) determines survival of even the hardiest genotypes, rather than ambient temperature alone, since snow cover is of critical importance when the air temperature exceeds -20°C (Link et al., 2010; Link and Bond, 2011).

The coldest air temperature experienced in our study was -14.5°C, which corresponded to a soil temperature of -3.1°C at seed depth. While winter temperatures were not extreme enough to distinguish between northern European materials, average survival was between 63-73% for the majority of European entries and there was sufficient selection pressure to separate them from the bulk populations. The bulk population’s mean survival (50-65%), however, was improved over their source, the original bulk (30%). This shows the efficacy of natural selection
for improving winter-hardiness. Continued selection for overwintering would be expected to improve these populations even further.

The majority of European entries tested were smaller seeded, later to flower, tended to have fewer branches, and were shorter at flowering, while taller at maturity than the bulk populations. The WF selected bulk did not follow this trend, however, supporting the claim that early spring regrowth and large seeds tend to limit winter-hardiness (Ney and Duc, 1997, Patrick and Stoddard, 2010). This association between hardiness, small seed size, late flowering, and yield may not be complete, however. The F3:5 population, for example, while still segregating for many traits did have the largest mean seed size of all entries tested, was the earliest to flower and mature, overwintered with a mean 70% survival, and had a mean plot yield >3000 kg ha\(^{-1}\). Having yields comparable to the most productive European materials suggests that these traits may have an advantage in our warmer and drier climate as compared to northern Europe. The advantages of these two traits were particularly apparent at CF and WF, where the F3:5 population was among the highest yielding entries. However, at SF, this population performed poorly, possibly due to shattering and height of pod set, since these traits were not under direct selection. Similar conclusions can be drawn about the CF, Dayton, and SF bulk populations. Whether or not adapted genotypes are present in these materials will require further testing and selection.

In addition to dehiscence and distribution of pod set, the large mean seed size of the F3:5 population may limit commercial adoption. Seed size is typically limited to 0.4-0.6 g seed\(^{-1}\) due to market preference and farm equipment restrictions (Lawes et al., 1983). Since this population is genetically diverse, composed of a range of seed sizes, there may be opportunity to select for
genotypes with appropriately sized seed, adequate winter-hardiness, and early spring regrowth. Similar possibilities likely exist for genotypes within the bulk-selected populations.

The two primary selection events overwinter occur at the beginning and end of winter (Link et al. 2010). The first period of selection coincides with the first autumn frosts and depends directly on the development of cold acclimation and extent of frost tolerance. Sufficient seedling development, storage of photosynthetic reserves, and cold acclimation prior to dormancy has been understood for some time to increase winter-hardiness in winter wheat (Janssen, 1929) and winter pea (Etève, 1985). Frost tolerance is a major component of winter-hardiness in faba bean as well (Arbaoui et al., 2008). Herzog (1987) showed the importance of establishment in that frost tolerance increased between the first and second leaf of the faba bean seedling during cold acclimation.

The second abiotic stress period encompasses the many vicissitudes of winter: inter and intracellular ice formation, frost heaving, water logging, wind injury, freeze-drying (inability to absorb water from the frozen soil), and loss of frost tolerance through dehardening (Herzog, 1988) and resumption of growth (Annicchiarico and Iannucci, 2007). Characterization of the cold acclimation period and its association with winter-hardiness has been limited to the accumulation of cryoprotectants and frost tolerance (Link and Bond, 2011), rather than energy reserves (Fukuta and Yukawa, 1999), which when sufficiently stored, would contribute to overwintering as shown in winter wheat (Janssen, 1929) and winter pea (Etève, 1985).

Precipitation in southeastern Washington is normally scarce during late-September and early-October when sowing winter faba bean would be ideal based on daily low air (<8°C) and
soil (<10°C) temperatures (Murray et al., 1988). Without adequate soil moisture, stand establishment is affected, reducing winter-hardiness, and, therefore, yield of faba bean (Herzog and Geisler, 1991) as is the case for winter wheat (Young et al. 1994). The earliest sowings were generally more appropriate than latest sowings for overwintering at the Pullman locations, even though low soil moisture delayed germination. Optimal cold acclimation would be expected if soil moisture supported prompt germination and seedling development (Herzog, 1989).

At the warmer CF location, where soil moisture was optimized for germination, a delayed sowing in mid-October avoided virus pressure on emerging seedlings. Without virus pressure, early-October sowing, when mean air temperature is still above 5°C, results in luxuriant growth susceptible to freezing. Ideally, seedlings going into winter should have two pairs of leaves and a strong root system (Hebblethwaite et al., 1983; Murray et al., 1988), which indicates optimal freezing tolerance and storage reserves.

Sowing date did not have a consistent effect on plot yield except at the CF location, where the second sowing generally out yielded the first, mainly as a result of virus pressure in 2012-13. Pod set was the main determinant of yield per plant as is commonly the case (Lawes, 1978; Chaieb et al., 2011), but advantages associated with early sowing (McEwen et al., 1988; Pilbeam et al., 1990) were inconsistent. We did not observe the increase in branch number in response to an early sowing date as shown by Herzog (1989), likely because of the shorter hardening period.

Seasonal effects on yield were more pronounced than sowing date. The colder winter and warmer summer of 2012-13 in Pullman likely led to a reduction in yield, not completely
attributable to percent survival. A late spring frost during April 2013 coincided with regrowth at the Pullman locations. While this did not reduce percent survival, it is unclear if it had limited yield potential through restricting branch number and height. Frost, once growth has resumed in spring, is just one of the many possible causes for loss of yield potential (Lawes, 1978; Bond et al., 1994). Generally drier and warmer conditions during flowering likely affected productivity. Below average winter temperatures and above average summer temperatures have been observed to reduce the productivity of winter faba bean (Soper, 1952), contributing to its notoriously unreliable yield.

Drought conditions usually limits stem elongation (Dantuma and Grashoff, 1984), biomass (Loss et al., 1997a), and yield (French, 2010), which may explain the general decrease in height and yield across entries observed in 2012-13. Gasim and Link (2007) testing many of the varieties included in this study were over 50 cm taller and generally yields over 4 t ha$^{-1}$, suggesting terminal drought limited the yield potential of our trial.

Shorter determinant spring-type varieties have been found to have more yield stability than indeterminant types under drought stress conditions (De Costa, 1997). Although not determinant, the early flowering and shorter $F_{3.5}$ population showed greater phenotypic stability in height, pod number, and yield per plant than taller northern European entries. Therefore, the combination of northern European winter-hardiness with Mediterranean geneotypes (Link, 1996) adapted (flowering, height, branching, pod number, and maturity) to the terminal drought environments characteristic of the Pacific Northwest would maximize yield potential. The introgression of the terminal inflorescence ($ii$) or stubby ($st$) alleles from the spring-type into
winter-type backgrounds may also improve developmental homeostasis in years with above average drought and heat stress (Bond et al., 1994).

Yield was suspected to be the result of not only an optimal sowing date, but also agronomic (biological vs. machine yield), morphological (number of pods, branches, and height), biotic (insect, pathogen, weed, and virus pressure), and abiotic (sustained temperatures above 28°C and moisture stress) effects and their interactions with the entry (Dantuma and Thompson, 1983; Planquaert and Raphalen, 1984). Further investigation into the agronomic aspects of yield, given a certain level of winter-hardiness, will provide insight into optimizing yield on an area basis, which ultimately depends on the yield and density of individual plants (Hay and Porter, 2006). Increasing sowing density may increase yield through compensation for individual plant losses overwinter (Hebblethwaite et al., 1983). Optimal sowing density and the effects of branch number on pod set per plant, and plot yield will require further investigation. Increasing the seeding rate to 200 kg ha\(^{-1}\) has been shown to increase plant and stem density, as well as height and leaf area, but reduces branch and pod number per plant, resulting in no yield increase (Soper, 1952; 1956; Poulain, 1984; Link et al., 2010). The competitive ability of individuals in and evaluation of populations under low and high-density sowings will indefinitely influence the characterization and selection of the ideal winter faba bean (Mayo, 1980).

Ultimately, a stable faba bean variety for southeastern Washington will need adequate winter-hardiness and productivity (i.e., pod set) under diverse conditions. Sufficient winter-hardiness appears to be present within northern European winter faba bean varieties, as well as in the USDA germplasm for this region. The majority of European entries expressed sufficient winter-hardiness (65%) and yield (3000 kg ha\(^{-1}\)), and were generally higher yielding when
autumn-sown versus spring-sown (Chapter 4). Mean yield was comparable to yields in southwestern Australia (Loss et al., 1997b) and across continental Europe where winter beans are sown (Arbaoui et al. 2008; Ghaouti and Link. 2009), but lower than studies from the UK (Link et al., 2010) and Germany (Gasim and Link, 2007). The yield of winter faba bean may respond to an even precipitation pattern throughout the growing season as experienced in northwestern Europe, indicated by the taller plant heights reported by Gasim and Link (2007) than observed under the low humidity/terminal drought conditions of the Palouse region. Measurement of leaf water potential (Hay and Porter, 2006) will be necessary to validate this claim.

Pure line selections from these open-pollinated composites will be important for increasing yield potential (Lawes et al., 1983). Population buffering by exploiting alleles through interlocus organization and/or intralocus overdominance (Allard and Bradshaw, 1964; Jensen, 1988; Allard, 1996; Link et al., 2010) should be possible within bulk populations (Becker and Léon, 1988), or through single plant selections (Rowland et al., 1982) via mass selection (Matzinger and Wernsman, 1968). However, characterization of prepotent pure lines and creation of synthetic cultivars may be more effective in combining positive alleles and exploiting heterosis (Bond, 1967; Gasim and Link, 2007; Link et al., 2010) than bulk selection. Comparing the response of synthetic and bulk populations to variation in environmental conditions would be important to elucidate the stabilizing effect of either method’s resulting genetic homeostasis.

Characterizing an ideal ecotype, which maximizes yield potential and winter-hardiness across the environments of southeastern Washington, will require more test environments and homogeneous populations than used here in order to minimize the error between locations and years and entry variance, respectively.
With climate change comes a general trend towards a warmer, longer growing season, and erratic precipitation, which may favor winter over spring pulse production (Singh et al., 2010). It would be wise of agriculturalists and plant breeders to invest in resilient crops and cropping systems to sustainably intensify agricultural production (Garnett et al., 2013).
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Washington State University. Pullman, WA.


TABLES AND FIGURES

Table 2.1. List of winter faba bean entries and origins, with percent winter survival for two years at three locations (CF- Central Ferry Farm, WF- Whitlow Farm, and SF- Spillman Farm).

<table>
<thead>
<tr>
<th>Entry name</th>
<th>Origin</th>
<th>Percent Survival 2011-2</th>
<th></th>
<th>Percent Survival 2012-3</th>
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<td></td>
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<td>CF</td>
<td>WF</td>
<td>SF</td>
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<td>WF</td>
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<tr>
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<td>59</td>
<td>60</td>
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<td>66</td>
<td>57</td>
<td>62</td>
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<tr>
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<td>70</td>
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<td>W6 12023</td>
<td>Bulgaria</td>
<td>92</td>
<td>68</td>
<td>59</td>
<td>70</td>
<td>53</td>
</tr>
<tr>
<td>W6 12024</td>
<td>Bulgaria</td>
<td>79</td>
<td>61</td>
<td>45</td>
<td>74</td>
<td>75</td>
</tr>
<tr>
<td>W6 12025</td>
<td>Bulgaria</td>
<td>88</td>
<td>64</td>
<td>57</td>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>W6 12028</td>
<td>Bulgaria</td>
<td>78</td>
<td>61</td>
<td>60</td>
<td>66</td>
<td>63</td>
</tr>
<tr>
<td>Wibo/I</td>
<td>Germany†</td>
<td>90</td>
<td>73</td>
<td>68</td>
<td>59</td>
<td>71</td>
</tr>
<tr>
<td>F3:5</td>
<td>Extra Precoce</td>
<td>88</td>
<td>74</td>
<td>55</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Violetto x Hiverna/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPGS bulk</td>
<td>Selection from</td>
<td>88</td>
<td>65</td>
<td>47</td>
<td>68</td>
<td>58</td>
</tr>
<tr>
<td>Whitlow</td>
<td>Whitlow Farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPGS bulk</td>
<td>Selection from</td>
<td>88</td>
<td>45</td>
<td>45</td>
<td>58</td>
<td>49</td>
</tr>
<tr>
<td>Central Ferry</td>
<td>Central Ferry Farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPGS bulk</td>
<td>Selection from</td>
<td>82</td>
<td>38</td>
<td>34</td>
<td>61</td>
<td>54</td>
</tr>
<tr>
<td>Dayton</td>
<td>Dayton, WA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPGS bulk</td>
<td>Selection from</td>
<td>82</td>
<td>31</td>
<td>33</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>Spillman</td>
<td>Spillman Farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPGS bulk original</td>
<td>460 accessions NPGS bulk unselected</td>
<td>69</td>
<td>9</td>
<td>28</td>
<td>35</td>
<td>9</td>
</tr>
</tbody>
</table>

† Seed obtained from Dr. Wolfgang Link, Georg-August-University.

*se ± 4.5*
Table 2.2. Phenological sampling and final harvest dates for Whitlow Farm (WF), Spillman Farm (SF), and Central Ferry Research Farm (CF) in 2011-12 and 2012-13.

<table>
<thead>
<tr>
<th>Year &amp; location</th>
<th>Sowing dates</th>
<th>Early branching, height, &amp; flowering</th>
<th>Branching &amp; height at maturity</th>
<th>Harvest</th>
<th>Date of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WF</td>
<td>30 Sept./14 Oct.</td>
<td>9 April</td>
<td>5 July</td>
<td>20 July</td>
<td></td>
</tr>
<tr>
<td>2012-13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>28 Sept./12 Oct.</td>
<td>18 April</td>
<td>12 July</td>
<td>19 July</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>5 Oct./19 Oct.</td>
<td>6 June</td>
<td>26 Aug.</td>
<td>16 Sept.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3. Minimum air temperatures at three locations (Central Ferry farm-CF, Whitlow Farm-WF, and Spillman Farm-SF) for two winter seasons (2011-13) and 30 year average minimum air and record low temperatures for Ice Harbor and Pullman, WA (NOAA, 2013). Ice Harbor was chosen for comparison with the Central Ferry Farm because of its geographic and meteorological similarities.

<table>
<thead>
<tr>
<th></th>
<th>30 yr. avg. min</th>
<th>Record low</th>
<th>Minimum temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice Harbor</td>
<td>Pullman</td>
<td>CF</td>
<td>2011-12</td>
</tr>
<tr>
<td>Oct. 5.1</td>
<td>2.3</td>
<td>-8.3 -13.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Nov. 1.6</td>
<td>-0.7</td>
<td>-18.9 -25.6</td>
<td>-1.3</td>
</tr>
<tr>
<td>Dec. -2.3</td>
<td>-4.0</td>
<td>-25.6 -35.6</td>
<td>-5.8</td>
</tr>
<tr>
<td>Jan. -1.9</td>
<td>-2.9</td>
<td>-25.6 -33.9</td>
<td>-5.9</td>
</tr>
<tr>
<td>Feb. -1.3</td>
<td>-2.2</td>
<td>-25.6 -31.1</td>
<td>-3.3</td>
</tr>
<tr>
<td>Mar. 1.8</td>
<td>-0.1</td>
<td>-12.2 -20.0</td>
<td>-3.1</td>
</tr>
<tr>
<td>April 4.9</td>
<td>2.1</td>
<td>-5.6 -10.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Table 2.4. Percent survival, branch number, height, pod number, yield per plant, and plot yield across 20 faba bean populations in 2011-12 and 2012-13 in response to two autumn sowing dates at three locations (CF- Central Ferry Farm, WF- Whitlow Farm, and SF- Spillman Farm). Letters separate significantly different LS-means ($P \leq 0.05$).

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Planting date</th>
<th>Percent survival</th>
<th>Branch number</th>
<th>Height (cm)</th>
<th>Pod number</th>
<th>Per plant yield (g)</th>
<th>Plot yield (kg ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-12</td>
<td>CF</td>
<td>30 Sept.</td>
<td>82.9 A</td>
<td>5.5 A</td>
<td>83.6 B</td>
<td>56.0 A</td>
<td>66.0 A</td>
<td>5961.0 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 Oct.</td>
<td>85.2 A</td>
<td>4.5 B</td>
<td>84.3 B</td>
<td>45.6 B</td>
<td>57.2 B</td>
<td>6618.2 A</td>
</tr>
<tr>
<td>2012-13</td>
<td></td>
<td>28 Sept.</td>
<td>59.1 CDEF</td>
<td>3.3 D</td>
<td>47.1 E</td>
<td>21.4 D</td>
<td>7.7 G</td>
<td>754.1 G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 Oct.</td>
<td>72.1 B</td>
<td>3.9 C</td>
<td>58.8 D</td>
<td>31.0 C</td>
<td>26.0 E</td>
<td>2065.1 EF</td>
</tr>
<tr>
<td>2011-12</td>
<td>WF</td>
<td>29 Sept.</td>
<td>62.0 CD</td>
<td>2.8 E</td>
<td>90.1 A</td>
<td>28.7 C</td>
<td>49.6 C</td>
<td>4518.7 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 Oct.</td>
<td>55.0 EF</td>
<td>2.7 EF</td>
<td>87.9 AB</td>
<td>27.7 C</td>
<td>47.5 C</td>
<td>3714.7 D</td>
</tr>
<tr>
<td>2012-13</td>
<td></td>
<td>25 Sept.</td>
<td>60.6 CDE</td>
<td>2.1 G</td>
<td>64.5 C</td>
<td>17.5 D</td>
<td>15.9 F</td>
<td>1853.8 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 Oct.</td>
<td>55.9 DEF</td>
<td>2.1 G</td>
<td>65.3 C</td>
<td>18.0 D</td>
<td>17.0 F</td>
<td>1964.2 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 Oct.</td>
<td>55.5 DEF</td>
<td>3.4 D</td>
<td>91.0 A</td>
<td>nd</td>
<td>nd</td>
<td>32.6 D</td>
</tr>
<tr>
<td>2011-12</td>
<td>SF</td>
<td>21 Oct.</td>
<td>52.6 F</td>
<td>3.0 DE</td>
<td>83.5 B</td>
<td>nd</td>
<td>29.9 DE</td>
<td>2450.7 E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 Oct.</td>
<td>63.9 C</td>
<td>2.3 FG</td>
<td>51.5 E</td>
<td>nd</td>
<td>7.2 G</td>
<td>880.8 G</td>
</tr>
<tr>
<td>2012-13</td>
<td></td>
<td>19 Oct.</td>
<td>70.8 B</td>
<td>2.2 G</td>
<td>50.5 E</td>
<td>nd</td>
<td>6.6 G</td>
<td>902.2 G</td>
</tr>
</tbody>
</table>

nd= no data
Figure 2.1. Daily low air temperature through the 2011-12 and 2012-13 winter seasons at Central Ferry (CF), Whitlow (WF), and Spillman (SF) Farms.
Figure 2.2. Scatter plot of plot yield for each of 20 faba bean populations by mean plot yield for each of twelve sowing date x location interactions. Regression lines are included for four entries.
CHAPTER THREE

Broadening the genetic base: Bulk selection for improving the winter-hardiness of faba bean (*Vicia faba* L.)

(To be submitted to Plant Breeding)

ABSTRACT

The successful adoption of autumn-sown faba bean in southeastern Washington is contingent upon adequate winter-hardiness, stable yield, and market demand. A lack of market demand has stifled interest by breeders and producers. However, there remains a need for winter-hardy legumes for green manure, cover crop, and specialty markets nationwide. The objectives of this research were to screen the NPGS collection for novel winter-hardy accessions and improve winter-hardiness via a bulk method of selection. Screening the germplasm *en masse* was efficient at increasing winter-hardiness over the original bulk population; although, the origin of selected genotypes is unknown. Winter-hardiness measured as percent survival overwinter increased annually for most populations tested. In general, northern European populations showed slower annual gains in percent survival (<5%) than NPGS bulks (>5%). Natural selection for overwintering reduced the frequency of large seeded genotypes (>80 g 100 seed⁻¹) over time, which is likely associated with delayed vernalization. Further alterations in morphological traits were ambiguous and genotypic variation was likely masked by phenotypic diversity confounded by environmental variation. The outcome of this research is the creation of broad-based germplasm stocks, which have been shown to tolerate up to -25°C when adequately cold acclimated.
The theory of evolution via natural selection is possible based on trait variation among individuals within a population that is associated with genetic fitness (Haldane, 1957; Fisher, 1958; Falconer and Mackay, 1996). If the trait is heritable and variants have a selective advantage, then the frequency of those individual’s alleles should increase over time. Since natural selection is operating on the phenotype, random environmental effects, in addition to available genetic variance (additive, dominance, and epistasis), will determine the heritability, evolutionary change, or selection response of a trait (Cleveland and Soleri, 2007). Cooperation and competition between individuals would also be expected to affect agricultural fitness, and, therefore, the frequency of individual genotypes within a population (Gustafsson, 1953; Mather, 1969; Denison, 2012).

Plant breeders via artificial selection employ the same basic principles as natural selection by altering allelic frequencies through phenotypic and genotypic based selection, but aim to produce a faster genetic gain by manipulating the mating design, population size, and environment (Allard, 1960). Sustained evolutionary change either through artificial or natural selection is ultimately contingent upon sufficient trait associated allelic diversity and the imposed selection intensity. The breeders equation $R = h^2S$, where $R$= response to selection, $h^2$= realized heritability (i.e., narrow sense heritability), and $S$= selection differential, or intensity of selection, functions to estimate genetic gain and predict future gain be estimating the realized heritability of a trait within a population over one generation (Hayes et al., 1955; Falconer and Mackay, 1996).

Winter-hardy faba bean (*Vicia faba* L.) from northern Europe originated from a rather narrow genetic background, which has limited selection gains for overwintering (Link et al., 2011). Cultivars routinely tolerate temperatures between -12 and -15˚C (Herzog, 1989) and ‘Côte d’Or’ has reportedly survived -25˚C with limited snow cover (Picard et al., 1985). Novel
genotypes with improved frost tolerance have been sought as one way of complementing existing alleles (Arbaoui et al., 2008). Link et al. (2011), using a frost-tolerant genotype from China, were able to select for inbred lines with comparable frost tolerance to Côte d’Or, but winter-hardiness results have not yet been published.

To increase winter-hardiness of autumn-sown faba bean, it is necessary to identify unrelated genotypes that increase the additive genetic variance for this quantitative trait. The Western Regional Plant Introduction Station (WRPIS) of the USDA’s National Plant Germplasm System (NPGS) is a readily available source of global faba bean germplasm. Mwengi (2011) identified a number of potential novel sources of winter-hardiness from the NPGS collection. To uncover additional sources, four heterogeneous bulk populations were created by combining 466 NPGS accessions and screening this original unselected bulk at four locations in southeastern Washington (Dayton, WA and Central Ferry, Spillman, and Whitlow Farms) for survival over the 2010-11 winter. The survival rate ranged from less than 1% at the Whitlow farm to >5% at the other locations. These results are comparable to those of Robertson and Saxena (1993) who found that 2.5% of the 5300 ICARDA germplasm accessions expressed frost resistance.

Little is known about the genetic components influencing winter-hardiness of faba bean. Arbaoui et al. (2008) and Link et al. (2010) suggested that more efficient screening for winter-hardiness could be achieved by frost tolerance testing using controlled environments, similar to that shown in pea (Cousin et al., 1993). However, due to the complex nature of winter-hardiness, field-testing remains essential for assessing vernalization and dehardening responses, waterlogging and desiccation tolerances, resistance to abiotic and biotic stresses, and capacity to tiller and yield (Murray et al., 1988; Bond et al., 1994; Link et al., 2010). To test whether natural selection would increase the frequency of winter-hardiness alleles within populations, while
maximizing the allelic variance across divergent populations of faba bean, a bulk method of breeding was applied *in vivo*. Nilsson-Ehle at Svalöf is credited with this method and used it to improve the winter-hardiness of wheat (Newman, 1912).

The selection intensity of natural selection for overwintering was expected to fluctuate in response to the environment and the genetic architecture of the population under selection. Generally, intense selection over winter should result in a rapid response over the first few generations and slow as major genes are fixed and additive variance decreases (Srd and Owen, 1957; Allard, 1960; Murphy et al., 2008). The number of generations it takes for the allele frequency to shift the population mean towards higher survival rates would depend on many factors, including: heritability, population size, outcrossing rate, initial allelic frequency-diversity, selection intensity, linkage, epistasis, assortative mating, environmental variance, maternal effects, and heterosis or dominant gene action (Srd and Owen, 1957; Allard, 1960; Muehlbauer et al., 1988; Falconer and Mackay, 1996). Estimating a reliable response to selection (R) for heterogeneous faba bean populations would be complicated by many of these factors.

Bulk selection is most often applied to breeding populations of self-pollinated species with highly heritable traits (Harlan and Martini, 1929), because selection within outcrossing species is difficult where heterosis masks the genotype. Faba bean is partially allogamous and outcrossing can range from almost complete inbreeding to complete outcrossing (Link and von Kittlitz, 1989; Hu et al., 2011). Link et al. (2010) described both maternal and overdominance effects present in the F1 generation for frost tolerance of faba bean. The response to positive winter-hardiness selection, i.e., adaptation, would ultimately depend on the allelic diversity, multilocus organization, outcrossing rate, and heterozygosity of individuals within the original populations (Allard, 1996).
Random mating within and between populations under the directional selection pressure of winter, would, over generations of recombination and segregation, free up potential variability for natural selection, allowing environmental adaptation (Allard, 1960; Frey and Maldonado, 1967), while maintaining genetic variation (Fisher, 1958; Simmonds, 1962). A working example of this is the Göttingen Winter Bean Population, which is composed of a diverse set of winterhardy populations that has proven winter-hardiness over its components (Gasim et al., 2002; Gasim et al., 2004; Gasim and Link, 2007). The use of heterozygosity and heterogeneity is one way to limit the negative effects of inbreeding and improve the stability of winter-hardiness over time and space (Hayes and Garber, 1919; Allard, 1961; Bond, 1966; 1982; 1987; Lawes et al. 1983; Bond et al., 1994), but makes the breeder’s equation less effective (Duc and Petitjean, 1995).

Bulk selection is either applied in the short-term as a cost effective method to sort segregating populations (Florell, 1929), or in long-term experiments exploiting the role of natural selection in evolution (Phillips and Wolfe, 2005). Evolutionary plant breeding (Suneson, 1956) relies on natural selection to improve adaptation of bulk hybrid populations derived from diverse seed stocks or composites. The major disadvantage is the number of generations (>12) suggested to achieve gains equivalent to pure line selection. However, genetic diversity is maintained at a much lower expense. If agricultural value is associated with survival, propagation en masse can be more efficient than the pedigree method (Allard and Hansche, 1964).

Evolutionary breeding, like bulk selection, is also most often applied to self-pollinated species; however, utilization of a partially allogamous species would allow both recombination and segregation to occur without much human intervention. Generations of selfing (exposing recessive alleles) and outcrossing (breaking linkage blocks) with recurrent natural selection
could concentrate genes that ultimately improve winter-hardiness, but progress would be expectedly slower than with strict inbreeding and manual crossing of complementary genotypes (Lawes et al. 1983; Bond et al., 1985; Muehlbauer et al., 1988). The creation of a bulk faba bean composite or gene pool that is subjected to long-term natural selection over contrasting environments (see dynamic management of Enjalbert et al., 1999) would be a cost effective way to promote adaptation, while maintaining a mass reservoir of genetic diversity for other traits of interest (Mak and Harvey, 1982; Danquah and Barrett, 2002; Duc et al., 2010).

Evaluation of and screening for winter-hardiness following a bulk method of breeding was practiced for three seasons (2011-14) at three locations in southeastern Washington. We expected that there would be significant improvement in overwinter survival across population due to outcrossing, recombination, and natural selection. Further, the NPGS bulk was expected to show a faster response to selection than the W6 accessions as the frequency of advantageous alleles increased due to overwinter selection, while and northern European entries would be slowest due to prior selection. The expected outcome of this research was the creation of dynamic winterhardy faba bean stocks by screening and incorporation of diverse sources of cold tolerance, facilitating future genetic gain.

**MATERIALS AND METHODS**

*Bulk selection of plant material*

An autumn-sown variety trial was conducted over the 2011-12 and 2012-13 seasons. In an effort to improve winter-hardiness and to increase the genetic variation of winter-type faba bean, a bulk method of selection was practiced each season. Seeds harvested from individuals that overwintered at the WF location were combined annually and autumn-sown (864 total seeds
or 48 seeds per 2.7m$^2$ plot) at three trial locations across two planting dates and three replications each season. Throughout the study all populations were cytoplasmically conserved, but were allowed open nuclear gene flow.

Fourteen of the twenty entries were winterhardy northern European cultivars, or breeding lines, and NPGS accessions that were previously identified from research conducted from 2008 to 2011 (Mwengi, 2011). The remaining six were five bulk populations and one HE$_{3:6}$ population derived from the cross ‘Extra Precoce Violett’/‘Hiverna/2’.

The original bulk served as a check entry and was resampled each season from remnant seed. The founder population of the original bulk was 466 accessions from the USDA faba bean germplasm collection, spring-sown on April 15, 2010 at Washington State University’s Whitlow Farm (WF), Pullman, WA (46°44’3.2”N; 117°7’25.8”W) in 1.5 m single row plots and bulk harvested by combine in August.

The NPGS bulk selections were derived from the original bulk after selection for overwintering during the 2010-11 winter at four southeastern Washington locations [WF, Dayton, WA, 46°38’8.1”N; 117°8’58.2”W, Central Ferry Research Farm (CF), Central Ferry, WA, 46°39’5.1”N; 117°45’45.4”W, and Washington State University’s Spillman Agronomy Farm (SF), 46°41’45.1”N; 117°9’2.7”W, Pullman, WA]. WF had the lowest survival (∼1%), whereas the other locations had moderate survival (>5%). Seed from the overwintered plants were harvested in bulk by location and served as source material for the variety trial.

**Estimation of response to bulk selection**

To test the selection gain of bulk selection on NPGS accessions, bulk selections, and northern European breeding lines and cultivars, pre-selected populations (Table 3.1) were compared to post-selections (Table 3.2) in 2013-14, in order to reduce the seasonal error that
affects estimates when comparing across years. The experimental design was a randomized complete block design (RCBD), n=3. Field operations, plot dimension, and plant and row spacing followed that of Chapter 2. Sowing date varied by location: WF 3 Oct., SF 10 Oct., and CF 24 Oct. 2013. All locations were hand harvested.

A total of forty entries were included, however, only fourteen paired comparisons between pre and post-selection were possible. Of these, NPGS accessions were obtained directly from the genebank and northern European materials were sourced from either the CF 2009-10 (no entries survived at WF) or the WF 2010-11 harvests seasons, depending on seed supply. Original seeds were not available. As a result, northern European pre-selected entries had two or three years of natural selection and outcrossing under southeastern Washington conditions, whereas NPGS accessions had a total of six generations of bulk selection.

In addition to NPGS bulk populations, four single plant selections from the SF bulk during the fall of 2011 were included after two seasons of bulk selection at WF (spring-sown in 2012 and autumn-sown in 2012-13). The cultivars, Banner, the most cold-tolerant commercially available cultivar in the Pacific Northwest (Sattell et al., 1998) and Gladice, a white flowered winterhardy cultivar, were included as checks.

Data collection and analysis

To compare the progress of individual populations a simple method to estimate the annual response to selection was used [(mean of trait post-selection – mean of trait pre-selection)/# of selection generations], which in effect is an average annual selection response and provides an estimate of the genetic variation available to improve winter-hardiness in subsequent generations of selection. Due to our focus on natural selection for winter-hardiness, rather than
artificial selection on a specific quantifiable trait, the traditional estimation of a selection
differential and therefore realized heritability was not used.

The following character traits of interest were collected:

- Percent flowering and survival (whole plot) and number of early branches and height
  (five representative plants per plot) were sampled on 5 May at CF and 16 May at SF and
  WF.

- Height of first pod and number of nodes to first pod were sampled from five
  representative plants per plot on 10 June at CF and 20 June at SF and WF.

- Height, branch number, pod number, hilum color, and 100 seed weight were scored from
  five representative plants per plot at harvest on 10 June at CF and 16 Aug. at SF and WF.

- Seed yield (g plant⁻¹) and plot seed yield (kg ha⁻¹).

Percent survival, phenological traits, and yield components were compiled and analyzed
using ANOVA with PROC MIXED (SAS Institute, 2008). Population and location effects were
treated as fixed effects and block was set as a random effect within the model following a
RCBD. Treatment means were comparisoned using Fisher’s Least Significant Difference (LSD)
test at \( P = 0.05 \). Standard deviation \( (s=\sqrt{\sigma^2}) \) and related coefficient of variation \( (100 \times s/\text{mean}) \)
statistics were calculated based on observations of individuals as an estimation of phenotypic
variation (McGill and Lonnquist, 1955; Mak and Harvey, 1982) along with bean plots of
frequency distributions (Kampstra, 2008).

**RESULTS**

The winter of 2013-14 was the coldest experienced since 2009-10 when there was
complete crop loss at WF. Minimum air temperatures (Fig. 3.1) coincided with minimal snow
cover, resulting in extreme lows at a 7.5 cm soil depth of -7.9°C at CF on 8 Dec. and -5.0°C at WF and -4.4°C at SF on 5 Jan. CF, which normally has higher survival than Pullman (Table 3.2), showed an almost complete crop loss. A mean survival of only 3.9% across entries was observed at CF, as compared to 42.0% and 57.6% at WF and SF, respectively.

Across all three locations, comparing post- and pre-selections, Gö-Wibo-Pop (post-selected) was the most winter-hardy entry (50.1% mean survival), as was observed in 2010-11 (Table 3.2). However, due to the low survival at CF and variation across replicate, Gö-Wibo-Pop did not have a significantly higher percent survival than any other northern European entry (Fig. 3.2A).

Selected northern European entries did, however, show enhanced winter-hardiness over their earlier generation counterparts. Across these ten entries there was a 29% increase in winter-hardiness. Some entries showed a greater gain in percent survival than others. Diva, Hiverna/2 and Wibo/1, originally inbred lines, showed the most gain, while Scout and Striker, both originally synthetic populations, lacked significant improvement. Across the four W6 accessions there was an 84% increase in winter-hardiness after bulk selection and were not statistically less hardy than northern European entries. Six selected entries (Gö-Wibo-Pop, Wibo/1, Karl/2-3, Hiverna, Côte d’Or /2-3, and Clipper) had greater survival than Banner.

Bulk selection was the most effective at increasing winter-hardiness among the bulk populations (Fig. 3.2B). The WF bulk, which was exposed to the most intense natural selection pressure, showed the greatest gain in winter-hardiness over the original bulk and exceeded Banner. Single plant selections did not appear to be more winterhardy than their source SF bulk, which was most apparent at the colder WF location. Finally, the HE$_3$6 population had winter-hardiness equivalent to Hiverna/2-5EP1, from which it was derived.
Estimated annual response to selection followed total gain in percent overwintering. The percent survival of northern European populations and W6 accessions increased at a rate below 5%. Côte d’Or /2-3 (4.2%), Diva (4.3%), and W6 12025 (4.0%) showed the highest annual gain. The responses of all bulk populations was above 5% per year.

In an attempt to further characterize the intra- and inter-population diversity, the standard deviation, coefficient of variation, and mean trait values were calculated for morphophysiological characters through the growing season. Due to low survival at CF, this location was omitted from further analyses. Across populations, early season height was the best, albeit weak predictor of flowering ($r = 0.40; P<0.001$). In general, earlier flowering was observed at SF where plants were taller earlier than at WF, likely due to a south facing field. Further, early branching ($r = 0.59; P<0.001$) and possibly early height ($r = 0.37; P<0.001$) may be important components of winter-hardiness, more so than time of flowering, although these relationships are likely genotype and environment specific.

The timing of flowering, early branching and height, and number of nodes and height to first pod did not shift appreciably across the fourteen pre- and post-bulk selection entries, except W6 12023, which shifted to later flowering. There was little separation between population means due to inter-plot variation, although all variables showed significant population.

The majority of northern European entries and W6 accessions showed less than 50% flowering by early May (Fig. 3.3). This was in contrast to the HE$_{3.6}$ population, bulk populations, and single plant selections, which all exceeded 50% flowering. The relationship between early height and branches ($r = 0.39, P<0.001$) is mainly represented by a gradient of entries. Those with early growth tended to have slightly more branches than those with later regrowth, with the majority trending in-between at ~35 cm, but having variable branch numbers (Fig. 3.4).
Furthermore, the early flowering entries tended to have taller plot means than later entries.

Number of nodes to first pod was rather consistent across populations (between six and seven) except for Gladice, W6 12023 (pre-selected), and SF Bulk six and five, which were below five nodes (Fig. 3.5). Height to first pod (30 cm) generally followed node number with these four entries. Standard deviations for mean node number were not distinguishable across entries except for the pre-selected W6 12023, which also had above normal uniform height to first pod. In contrast, the HE3.6 population was one of the most diverse entries for height to first pod.

A general shift in height, branch and pod number, and 100 seed weight at maturity was not apparent between pre- and post-selected entries (Table 3.3). Height was variable across populations with northern European entries (60.6 cm) exceeding bulks (53.6 cm), on average. In general, height as a measure of vigor was lower than in previous years when survival was higher. Branching was also slightly lower than in previous years, however, SF bulk selection five had a mean branch number greater than three and an appreciable standard deviation suggesting above average variability. Pod number, generally a function of branch \((r = 0.62; P<0.0001)\) and height \((r = 0.58; P<0.0001)\), was extremely variable, which limited separation between the majority of entries.

The high standard deviation for pod number between individuals of SF bulk selections five, six, and ten, along with W6 12025 post-selected, was shown as bean plots (Fig. 3.6A) to highlight the extreme variability in pod number and per plant yield these populations had compared to others (Fig. 3.6B).

Another untapped source of variation among winter faba bean is seed size. The northern European lines tested were all less than 62 g 100 seed\(^{-1}\), whereas the largest of the trial was SF bulk selection five at 88 g 100 seed\(^{-1}\). Over time, however, seed size among many of the bulk
populations declined (Fig. 3.7), whereas northern European and W6 accessions remained relatively constant. It would appear that the greatest reduction in seed size was for the source populations with the largest seeds.

Plot yield was higher on average for the post-selected (2.0 t ha\(^{-1}\)) as compared to pre-selected (1.5 t ha\(^{-1}\)) populations. The majority of this yield difference can be explained by percent survival \((r = 0.65; P<0.0001)\) rather than yield components of individual plants, since there was no general shift in single plant yield components through selection. A scatter plot of mean plot yield by CV of plot yield was used to assess individual population stability (Fig. 3.8). Gö-Wibo-Pop post-selected, had both a high mean and stable yield across sites, while post-selections of Clipper and Scout, had similar mean yield but higher CV, indicating more variability across blocks and locations. The WF and SF bulks were also reasonably high yielding with intermediate variability, while the HE\(_{3.6}\) population and W6 12025 accession had appreciable yield instability.

**DISCUSSION**

Bulk selection as used here is slightly different than its conventional use in managing segregating progenies from artificial hybridization (Jensen, 1988). Instead, it followed natural selection within a partially outcrossing species (Cubero, 1993). Our modified bulk selection method is reminiscent of Nilsson-Ehle’s seminal application to improve the winter-hardiness of wheat (Newman, 1912). Since faba bean is partially allogamous, our version may be interpreted as a combination of recurrent and mass selection of composite cross populations. Recurrent selection typically involves cycles of self- and cross-pollination focusing on progeny selection (Muehlbauer et al., 1988), while mass selection is based on open pollination and phenotypic
selection (Matzinger and Wernsman, 1968). Desirable individuals in both cases are selected from populations (positive selection) or culled (negative selection) (Jensen, 1988). Since selection occurs prior to flowering, only successful alleles and allelic combinations are transmitted to the next generation.

Self- and cross-pollination increases recombination and genetic variance, while natural selection tends to reduce genetic variation (Mayo, 1980). The inclusion of multiple pedigrees that were maternally independent with open access to paternal gene flow, genetic drift, potential transgressive segregation, and allelic frequency shift, i.e., microevolution, was our method to improve winter-hardiness (Wright, 1940; Dobzhansky, 1941).

Artificial selection enables the breeder to select for extreme phenotypes, whereas natural selection is often less stringent, as it is intrinsically tied to environmental variance (Jain and Suneson, 1966). With selection for winter-hardiness under field conditions, selection for superior phenotypes would be expected to be dependent upon natural conditions, unless indirect selection for regrowth is also practiced. Therefore, our approach was to rely on a negative culling approach via directional natural selection and outcrossing to maximize recombination and genetic variance, while creating resilient populations adapted to autumn sowing.

Differential survival was observed between pre- and post-selection populations, as well as between the original and selected bulk populations. The response to bulk selection for winter-hardiness was apparent for most entries ranging from an average gain of 0.5% to 10.9% in overwintering per cycle. Mayo (1980) and Cubero (1993) stressed the use of response to selection as descriptive rather than to predict population change. Regardless, natural selection can be a significant evolutionary force, even over short duration breeding cycles (Dawson and...
Goldringer, 2012) and would likely lead to future gains from selection in these faba bean populations.

Single plant selections from the SF bulk population showed less winter-hardiness than their source population. Environmental slippage, as a result of growing these single plant selections in the spring of 2012, rather than overwinter, may have led to this lower performance. The HE3.6 population maintained as high a winter-hardiness as its most tolerant parent, because of continual directional selection pressure. Selection gains from SF bulk single plant selections would be expected to continue with further seasons of autumn-sowing, however, any additional gains in the HE3.6 population would likely be more incremental due to a longer and consistent period of selection.

The 2013-14 winter was similar to the 2008-09 and 2009-10 harsh winters with an extreme low of ~-25°C in Pullman. This is far below the extreme low of -16°C, which caused almost complete loss at Göttingen, Germany (Link, et al., 2010) and matches the temperature reported by Picard et al. (1985). Although these three winters experienced a similar extreme low temperature, survival was likely also dependent upon snow cover, and therefore, soil temperature at the seed (Kinbacher and Jensen, 1959). After the winter of 2009-10 at WF, overwintering was the lower, soil temperature (15 cm soil depth) below -7°C for four days, than in 2008-09 and 2013-14, soil temperature above -5°C. Further evidence of the importance of soil temperature in freezing tolerance is provided by the near complete loss of the CF planting in 2013-14, which experienced a temperature of -7.5°C at seed depth, even though air temperature did not fall below -20°C. This was likely due to a lack of snow cover and a lighter textured soil than in Pullman. While Murray et al. (1988) stated an extreme tolerance of -9°C for faba bean at the rhizosphere, a temperature of -7°C was sufficient to result in almost complete crop loss at CF.
As a result of freezing stress on above and belowground tissues, intercellular ice
formation limits transpiration (Thomashow, 1998) and translocation (Murray et al., 1988) from
the root system, leading to the frost-drought or freeze-drying symptoms described by Herzog
(1989) and Link et al. (2010). The critical period at CF during the beginning of Dec. with soil
temperature below -5°C, air temperature below -10°C, and wind gusts over 30 kph was probably
a leading cause of death to the majority of emerging seedlings. Further, the late planting resulted
in delayed emergence, limiting cold acclimation.

Côte d’Or is known to be the hardiest of entries, followed by Wibo and Hiverna, while
Banner is more prone to freezing stress (Herzog, 1989). Further, Hiverna/2, Karl, and Gö-Wibo-
Pop should also have superior winter-hardiness compared to Diva, Webo, Clipper, and Striker
(Arbaoui et al., 2008). We expected to have less clear distinctions for winter-hardiness between
entries than other studies, because outcrossing was not controlled. However, due to probable
positive allelic interactions and cytoplasmic effects (Etève, 1985) carried over the selection
period, we did observe similar results, although not definitive.

Adequate population size is important to reduce genetic drift and increase the probability
of obtaining desirable recombinants (Haldane, 1957; Cubero, 1993; Enjalbert et al., 1999).
Florell (1929), using a bulk population method for cereal hybrids, suggested 16,000 plants based
on the expression \(4^n\) (Babcock and Clausen, 1918), where \(n\) equals the number of segregating
factors. In this case, seven traits following independent segregation would require upwards of
16,000 plants to obtain all recombinants, highlighting the importance of large population sizes
with bulk breeding. To maintain genetic diversity and limit drift, Eberhart et al. (1967) suggested
that upwards of 1,000 plants should be saved, while Rawlings (1980) figured an effective
population size of 30 is more reasonable. Ultimately, there is a trade-off between short-term gain
and long-term response (Mayo, 1980). To conserve genetic potential one must maintain a large reservoir of genetic variance, where elite selections may then be derived from (Muehlbauer et al. 1988).

The use of hand harvesting limited plot size to 48 plants per plot and restricted the effective population size to less than 144 individuals depending on percent overwintering. The relatively small population size likely reduced potential genetic variance, increasing genetic drift (Mayo, 1980; Arterbrun et al., 2012). Larger plot size and access to a combine would allow full implementation of this method, in order to conserve a dynamic gene pool (Allard and Hansche, 1964; Simmonds, 1993) and exploit favorable intra- and inter-locus interactions (Allard, 1990) within future populations. Alternatively, selection from these early generation bulk populations under various local conditions could potentially increase genetic diversity overall (Wright, 1931; Ceccarelli, 1996; Dawson and Goldringer, 2012).

It is common across adaptation studies to have desirable or positive changes in evolutionary fitness, yet undesirable negative changes in agricultural or end-use traits like seed quality (Dawson and Goldringer, 2012; Denison, 2012) and fecundity, i.e., agricultural fitness (Jain and Suneson, 1966). Indirect shifts in traits of the base population were limited in this study. We found that the recessive clear hilum trait associated with low vicine-convicine (Crépon et al., 2010) present in some northern European lines decreased, while in others it increased to varying extents over time. This may be explained by consistent inbreeding of the original inbred lines, assortative mating, or outcrossing and subsequent segregation for this recessive trait.

Bond and Pope (1987) studied outcrossing over seasons within a population of faba bean and found relatively stable (~45%) dark hilum progeny from clear hilum seed. If these dark hilum individuals express heterotic autofertility (Drayner, 1956, 1959), then upon inbreeding
clear and dark hilum individuals, traits should segregate, theoretically maintaining ~25% clear hilum within the population at equilibrium (Wright, 1977; Bond and Pope, 1987). One method of increasing the frequency of recessive quality traits in the future would be through clustering in the form of selected bulks (Florell, 1929).

True winter types (winterhardy types from northern Europe) are small seeded (var. equine and minor) and are more tolerant to freezing and show some vernalization requirement for early-node flowering (i.e., late flowering) as compared to overwintering broad bean (var. major) (Saxena, 1982; Ney and Duc, 1997; Link and Bond, 2011). Over time populations with a mean 100 seed size >80 g, generally classified as var. major, decreased to < 80 g 100 seed⁻¹. Interestingly, SF bulk five had the largest seeds on average (>80 g 100 seed⁻¹) and was one of the earliest populations to flower, however the winter survival was comparable to smaller seeded and later flowering stocks.

Bulk populations were, in general, earlier flowering than northern European populations. The association between flowering and winter-hardiness was clear for W6 12023, which showed a substantial increase in vernalization and winter-hardiness. Yet other W6 accessions, with comparable increases in winter-hardiness, lacked alteration in flowering over the selection period. While there is likely an association of winter-hardiness with late flowering, they appear to be under separate mechanisms of genetic control (Lawes, 1983).

Early flowering genotypes with vigorous regrowth may be advantageous in southeastern Washington where terminal drought and temperature stress limit yield, yet late spring frost can result in plant loss and flower injury (Stoddard, 1986). A trade-off between early flowering and pod set is also a concern. The height of the first pod should be at least 20 cm high if machine harvested (Lawes, 1983). Some of the observations, mainly from bulk populations were below
20 cm with means trending higher. Areas of the plots with high plant density tended to have
taller first pods than less dense areas.

A need for early flowering and maturing genotypes is most apparent when mature height
and pod set are reduced in water limiting years (Duzdemir and Ece, 2011). Taller northern
European types were especially susceptible to the terminal drought conditions of southeastern
Washington, limiting yield potential. Measurements of harvest index under varying levels of
water stress would identify genotypes adapted to terminal drought conditions (Jain and
Kulshrestha, 1976).

Wrinkled indehiscent pods are more common from genotypes of arid origin (Lawes,
1983). A wide diversity of pod shape, size, and type were observed including wrinkled
indehiscent types, which were prevalent in bulk populations. Directed hybridization between
north European x Mediterranean winter-types may combine winter-hardiness and morphological
traits (EUFABA, 2004) adapted to southeastern Washington conditions, as has been observed
within the HE$_{3:6}$ population. Further characterization of the correlation between flowering and
maturity, with an extended reproductive growth phase before late season stresses appear, should
be sought as the ideal ideotype.

Some work has addressed heterotic germplasm pools of spring faba beans (Link, 1995;
Link et al., 1996), but heterotic groups of true winter faba beans are mainly restricted to minor
and equina germplasm from northern Europe. Access to germplasm from milder winter climates
like southern Australia (Gnanasambandam et al., 2012) and Europe (Schill et al., 1995; Bozzini
and Chiaretti, 1997) could exploit heterosis. Combinations of unrelated, but adapted, germplasm
could lead to overdominance of multiple productivity traits (Ghaderi et al., 1984; Schill et al.,
1995) and improve breeding values as synthetic components (Link, 2012).
Variance is an indicator of genetic diversity and is what natural selection acts upon during adaptation (Fisher, 1958). However, numerous reports have noted the difficulty of increasing winter-hardiness, yield, seed size, vigor, and plant height due to overdominance (Gasim and Link, 2007; Link et al., 1994; Stelling et al., 1994). It is common upon inbreeding hybrids exhibiting heterosis that the variance of these traits increases, while the mean decreases. The original single plant selections from the SF bulk populations were likely hybrids exhibiting overdominance for vigor and yield. Original selections all had seed yields greater than 50 g per plant, whereas the F₃ generation means were all less than 50 g per plant. However, the above average standard deviations for single plant yields indicated variation with some individuals yielding over 200 g per plant. This transgressive increase in variability may be explained by the heterozygosity of individuals within the populations.

Jensen (1988) stressed that for a breeding population, between-line variance was more important than within-line variance. This concept is applicable to both bulk populations and pedigree families. Heterozygosity assumed, single plant selections from bulk populations with desirable traits may be useful for short-term improvement via pedigree selection (Bakheit and Metwali, 2011; Yassien et al., 2012), as well as, long-term pre-breeding strategies based on intravarietal diversity and in situ conservation of biodiversity. These bulk mass-reservoir or broad-based genetic germplasm stocks are conducive to on-farm conservation and participatory breeding (Ghaouti et al., 2008; Ghaouti and Link, 2009; Duc et al., 2010), dual population, or supplemental breeding strategies (Muehlbauer et al. 1988), and evolutionary breeding (Jensen, 1988) across diverse environmental contexts.

Estimating within-family variability by calculating the standard deviation and frequency distributions (Jain and Suneson, 1966) of individual plant height, seed size, pod and branch
number, and yield highlighted the potential for individual plant selections from families. Estimation of between-family differences, based on means, was made difficult by this extreme variability for most traits due to a decay of discontinuous variability, via gene flow between populations (Dobzhansky, 1941). The use of the coefficient of variation statistic (Bowley, 1999) as a normalized measure of population variability in response to the environment allowed for the comparison of stability between families for plot yield. From these data Gö-Wibo-Pop, Clipper, and Scout should be studied further. These three lines were relatively high yielding, which is likely a function of winter-hardiness, as well as, early season vigor. Furthermore, they are derived from selected synthetic populations, which may explain their stable performance (Bond, 1982; Link et al., 1996).

One of the major concerns when using a bulk method of breeding is whether yield and competition are correlated (Mak and Harvey, 1982). Often individuals in a mixture exposed to natural selection will be highly competitive, but not necessarily high yielding in a pure stand. However, if yield is associated with adaptation (i.e., winter-hardiness, drought resistance, and photoperiod sensitivity), then selected individuals will tend to outperform the unselected individuals (Jennings and Aquino, 1968). Examining the effects of competition or cooperation on yield (Gustafsson, 1953) will help to inform future studies of an ideal planting density to optimize heritability and genetic gain from selection.

Following Jensen’s (1988) method of exploding, it may be useful to make selections under both spaced and crop density conditions to maximize the range in selection matrices, while distinguishing developmentally homeostatic, competitive, and productive lines. Selections from different densities may then be recombined, following reciprocal recurrent selection, into a synthetic exhibiting heterosis under the variable conditions of commercial production (Ferwarda,
Commercial sowing density based on average winter conditions would likely be at least 200 kg ha\(^{-1}\) (Poulain, 1984), double that used herein depending on the population, further increasing seed costs.

**Summary**

Sufficient directional selection for ability to overwinter has created diverse faba bean populations amenable to other improvement strategies by public and private stakeholders. The cryptic effects of heterosis and its nonadditivity are likely a component of the observed improvement in winter-hardiness and extreme variance for some morphological traits attributable to certain populations (Bond, 1966; Jensen, 1988; Link et al., 1994; Stelling et al. 1994).

Populations derived from NPGS bulked accessions were clearly morphologically diverse, with individuals expressing novel flowering, pod, and seed traits that may fulfill future breeding objectives. These bulk derived populations did not, however, outperform proven entries from northern Europe based on mean percent survival and yield traits, suggesting the need for further improvement via selection.

Northern European populations showed less overall gain from selection for winter-hardiness than NPGS bulks or W6 accessions. Substantial short-term gains are often the result of simple selection for individuals possessing favorable genotypes (Rawlings, 1980). Additional cycles of truncation selection would be expected to lead to further population divergence via fixation of favorable alleles and allelic combinations (Mayo, 1980). Further gains in winter-hardiness of northern European populations would be expected to continue at a slow rate, while NPGS bulks and W6 accessions may still see appreciable advances in the short-term via bulk selection.
Overall, the faba bean populations assembled via bulk selection would be conducive to participatory variety development, because they possess a wide range of phenotypic diversity that is clearly observed (Myers et al., 2012), and much genetic variation is expected to remain. Link and Ghaouti (2012) identified seed size, clear hilum, and a clean and light seed color as the main market trait requirements for the Egyptian export market. These would likely be similar to requirements of a domestic U.S. market. Trade-offs between seed size and winter-hardiness will have to be addressed, as well as, regrowth vigor and early maturity if faba bean is to be a successfully autumn-sown pulse crop in southeastern Washington. The requirements for a successful winter green manure or cover crop are primarily winter survival and abundant spring regrowth. Distributing these populations nationally and making comparisons with other winter annual legumes remains necessary for assessing the potential of winter faba bean.
REFERENCES


TABLES AND FIGURES

Table 3.1. Pre-selected populations and their sourcing year and location [Central Ferry (CF) and Whitlow (WF) farms].

<table>
<thead>
<tr>
<th>Population</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPGS Bulk original</td>
<td>2010 WF 466 accessions</td>
</tr>
<tr>
<td>13 Karl/2-3</td>
<td>2009-10 CF harvest</td>
</tr>
<tr>
<td>Clipper</td>
<td>2009-10 CF harvest</td>
</tr>
<tr>
<td>Côte d’Or /2-3</td>
<td>2010-11 WF harvest</td>
</tr>
<tr>
<td>13 Diva</td>
<td>2009-10 CF harvest</td>
</tr>
<tr>
<td>Wibo/1</td>
<td>2009-10 CF harvest</td>
</tr>
<tr>
<td>Hiverna</td>
<td>2009-10 CF harvest</td>
</tr>
<tr>
<td>Hiverna/2-5EP1</td>
<td>2009-10 CF harvest</td>
</tr>
<tr>
<td>Gö-Wibo-Pop</td>
<td>2009-10 CF harvest</td>
</tr>
<tr>
<td>Striker</td>
<td>2010-11 WF harvest</td>
</tr>
<tr>
<td>Scout</td>
<td>2010-11 WF harvest</td>
</tr>
<tr>
<td>W612023, W612024, W612025, W612028</td>
<td>NPGS accessions</td>
</tr>
</tbody>
</table>
Table 3.2. Post-selected populations and their country of origin, original population structure, seed hilum color, percent survival, and individual plant yield (g plant\(^{-1}\)) at the Whitlow (WF) and Central Ferry (CF) research farms in 2010-11.

<table>
<thead>
<tr>
<th>Population</th>
<th>Origin/population structure/seed hilum color</th>
<th>% survival WF/CF</th>
<th>Yield WF/CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 Diva</td>
<td>Germany/inbred line/clear</td>
<td>45/76</td>
<td>88/70</td>
</tr>
<tr>
<td>13 Karl/2-3</td>
<td>France/inbred line/black</td>
<td>73/90</td>
<td>57/68</td>
</tr>
<tr>
<td>Clipper</td>
<td>UK/population/mix</td>
<td>40/62</td>
<td>87/92</td>
</tr>
<tr>
<td>Côte d’Or /2-3</td>
<td>France/inbred line/black</td>
<td>20/77</td>
<td>33/64</td>
</tr>
<tr>
<td>Gö-Wibo-Pop</td>
<td>Germany/population/mix</td>
<td>90/92</td>
<td>73/69</td>
</tr>
<tr>
<td>Hiverna</td>
<td>Germany/population/black</td>
<td>62/82</td>
<td>61/84</td>
</tr>
<tr>
<td>Hiverna/2-5EP1</td>
<td>Germany/inbred line/black</td>
<td>55/84</td>
<td>77/69</td>
</tr>
<tr>
<td>Scout</td>
<td>Germany/selection from Gö-Wibo-Pop/black</td>
<td>83/85</td>
<td>72/72</td>
</tr>
<tr>
<td>Striker</td>
<td>UK/population/mix</td>
<td>20/73</td>
<td>72/76</td>
</tr>
<tr>
<td>W6 12023</td>
<td>Bulgaria/accession</td>
<td>37/72</td>
<td>69/84</td>
</tr>
<tr>
<td>W6 12024</td>
<td>Bulgaria/accession</td>
<td>37/77</td>
<td>126/63</td>
</tr>
<tr>
<td>W6 12025</td>
<td>Bulgaria/accession</td>
<td>68/80</td>
<td>78/71</td>
</tr>
<tr>
<td>W6 12028 ‘Webo’</td>
<td>Bulgaria/accession</td>
<td>27/77</td>
<td>79/67</td>
</tr>
<tr>
<td>Wibo/1</td>
<td>Germany/inbred line/clear</td>
<td>67/83</td>
<td>78/67</td>
</tr>
<tr>
<td>HE(_3)</td>
<td>Hiverna/2 x Extra Precoce Violettoblack</td>
<td>27/50</td>
<td>42/82</td>
</tr>
<tr>
<td>WF, CF, Dayton, Spillman bulks</td>
<td>NPGS Bulk selected populations</td>
<td>nd.</td>
<td>nd.</td>
</tr>
<tr>
<td>Spillman Farm bulk #2, 5, 6, and 10</td>
<td>Single plant selection 2010-11</td>
<td>nd.</td>
<td>nd.</td>
</tr>
</tbody>
</table>

*nd= no data*
Table 3.3. Mean (±s) height, branch number, pod number, and 100 seed weight at maturity across two locations. The standard deviation was calculated for each population based on five representative plants per plot.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Height (cm)</th>
<th>Branch s</th>
<th>Pod s</th>
<th>100 seed weight (g)</th>
<th>100 seed weight s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clipper</td>
<td>62.7</td>
<td>6.4</td>
<td>2.4</td>
<td>18.3</td>
<td>6.8</td>
</tr>
<tr>
<td>Clipper *</td>
<td>60.1</td>
<td>8.4</td>
<td>1.9</td>
<td>16.5</td>
<td>8.1</td>
</tr>
<tr>
<td>Côte d’Or /2-3</td>
<td>56.3</td>
<td>9.9</td>
<td>1.8</td>
<td>19.4</td>
<td>7.0</td>
</tr>
<tr>
<td>Côte d’Or /2-3 *</td>
<td>67.8</td>
<td>8.5</td>
<td>1.8</td>
<td>21.4</td>
<td>9.8</td>
</tr>
<tr>
<td>13 Diva</td>
<td>58.0</td>
<td>7.5</td>
<td>2.2</td>
<td>21.0</td>
<td>10.2</td>
</tr>
<tr>
<td>13 Diva *</td>
<td>57.6</td>
<td>10.5</td>
<td>2.4</td>
<td>20.1</td>
<td>9.4</td>
</tr>
<tr>
<td>Gö-Wibo-Pop</td>
<td>64.2</td>
<td>8.3</td>
<td>1.9</td>
<td>16.9</td>
<td>8.1</td>
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*= Pre-selected population
Figure 3.1. Daily low air temperatures (°C) through the 2013-14 winter season at Central Ferry (CF), Whitlow (WF), and Spillman (SF) Farms.
Figure 3.2. Mean (±se) percent survival over the winter of 2013-14 across three locations (Whitlow, Central Ferry, and Spillman Farms); A) pre- and post-selection and B) NPGS bulk populations.
Figure 3.3. Mean (±se) percent flowering of pre- and post-selected entries. The remaining 12 populations are separated for comparison. The number of flowering plants per plot was scored on 16 May for both Whitlow and Spillman Farm locations.
Figure 3.4. Scatter plot of mean branch number by height on 16 May across two locations (Whitlow and Spillman Farms). Pre-selection entries are denoted with sourced year or NS (not selected) in parentheses.
Figure 3.5. Mean (±s) node and height to first pod across two locations (Whitlow and Spillman Farms). Pre-selected entries are denoted with an asterisk. Node and height to first pod standard deviation for each population were based on five representative plants per plot.
Figure 3.6A. Bean plots of pod number and single plant yield from five individuals per plot across two locations (Whitlow and Spillman Farms). B) The comparison of pre- and post-selection single plant yield frequency distributions. Dotted line represents the grand mean and each solid black line corresponds to the population mean.
Figure 3.7. Mean 100 seed weight over three or four seasons of bulk selection.
Figure 3.8. Scatter plot of mean plot yield by its coefficient of variability (CV) across two locations (Whitlow and Spillman Farms). Pre-selected populations are denoted with an asterisk.
CHAPTER FOUR

Agronomic performance of spring-sown faba bean (*Vicia faba* L.) in southeastern Washington

(Accepted to Agronomy Journal)

ABSTRACT

Faba bean (*Vicia faba* L.) is the world’s fourth most important cool-season pulse crop. Currently commercial faba bean cultivars are unavailable in the United States specifically selected for grain production; therefore, a spring-sown variety trial with eleven populations was conducted for two seasons at two contrasting locations in southeastern Washington. Early seeding was expected to result in optimal grain yield. In 2012, the warmer Central Ferry location was sown a month earlier than at Pullman (April vs. May), resulting in earlier flowering, higher grain yield, and an earlier maturity across populations. A drier March-April in 2013 allowed similar sowing dates across locations, synchronizing phenological development and improving grain yields at Pullman. However, at Central Ferry, yield was reduced due to high temperatures (>25 °C) and subsequent flower and pod shedding. The NPGS accession W6 12023 and the ‘Extra Precoce Violettö’ x ‘Hiverna/2’ F$_3$:5 breeding population were the highest yielding populations and earliest to flower and mature in 2012 and 2013, respectively. Across four site years, W6 12023 proved to have superior general adaptability ($b_1 = 1.16$) with high mean plant yield (22.6 g plant$^{-1}$). Yield data compared favorably to other spring sown pulses currently grown in the region; therefore, a multi-environment trial with earlier maturing spring-type cultivars is warranted.
The agroecosystem benefits of cool season pulses in rotation with cereals supports agronomic sustainability via improvement of cereal yield through residual nitrogen, residue management, weed control, and by providing a disease break (Veseth, 1990). Other benefits of pulse integration into a cereal dominated cropping system include: increased microbial diversity, soil sanitation, improvement of soil structure, and soil moisture retention (Jensen et al., 2010).

Faba bean could support the sustainability of cereal-cool season pulse cropping systems in the United States, as shown in Europe and Australia (Champ, 2001; Kopke and Nemecek, 2010). Most studies have supported the benefits of biological nitrogen fixation and residual nitrogen to the following cereal (McEwen et al., 1990). Herridge et al. (1994) showed faba bean to have one of the highest N₂ fixing capacities when compared to other cool season pulses. Although the extent of this capacity has not been conclusively determined in the inland Pacific Northwest, a positive nitrogen economy would be expected based on studies throughout the Northern Great Plains (Walley et al., 2007). Based on available literature, Jensen et al. (2010) estimated that the global average nitrogen fixation of faba bean was 153 kg shoot N ha⁻¹ was higher than average lentil (Lens culinaris Medik.) (71 kg shoot N ha⁻¹), pea (Pisum sativum L.) (86 kg shoot N ha⁻¹), or chickpea (Cicer arietinum L.) (86 kg shoot N ha⁻¹).

Thomas et al. (2012) examined different crop rotations that would improve the sustainability of dryland grain production in Australia and found that even though faba bean provided agroecological services, chickpea was economically advantageous providing a higher gross margin of return. According to Pulse Australia, 2013 prices for faba bean and chickpea were similar. However, it is uncertain if faba bean production in southeastern Washington would have an agronomic or economic competitive advantage compared with countries, such as
Australia, Canada, France, or the United Kingdom, that export faba bean (FAOSTAT, 2011). There are limited markets for human consumption of faba bean in the United States; however a regional source of protein rich animal feed could supplement feed pea and mustard seed/canola oil cake.

The purpose of this research was to evaluate spring-sown faba bean across two contrasting environments in southeastern Washington. Assessment of phenological development and grain yield would identify key areas of additional research necessary for the development of this crop to satisfy regional economic and agroecological sustainability.

MATERIALS AND METHODS

A spring-sown variety trial with ten European breeding lines, cultivars, and USDA accessions (Table 4.1) was conducted for two seasons (2012 & 2013) across two locations (Central Ferry (CF) Research Farm in Central Ferry, WA, 46°39’5.1’’N lat; 117°45’45.4’’W long; Washington State University’s Whitlow Farm (WF) in Pullman, WA, 46°44’3.2’’N lat; 117°7’25.8’’W long) following a randomized complete block (RCB) experimental; n=3. Treflan (Trifluralin; Dow Chemical) was applied to control monocot weeds and Thio-Sul (NH₄)₂S₂O₃; Texas Sulfur Co.) to reduce bird damage to seedlings post-plant pre-emergent. Warrior (Lambda-cyhalothrin; Syngenta) was used to control pea leaf weevil (Sitona lineatus L.) as necessary. Field sites characterized extremes in elevation, moisture, and temperature (Table 4.2) of southeastern Washington, as well as field management. The CF location has a Chard silt loam soil and is characterized as low elevation (198 m), warmer temperature, less rainfall, and is irrigated with subsurface drip irrigation (10 min d⁻¹), while WF has a Palouse-Thatuna silt loam,
is higher elevation (790 m), and dryland managed. The 2013 season, at both locations, was cooler and drier in April than in 2012, but warmer for the remainder of the year. The weather in 2012 was very similar to the 30 yr average; however, 2013 temperatures during the main growing season were above average at both locations.

Plot dimension varied by location due to irrigation constraints at CF, but area was 2.7 m$^2$. Plots at WF had four rows, while CF had two. Rows were spaced ~35 cm apart at each location. Plot dimensions were 1.5 x 1.8 m at WF and 0.75 x 3.6 m at CF, with a 40 cm space separating plots within blocks at both locations. A Hege 120 planter was set for 48 seeds per plot, equivalent to 80-120 kg of seed ha$^{-1}$ depending on seed size. Seeding date varied according to prevailing weather. For 2012, sowing was 4 April at CF and 7 May at WF, whereas in 2013, sowing was 26 March at CF and 3 April at WF. Furthermore, in 2013, ‘Striker’, one of the lowest yielding and latest flowering 2012 populations, was replaced with a F$_3$:5 population derived from a cross between cvs. Extra Precoce Violetto and Hiverna/2 (spring-type x winter-type) in an attempt to distinguish late flowering populations from early.

Phenological development was partitioned to characterize vegetative and generative stages. Percent flowering and early branching and height measurements were taken when plants started to bloom (2012: 16 July at WF and 11 June at CF; 2013: 10 June at WF and 7 June at CF), late branching and height were measured prior to leaf shedding (2012: 19 Sep. at WF and 17 July at CF; 2013: 26 Aug. at WF and 10 July at CF), and harvest was between the 409 and 410 maturity stage (Knott, 1990). Number of branches and height measurements were based on 20 representative plants from each plot. The percentage of flowering plants was based on plot stand counts averaged across blocks and locations. Plot yield was converted to kg ha$^{-1}$ and g
plant\textsuperscript{1} was based on stand counts at harvest. Yield adaptability (b\textsubscript{i}), based on g plant\textsuperscript{1}, was estimated according to Finlay and Wilkinson (1963) as the regression coefficient of an individual population on the mean site year yield across testing locations. A further stability identifier ‘ecovalence’ (W\textsubscript{i}\textsuperscript{2}) was computed and transformed into a variation coefficient (VCW\textsubscript{i}\textsuperscript{2}) according to Stelling et al. (1994) to weight variation according to yield.

Phenological characteristics and yield components least squares means (LS-means) and Pearson correlation coefficients were compiled and analyzed using ANOVA with PROC MIXED and PROC CORR (SAS Institute, 2008), respectively. Population, location, and year were treated as fixed effects and block was set as a random effect within the model following an RCB experimental design. Treatment mean comparisons were assessed based on Fisher’s Least Significant Differences (LSD) test at $P = 0.05$.

RESULTS

There was a significant year $\times$ location effect for all dependent variables; therefore, data were analyzed separately by year. Both Striker and the F\textsubscript{3:5} populations were removed prior to the combined analysis and were only included within their respective year of cultivation.

The date of transition from vegetative to generative phase was later at WF in 2012, likely due to a later seeding date than in 2013. An earlier seeding date for WF in 2013 improved the alignment of flowering between the two locations. The W6 12023 accession was consistently early flowering across seasons and locations and was comparable to the F\textsubscript{3:5} population in 2013 (Table 4.1).
There was no clear difference between populations or locations for branching (2.0) at flowering in 2012; however, in 2013 there was an unexpected disparity between locations with populations generally having a higher branch number at CF (2.8) than at WF (1.1) (Table 4.3). The earlier seeding at WF in 2013 resulted in shorter plants with fewer branches at flowering than in 2012 suggesting that the timing of sowing affects early development.

Branching and height at flowering did not necessarily predict height and branching at maturity. Correlations between early and late branch number were consistently positive for each year (2012: \( r = 0.59 \); 2013: \( r = 0.87, P<0.0001 \)), whereas early and late height was positive in 2012 (\( r = 0.32; P=0.02 \)) and negative in 2013 (\( r = -0.80, P<0.0001 \)), likely as a result of environmental stress at CF in 2014. Both branching and height at maturity were higher across populations in 2012 than in 2013; however, across site years plant height was severely stunted at CF in 2013 (Table 4.3).

Populations were very similar in their height and numbers of branches when averaged across locations within each year (Fig. 4.1). Since the W6 12023 accession was consistently early flowering it may be informative to compare this population against the others tested. In 2012, W6 12023 was the tallest population at flowering, but shortest at harvest, across location. In 2013, W6 12023 did follow this same trend, but was not as strongly observed as in 2012, because of environmental stress at CF in 2013. The F_{3:5} population, also early flowering in 2013, was, however, the tallest at flowering and among the shortest at harvest.

Grain yield components, i.e., plant and plot yield, were also inconsistent across locations and growing seasons (Table 4.3). There was a severe incidence of pea enation mosaic virus
(PEMV) at WF in 2012 that decreased plot yield as compared to 2013. Heavily infected barren plants were culled prior to harvest at an average of 16% across populations. Yield per plant was higher across year at WF than at CF, but because of the high incidence of virus at WF in 2012, plot yield did not follow this same trend.

There were only differences among the highest and lowest yielding populations in 2012 and 2013 (Fig. 4.2). Côte d’Or/2-3 and W6 12023 had the lowest and highest plot yields in 2012, respectively. However, poor production across populations in 2013 at CF reduced the resolution of differences between populations. Other than the reduction in plant height and yield at CF in 2013 mean 100 seed weight was significantly lower (43.3 g 100 seed\(^{-1}\)) than across the other site years (53.8 g 100 seed\(^{-1}\)), indicating severe stress during seed production.

The significant location \(\times\) population interaction for per plant yield may help to resolve discrepancies observed at the whole plot level (Fig. 4.3). Per plant yield of each population was plotted against site means (average per plant yield across populations) and regression coefficients \((b_i)\) and coefficient of variation or ecovalence \((VCW_i^2)\) were calculated. Similar to plot yield, Côte d’Or/2-3 and W6 12023 had the smallest and greatest mean per plant yield across location and year (data not shown). Côte d’Or/2-3 had a \(b_i\) of 0.55 and a \(VCW_i^2\) of 31.8, while W6 12023 had a \(b_i\) of 1.16 and a \(VCW_i^2\) of 13.8. These data indicate that W6 12023 was more responsive or dynamically stable than Côte d’Or/2-3 over the growing environments tested, possibly through avoiding of heat stress.

The \(F_{3:5}\) population had the greatest per plant and plot yields in 2013. Further, the yield per plant was above average for stability with a \(b_i\) of 0.52. Therefore, the \(F_{3:5}\) population fit the
static concept of phenotypic stability and also appeared to avoid heat stress through early maturity. Since the F$_3$:5 was only tested in 2013, it would be important to confirm how this population is adapted to seasonally high and low yielding environments.

**DISCUSSION**

Faba bean has been neglected by researchers in the United States due to yield instability and market saturation from other cool season pulses (D.F. Bezdicek, personal communication, 2012). Our data has confirmed the environmental yield instability facing faba bean production; however, collaboration between developed breeding programs in Europe, Australia, and Canada with USDA pulse breeders and germplasm could improve the regional visibility of the world’s fourth most important food legume (FAOSTAT, 2012). Stability trials in Europe have suggested that wide adaptation can be bred for once stable genotypes are identified (Link et al., 1996). The presented research suggests selection for early flowering and more determinant maturity are two traits that would improve the stability of yield, i.e. adaptation, of spring-sown faba bean in southeastern Washington.

Mean plot yield of populations across site years ranged from 1257.2 to 2897.7 kg ha$^{-1}$, or an average across populations of about 2000 kg ha$^{-1}$, comparing favorably with a faba bean variety trial in Oregon (James et al., 1984), the average yield of chickpea in Washington state of 1,800 kg ha$^{-1}$ (NASS, 2012), and the global average yield of faba bean at just over 1,600 kg ha$^{-1}$ (FAOSTAT, 2012). Yet there was no differentiation between yields of individual populations, except between W6 12023 and the F$_3$:5 population, which were both notably earlier flowering and maturing than the other populations tested. W6 12023 had the highest mean per plant yield.
across site years with one of the lowest VCWIi2, while as a comparison, Côte d'Or/2-3 was the lowest yielding and had the highest VCWIi2. Additionally, in 2013, the F3:5 population had an above average stability (low bi) coupled with the greatest mean yield across locations. Therefore, both W6 12023 and the F3:5 population would be candidates for future testing.

Key issues for successfully spring seeding faba bean include identification of adapted cultivars, optimizing seeding date, and management of pests and weeds. Based on the comparisons between the winter-type populations tested here and adjacent observational plots with more spring-type materials, faba bean has an extensive variation for timing of flowering and maturity. Comparing the winter faba bean ‘Webo’ to other spring faba beans, Herzog (1989) did not observe a significant developmental delay when spring sown. However, direct comparisons were not made and could have been influenced by the location or year of observation. Winter-type faba bean shows a vernalization response and is typically later flowering and maturing when spring-sown than spring-type stocks under a given temperature and photoperiod regime (Evans, 1959).

Future studies exploring the sowing date × cultivar interaction for growth and yield of spring sown faba bean in southeastern Washington will help to establish an optimal seeding date (Thompson and Taylor, 1977). It appears early spring seeding generally outperforms later through earlier flowering, higher branching, less pod shedding, and earlier maturity. The earlier 2013 seeding at the dryland managed WF location resulted in earlier flowering and maturity, likely contributing to higher plot yields than in 2012. A modest improvement in yield per plant compared with the gain in plot yield at WF in 2013 suggests that the incidence of virus in 2012
may have been an important factor affecting plot yield. Due to limited site year testing further studies on earlier sowing dates using spring genotypes would be advisable.

Heat (Bond et al., 1994) and associated moisture stress during flowering (Lawes, 1978) were limiting environmental factors of plant performance at the irrigated CF location. Flower abortion, pod shedding, reduced seed size, stunting, and early maturity (i.e. ‘fire’ see Khan et al., 2007) observed at CF in 2013, commonly attributed to drought stress (Kambal, 1969; Link et al., 1999), were likely the result of a high water demand due to low humidity and temperatures above 25 °C. Therefore, genotypes with a prolonged vegetative phase would not be recommended where average temperatures exceed 10 °C at sowing or >25 °C at flowering, even if irrigation is available. The exceptional performance of the F\textsubscript{3}:F\textsubscript{5} population under adverse conditions at CF in 2013, however, should indicate the possibility for improving adaptation where early developmental heat stress is likely.

Based on these yield data and its nutritional and agronomic values (Guillon and Champ, 2002; Crépon et al., 2010), the potential of faba bean as a rotational crop should be explored further. Future research should focus on evaluating seeding dates and rates, testing additional field locations across the precipitation zones of southeastern Washington, and selecting early flowering and maturing spring-type materials. Spring sown cultivars in Canada and Europe yield on average >3000 kg ha\textsuperscript{-1} (Rowland et al., 1982) and can exceed 5000 kg ha\textsuperscript{-1} across a broad range of environments (Dantuma et al., 1983). Ultimately, selecting adapted material with optimized growth and development will depend on developing and screening diverse lines within the agroecosystems of intended production.
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TABLES AND FIGURES

Table 4.1. Faba bean populations tested, their country of origin, 100 seed weight (g), and percentage of total flowering plants across two locations (Whitlow Farm and Central Ferry Research Farm) for two seasons (2012-2013).

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<td>W6 12025</td>
<td>Bulgaria</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>W6 12028</td>
<td>Bulgaria</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>Scout†</td>
<td>UK</td>
<td>48</td>
<td>25</td>
</tr>
<tr>
<td>Striker†</td>
<td>Germany</td>
<td>49</td>
<td>16</td>
</tr>
<tr>
<td>Wibo/1†</td>
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<td>51</td>
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<tr>
<td>F3:5</td>
<td>Pullman, WA</td>
<td>69</td>
<td>nd</td>
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</table>

†Seed obtained from Dr. Wolfgang Link, Georg-August-University.
nd= no data.
Table 4.2. Average monthly temperature and total precipitation at two locations (Central Ferry farm-CF and Whitlow Farm-WF) for two seasons (2012-13) and 30 year average temperature and precipitation for Ice Harbor and Pullman, WA (NOAA, 2013). Ice Harbor was chosen because of its geographic and meteorological similarities with CF.

<table>
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<tr>
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<td>°C</td>
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<td>°C</td>
<td>cm</td>
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<td>2.3</td>
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<td>15.5</td>
<td>1.8</td>
<td>16.4</td>
<td>2.1</td>
<td>15.8</td>
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<td>24.7</td>
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<td>26.3</td>
<td>0.2</td>
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<td>25.2</td>
<td>0.5</td>
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<td>Sep.</td>
<td>19.2</td>
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<td>20.3</td>
<td>3.8</td>
<td>18.4</td>
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</tbody>
</table>

Ice Harbor was chosen because of its geographic and meteorological similarities with CF.
Table 4.3. Early branching and height (cm), late branching and height (cm), yield per plant (g), and plot yield (kg ha\(^{-1}\)) marginal means across nine faba bean populations grown at Central Ferry Research Farm (CF) and Whitlow Farm (WF) for two years (2012-13). Letters separate significantly different LS-means (\(P \leq 0.05\)).

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Early branch</th>
<th>Late branch</th>
<th>Early height</th>
<th>Late height</th>
<th>g plant(^{-1})</th>
<th>kg ha(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>2012</td>
<td>2.0b</td>
<td>4.3a</td>
<td>28.9b</td>
<td>83.8b</td>
<td>21.0b</td>
<td>2650.8a</td>
</tr>
<tr>
<td>CF</td>
<td>2013</td>
<td>2.8a</td>
<td>3.4c</td>
<td>41.6a</td>
<td>55.3c</td>
<td>7.3c</td>
<td>799.8d</td>
</tr>
<tr>
<td>WF</td>
<td>2012</td>
<td>2.0b</td>
<td>3.8b</td>
<td>40.5a</td>
<td>90.2a</td>
<td>24.5a</td>
<td>2041.0c</td>
</tr>
<tr>
<td>WF</td>
<td>2013</td>
<td>1.1c</td>
<td>2.1d</td>
<td>26.8c</td>
<td>85.4b</td>
<td>22.1b</td>
<td>2418.6b</td>
</tr>
</tbody>
</table>
Figure 4.1. Early branching and height and late branching and height (±) of ten faba bean entries across two locations (Central Ferry Research Farm-CF and Whitlow Farm -WF) and for two years (2012-13).
Figure 4.2. Plot seed yields (converted to kg ha\(^{-1}\)) of 11 entries for two years (2012-13) across two locations (Central Ferry Research Farm and Whitlow Farm). The provided LSD bars, included below their respective year, separate significantly different populations by year (\(P \leq 0.05\)).
Figure 4.3. Scatter plot of per plant grain yield for each of 11 entries by mean per plant grain yield for each of four site years. Regression lines are included for only 3 of the 11 entries for clarity and comparison.
CHAPTER FIVE

Registration of four winterhardy faba bean germplasm lines for use in winter pulse and cover crop development

(To be submitted to Journal of Plant Registration)

ABSTRACT

Faba bean (Vicia faba L.) is a versatile crop grown as food, feed, vegetable, or as a cover crop in many countries. In response to the growing demands for winter annual legumes for cover crop development in the U.S., we developed four winterhardy faba bean germplasm lines, WH-1 (Reg. No. __, PI__), WH-2 (Reg. No. __, PI__), WH-3 (Reg. No. __, PI__) and WH-4 (Reg. No. __, PI__) at the USDA-ARS, Western Regional Plant Introduction Station, Pullman, WA, after six consecutive field seasons selecting for overwintering ability in two locations; Pullman and Central Ferry, WA. These four accessions were identified after the initial screening of 175 accessions from the USDA faba bean germplasm during the 2008-09 winter in Pullman, WA. In the following years, these populations were compared with several winter-type cultivars and breeding lines from Europe. During the final testing season, in 2013-14, selected and original accessions were compared. The average increase in percent survival was 84% across the four accessions. Furthermore, the winter-hardiness of the selected accessions was comparable to European materials. These four lines are potential reservoirs of useful diversity accessible for the development of winter annual cover crops for the U.S. Pacific Northwest and other regions up to USDA plant hardiness zone 6b.
Sustainable farming systems often feature crop rotations that maintain soil fertility and plant health. Among the different crop species, grain legumes are well suited for rotations with cereals (Karlen et al. 1994). Numerous reports have documented that grain legumes have positive effects on the yield of the succeeding crop (Chalk, 1998; Plancquaert and Desbureaux, 1985). The main reasons for these positive effects include: 1) grain legumes improve soil fertility via symbiotic nitrogen fixation (Mayer et al., 2003), 2) grain legumes diversify cropping systems and mitigate the phytotoxic and allelopathic effects of cereal residues, and 3) grain legumes improve soil structure and break pest and disease cycles (Chalk, 1998). In addition to conventional cash cropping, the use of grain legumes applied as green manure has the potential to improve soil health even further (Pieters, 1927; Köpke and Nemecek, 2010).

Faba bean (Vicia faba, L.) is one of the earliest domesticated crops and has a wide global distribution today. Its origin is thought to be in the Near East, and remnant seed dating to the late 8th millennium BC has been documented in northwest Syria (Tanno and Willcox, 2006). Faba bean subsequently spread to Europe, North Africa, and China and has been cultivated in these regions for centuries (Cubero, 1974, Duc, 1997). It was introduced to South America by the Spanish circa 1400 AD and was established as a crop in Australia as recently as the 1980s (Paull et al., 2011). Faba bean is a versatile legume crop grown as food, feed, vegetable, and forage in many countries (Rubiales, 2010). The world production of faba bean was 3.4 MT in 2013 (FAOSTAT, 2014).

The importance of faba bean as a specialty crop in the USA, especially for certain immigrant and ethnic groups, is augmented by its use as a dual-purpose cover crop or green manure. Faba bean has been noted for its generally high capacity for biological nitrogen fixation
(Herridge et al., 1994, Jensen et al., 2010) and high biomass production (Hickman and Canepari, 2012) among the major cool season food legumes. In areas where winter rains usually lead to soil compaction, especially in hard clay soils, planting faba bean as cover crop is ideal for opening up compacted soils and preventing possible soil erosion. In addition, faba bean attracts beneficial insect pollinators (Bugg et al., 1989).

The majority of faba bean germplasm is tolerant of cool conditions and light frost at the seedling stage, but is not necessarily winterhardy. True winter-type faba bean was reportedly introduced to England during the early 19th century, possibly via Russia (Lawes et al., 1983; Bond and Crofton, 1999). True winter-type faba bean germplasm from northern Europe is small seeded (<80 g·100 seed⁻¹) and shows a vernalization requirement for early-node flowering, atypical of spring-types (Saxena, 1982; Ney and Duc, 1997; Link and Bond, 2011). A protracted rosette appears to be associated with an increase in freezing tolerance (<-10°C) (Herzog 1988; Arbaoui et al. 2008). ‘Côte d’Or’ is reportedly the ultimate source of winter-hardiness, with 61% survival after an extreme low of -25°C (Picard et al., 1985). We report here, the development of faba bean germplasm tolerant to -25°C after six cycles of natural selection for overwintering in Pullman, WA.

MATERIALS AND METHODS

In October 2008, 43 faba bean accessions (USDA-ARS, Western Regional Plant Introduction Station, Pullman, WA.) from six countries, along with three commercial varieties and 12 European winterhardy cultivars and breeding lines were sown in the field to test their tolerance to survive local winters (Hu et al., 2009). In 2009, an additional 132 accessions were sown along with the seeds harvested from the plants that survived the previous winter. These
experiments identified several PI accessions that had winter-hardiness scores comparable to commercial European checks (Mwengi, 2011). Among them were four accessions with temporary ID numbers W6 12023, W6 12024, W6 12025, and W6 12028. The passport data indicated that these accessions were originally designated as ‘347-2’, ‘A1’, ‘A4’, and ‘Webo’, respectively, and were donated by the K. Malkov Agricultural Experiment Station of the Institute of Introduction and Plant Genetic Resources Center in Sadovo, Plovdiv, Bulgaria in 1987. Bulk selection for overwintering was continued annually for each accession till the final 2013-14 season.

The experimental design for the 2008-09 season followed Hu et al., (2009), where two selection locations in southeastern Washington were utilized based on contrasting mild [Central Ferry Farm (CF), Central Ferry, WA- 46°43’52‘’N; 117°39’52‘’W] and severe [Whitlow Farm (WF), Pullman, WA- 46°43’28‘’N; 117°08’07‘’W] winter selection pressures. Seed for the next cycle of selection was always sourced from WF, except in 2008-09 when there was a complete crop loss (Table 5.1) and seed was harvested from CF.

The experimental design for the 2009-10 and 2010-11 seasons was slightly different from that of 2008-09. Three replications of single row plots 4.6 m long were sown by hand at 10 seeds·m$^{-1}$ on 15 and 16 September for WF and CF, respectively. The seeding dates were 22 September for WF and 8 October for CF in 2010 and the seeding rate was the same as the previous year.

Slight alterations were made for the 2011-14 seasons, and an additional field site in Pullman, WA was included [Spillman Agronomy Farm (SF), 46°41’45.1‘’N; 117°9’2.7‘’W] (Chapter 2). Plot dimension varied by location, due to irrigation constraints at CF, but area was
consistent. Plots at WF and SF had four rows, while CF had two. Rows were spaced 35 cm apart at each location. Plot dimensions were 1.5 x 1.8 m at WF and SF and 0.75 x 3.6 m at CF, with a 40 cm space separating plots. A Hege 120 planter was set for 48 seeds per 2.7 m² plot, equivalent to 80-120 kg of seed ha⁻¹, depending on seed size.

In 2013-14 original NPGS accessions were obtained directly from the genebank and compared to their selected counterparts to reduce the seasonal error when comparing across years (Chapter 3). Pre-selected (2009-10 CF harvest) and post-selected Wibo/1 were included for comparisons to W6 accessions and WH-selections, as they are likely related (Kwon et al., 2010). Sowing date varied by location: WF 3 Oct., SF 10 Oct., and CF 24 Oct. 2013. All locations were hand harvested on 10 June (CF) and 16 August (SF & WF).

For comparison with the autumn-sown trial, a spring sowing was established on 24 April at WF using both original W6 accessions and WH-selected populations. The experimental design was an RCBD with four replications. Fifteen seeds were sown equidistant per single 1.5 m row plot and thinned to nine seedlings. The two seedlings on each end of the row served as borders and the five remaining plants were averaged. Rows were spaced 35 cm apart. Individual plants were hand harvested on 5 Sept.

Percent survival and flowering, 100 seed weights, and per plant yields are presented for the 2013-14 season. An analysis of variance was constructed with the data using PROC MIXED (SAS 9.2) and significance was set at \( P < 0.05 \) using the Tukey’s honest significant difference test for means separation. Population and location effects were treated as fixed effects and block was set as a random effect within the model following an RCBD.

CHARACTERISTICS
The winter-hardiness of four germplasm lines was enhanced after six consecutive cycles of natural selection by an average of 84%. We selected winterhardy 1 (WH-1), winterhardy 2 (WH-2), winterhardy 3 (WH-3), and winterhardy 4 (WH-4) bulk populations from W6 12023, W6 12024, W6 12025, and W6 12028, respectively.

Mature branching and height and per plant yield, across the WF and SF locations in 2013-14, were very similar between selected and unselected populations (Table 5.2). In general plant performance was less than previous because winter injury, seasonal soil moisture, and temperature fluctuation during flowering limited branching, height and pod set. Under the mild winter and spring of 2011-12 the mean branch number at maturity, height at maturity and plant yield of these populations averaged across WF, SF, and CF was over 3.5, 90 cm, and 50 g, respectively. Further, higher survival and favorable early season conditions at the irrigated CF location supported the highest recorded plot yields from 6.9 to 8.5 t ha\(^{-1}\) depending on population.

The height at flowering of spring-sown populations was similar to autumn-sown populations. However, the autumn-sown trial was scored 45 days prior to the spring-sown trial. Mature plants were substantially taller when spring-sown than if autumn-sown (Table 5.2). Generally, mature height and branching increase when winter-type populations are spring-sown, possibly by avoiding overwintering injury. Further, harvest was delayed (16 Aug. vs. 5 Sept.) and yield is often reduced (<20 g plant\(^{-1}\)), due to a later flowering period (June-July), than if autumn-sown (May-June), predisposing spring sowings to yield limiting environmental conditions.

**DISCUSSION**
The successful survival of overwintered faba bean grown in southeastern Washington is highly influenced by environmental fluctuations from year to year (Table 5.1). The crop failure of 2009-10 was likely the result of minimal snow cover, allowing frost to kill susceptible below ground tissues (Saxena, 1982; Mwengi, 2011). The 2010-11, 2011-12, and 2012-13 winters were relatively mild. The 2013-14 winter, however, had limited snow cover and was sufficiently cold enough to distinguish differences between selected and unselected populations.

Complicating the influence of prevailing environmental conditions, planting date can also affect overwintering. Late sowings, as was the case at CF in 2013-14 resulted in crop failure, because of inadequate hardening-off and accumulation of storage reserves. A general recommendation for much of southeastern WA is to sow by the first week of October. Generally this coincides with daily low air (<8°C) and soil (<10°C) temperatures (Murray et al., 1988).

Mean percent survival at WF and SF was not different between selected populations. However, there was a general increase observed between original W6 accessions and their counterpart WH- populations, whereas Wibo/1 showed no notable improvement (Table 5.1).

WH-1 was earlier flowering than W6 12023 (Table 5.3). Furthermore, 100 seed weight was reduced 13%. This shift in flowering and seed weight corresponded to an increase in winter-hardiness (Table 5.1). Since other genotypes did not experience the same alterations, however, these were likely indirect effects. There was no general shift between selected and unselected populations for branching, height, and yield traits. WH-1 did have a higher plot yield than W612023, as a result of winter survival.
In general, WH-populations (99 g plant\(^{-1}\)) out yielded W6 accessions (37 g plant\(^{-1}\)) when spring-sown. The lower yields of the W6 accessions may be the result of less autofertility than the WH-selections, since pollinators were excluded (Wright, 1977; Link, 1990).

Substantial outcrossing of faba bean (Hu et al., 2011) impedes inbreeding and the stabilization of breeding populations, limiting selection efficiency and genetic gain. Populations are expected to have had considerable inter-accession gene flow overtime likely resulting in heterogeneity and heterotic trait responses. The improved performance of these composite populations could be the result of recombination and incorporation of favorable alleles or simply the selection of favorable alleles present within the original accessions (Allard, 1960). WH-populations have at least maintained seed morphological diversity, and in the case of WH-1, have increased seed morphological diversity based on seed coat color and the decrease in frequency of the recessive clear hilum trait through selection and outcrossing.

We would suggest that researchers interested in this novel source of winterhardy germplasm to identify an optimal planting window for overwintering faba bean in zone 6b climates and warmer. Alternatively, since these four winter-type faba bean selections are facultative, early spring sowing is also feasible. Spring plantings in southeastern WA are detrimentally impacted by water and heat limiting biomass and grain yield. Further, earlier regrowth is achieved via autumn sowing. Yet spring-sown winter-type faba bean may still hold promise as a green manure, or cover crop, in environments where moisture and temperature are conducive i.e., high temperature below 30°C during growth and development, because of upright indeterminate growth. Further research should explore the potential of winter-type faba bean as a spring, late summer, and winter green manure-cover crop for cool season environments.
AVAILABILITY

Small quantities of seed WH-1, WH-2, WH-3, and WH-4 are available for breeders, geneticists, and other research personnel on written request to Jinguo Hu or Clarice Coyne, USDA–ARS, Western Regional Plant Introduction Station, 59 Johnson Hall, Washington State University, Pullman, WA 99164, or through the National Plant Germplasm System’s GRIN (Germplasm Resources Information Network) database at http://www.ars-grin.gov/npgs/orders.html. It is requested that appropriate recognition of the source be made when these germplasm lines are used in the development of novel breeding lines or commercial releases.

CONCLUSIONS

WH-1, WH-2, WH-3, and WH-4 are the only characterized source of true winterhardy faba bean available through the USDA-ARS, Western Regional Plant Introduction Station. These released faba bean lines are expected to complement previously characterized and currently available materials on the seed market. These lines will be useful for breeding improved pulse and green manure or cover crop faba bean cultivars adapted to the Plant Hardiness Zones of 6b and above.
REFERENCES


USDA-ARS, 2012. USDA Plant Hardiness Zone Map. Accessed from

http://planthardiness.ars.usda.gov/PHZMWeb/#

Table 5.1. Percent survival of faba bean and the coldest recorded temperature overwinter at Whitlow Farm, Pullman, WA; 2008-2014. Unselected entries for the 2013-14 season were germplasm stocks of their representative WH selections and 2009-10 seed sourced from the Central Ferry Farm in Central Ferry, WA for Wibo/1.

<table>
<thead>
<tr>
<th></th>
<th>W6 12023</th>
<th>W6 12024</th>
<th>W6 12025</th>
<th>W6 12028</th>
<th>Wibo/1</th>
<th>Coldest air temperature °C</th>
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<tr>
<td>2008-09</td>
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<td>83</td>
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<td>2009-10</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>2010-11</td>
<td>37</td>
<td>17</td>
<td>70</td>
<td>77</td>
<td>67</td>
<td>-5.4</td>
</tr>
<tr>
<td>2011-12</td>
<td>68</td>
<td>61</td>
<td>64</td>
<td>61</td>
<td>73</td>
<td>-11.7</td>
</tr>
<tr>
<td>2012-13</td>
<td>53</td>
<td>75</td>
<td>65</td>
<td>63</td>
<td>71</td>
<td>-14.5</td>
</tr>
<tr>
<td>2013-14 (selected)</td>
<td>45</td>
<td>57</td>
<td>61</td>
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<td>27</td>
<td>37</td>
<td>37</td>
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Table 5.2. Early height and branching at flowering (16 May), late height and branching at maturity (16 Aug.), 100 seed weight, per plant yield, and plot yield of unselected and selected autumn-sown populations at two locations (Whitlow and Spillman Farms, Pullman, WA). Only early height at flowering (20 June) and late height and maturity (5 Sept.) are presented for a spring sowing at Whitlow Farm. Unselected entries were original germplasm stocks of their representative WH- selections and 2009-10 seed sourced from the Central Ferry Farm in Central Ferry, WA was used for Wibo/1. Means followed by different letters are significantly different ($P\leq 0.05$).

<table>
<thead>
<tr>
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<th>Spring-sown</th>
<th>Autumn-sown</th>
<th>Yield per plant (g)</th>
<th>Plot yield (kg ha$^{-1}$)</th>
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<tr>
<td></td>
<td>Early height (cm)</td>
<td>Late height (cm)</td>
<td>Branch number</td>
<td>Height (cm)</td>
</tr>
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<td>1.7 AB</td>
<td>2.3 A</td>
</tr>
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<td>1.3 B</td>
<td>1.7 A</td>
</tr>
<tr>
<td>W6 12024</td>
<td>40.9 A</td>
<td>93.2 AB</td>
<td>1.7 AB</td>
<td>1.7 A</td>
</tr>
<tr>
<td>WH-2</td>
<td>40.6 A</td>
<td>98.7 B</td>
<td>1.5 AB</td>
<td>1.6 A</td>
</tr>
<tr>
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<td>41.4 A</td>
<td>98.4 AB</td>
<td>2.1 A</td>
<td>2.2 A</td>
</tr>
<tr>
<td>WH-3</td>
<td>36.6 A</td>
<td>100.9 A</td>
<td>1.5 AB</td>
<td>1.9 A</td>
</tr>
<tr>
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<td>87.4 B</td>
<td>1.8 AB</td>
<td>2.0 A</td>
</tr>
<tr>
<td>WH-4</td>
<td>35.4 A</td>
<td>91.5 AB</td>
<td>1.7 AB</td>
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</tr>
<tr>
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<td>nd</td>
<td>nd</td>
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</tr>
<tr>
<td>2009-10</td>
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<td>nd</td>
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<td>nd</td>
<td>nd</td>
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<td>2.1 A</td>
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Table 5.3 Percent flowering of spring-sown (30 June) and autumn-sown (16 May) and 100 seed weight for Wibo/1, W6 accessions, and WH-selections. Means followed by different letters are significantly different ($P \leq 0.05$).

<table>
<thead>
<tr>
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<th>Percent flowering</th>
<th>100 seed weight (g)</th>
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<tr>
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<td>Autumn-sown</td>
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<td>79 A</td>
</tr>
<tr>
<td>WH-1</td>
<td>60 C</td>
<td>37 B</td>
</tr>
<tr>
<td>W6 12024</td>
<td>95 A</td>
<td>6 C</td>
</tr>
<tr>
<td>WH-2</td>
<td>75 B</td>
<td>20 BC</td>
</tr>
<tr>
<td>W6 12025</td>
<td>85 AB</td>
<td>34 B</td>
</tr>
<tr>
<td>WH-3</td>
<td>90 AB</td>
<td>37 B</td>
</tr>
<tr>
<td>W6 12028</td>
<td>80 B</td>
<td>34 B</td>
</tr>
<tr>
<td>WH-4</td>
<td>45 D</td>
<td>26 BC</td>
</tr>
<tr>
<td>Wibo/1 2009-10</td>
<td>nd</td>
<td>13 BC</td>
</tr>
<tr>
<td>Wibo/1 selected</td>
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<td>25 BC</td>
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*nd* = no data
CHAPTER SIX

Low molecular weight carbohydrates from mature and immature seeds of *Vicia faba* L.

(Submitted to HortScience)

ABSTRACT

Mature and immature seeds from faba bean (*Vicia faba* L.), with a broad range of seed size (26.2-172 g 100 seed\(^{-1}\) DW), were examined for low molecular weight carbohydrates (LMWC): glucose, fructose, and sucrose (GFS) and raffinose, stachyose, and verbascose (α-galactosides). Sucrose was the main constituent LMWC of immature (105.6 mg g\(^{-1}\) DW) and mature seeds (23.7 mg g\(^{-1}\) DW) across entries. Total α-galactoside concentration exceeded sucrose within mature seeds (46.3 mg g\(^{-1}\) DW), but was lower within immature seed (7.5 mg g\(^{-1}\) DW). Across entries, there appeared to be a positive correlation between mature seed size and total GFS concentration of immature seeds; however the rank order of GFS from mature seed was not necessarily predictive of GFS at the immature stage. LMWC deposition appeared characteristic for many tissues and seed classes. Based on correlations between LMWCs and seed size, historical selection for large seeds may have indirectly increased sucrose of immature seeds and α-galactoside content of mature seed. Genotypes such as De Monica that have relatively low α-galactoside (2.5%) within mature seed, but high GFS (>15%) in immature seed should be sought for dual purpose consumption. Modern breeding methodologies and analytical testing of immature and mature seeds will be necessary to further characterize and improve the culinary quality of faba bean.
Faba bean (*Vicia faba* L.) is a dynamic cool-season pulse crop utilized for human and animal consumption, as well as soil improvement (Crépon et al., 2010). Pulses serve as a healthy alternative to meat, potentially reducing the risk of type II diabetes, obesity, colon cancer, and cardiovascular disease (Champ, 2001; Kozłowska, 2001). The immature and mature seed, including foliage, are primarily consumed in China, the Middle East, and the Mediterranean (Hawtin and Hebblethwaite, 1983). Production estimates indicated China (882,000 ha) as the world leading producer and consumer, with Ethiopia (512,000 ha) and Australia (160,000 ha), responsible for the bulk of world trade (FAOSTAT, 2010). Production and consumption in North America is limited in scale.

There are three main botanical varieties of faba bean: var. minor (<40 g 100 seed$^{-1}$), var. equina (40-80 g 100 seed$^{-1}$), and var. major (>80 g 100 seed$^{-1}$) (Duc, 1997). Depending on the cultural context, minor and equine types are used as animal and human feed stock at their mature or immature stage. The immature seeds of broad beans (var. major) are most often consumed as fresh vegetables (Hawtin and Hebblethwaite, 1983) and considered sweeter than smaller classes (Torres et al., 2012).

The main energy components of faba bean seed are starch (~40% DW) and protein (~30% DW) (Pritchard et al., 1973). Soluble carbohydrates, i.e., low molecular weight carbohydrates (LMWC: glucose, fructose, sucrose, raffinose, stachyose, and verbascose) have also been quantified with varying specificity and breadth (White, 1966; Pritchard et al., 1973; Cerning et al., 1975; El-Shimi et al., 1980; Frauen et al., 1984; Quemener, 1988; Dini et al., 1989; Barratt, 1992; Freijnagel et al., 1997; Ruperez, 1998; Duc et al., 1999; Guillon and Champ, 2002). Verbascose is the principle α-galactoside of mature seeds and ranges from 0.8-3.1% DW,
followed by stachyose 0.2-1.6% DW, and raffinose 0.1-1.5% DW (Lattanzio et al., 1986; Quemener, 1988; Dini et al., 1989; Frias et al., 1996; Kozlowska, 2001; Guillon and Champ, 2002). As a reference, dry pea \( (Pisum sativum \, L.) \) has a similar range of verbascose (1.9-3.2% DW), but more raffinose (1.6-1.9% DW) and less stachyose (0.3-0.6% DW) (Quemener, 1988), with extremes found outside these ranges (Jones et al., 1999; Kozlowska, 2001). The monosaccharides fructose and glucose are generally negligible in mature seed, but sucrose content has a wide distribution, 0.02-5.23% DW (Pritchard et al., 1973; Cerning et al., 1975; Frauen et al., 1984; Lattanzio et al., 1986; Quemener, 1988; Frias et al., 1996).

Specific interest in oligosaccharides (\( \alpha \)-galactosides) has been due to a presumption that they are anti-nutritional (Frauen et al., 1984; Ruperez, 1998), or non-nutritional (Torres et al., 2012). However, alternative opinions suggest that these flatulence-causing carbohydrates may also serve as important health promoting prebiotics (Tomomatsu, 1994; Frias et al., 1996; Champ, 2002; Martinez-Villaluenga et al., 2008). Hayakawa et al. (1990) showed that low doses \((3 \, \text{g day}^{-1})\) of purified \( \alpha \)-galactosides could increase bifidobacteria. Kozlowska (2001) suggested 0.1-0.3 g of \( \alpha \)-galactosides per portion would not likely cause digestive issues. Further testing is necessary to establish what the therapeutic effects of \( \alpha \)-galactosides are (Champ, 2002; Di Bartolomeo et al., 2013). Processing methods such as germination, fermentation, soaking with \( \text{NaHCO}_3 \), and cooking all lower the \( \alpha \)-galactoside content of mature seed (Pridham, 1958; Khokhar et al., 1996; Ruperez, 1998; Granito et al., 2003; Martinez-Villaluenga et al., 2008).

Compared to mature faba bean seed, less information is available for the LMWC content of immature faba bean seed (Lattanzio et al., 1986; Ziena et al., 1987; Frias et al., 1996). The sucrose concentration of immature seed (\(<30\% \, \text{DW}, \) or prior to the color of the cotyledon
changing from green to yellow) is much higher than in mature seed rapidly decreasing from 15% to 4% DW, while α-galactosides are negligible early in seed development and accumulate through maturation (Lattanzio et al., 1986; Frias et al., 1996; Weber et al., 1996). Frias et al., (1996) observed that the sucrose content of immature pea seed was considerably higher (22% DW) compared to immature seeds from a minor type faba bean (6.3% DW).

Lattanzio et al., (1986) reported no relationship between faba bean seed weight and sucrose content. However, Barratt (1992) speculated that ‘Gillett’s long pod’, the largest-seeded line (1.98 g seed⁻¹) of 30 tested, had the highest ethanol-soluble sugar concentration and lowest starch content due to previous selection. Furthermore, a study by Weber et al., (1996) confirmed that large seeded types have a maximum sucrose content of 10%-15% when measured at 25-40 days after flowering (DAF) compared to 5%-10% for small seeded types at 20-30 DAF.

Sucrose is considered a critical component of many foods, as it enhances natural flavors and improves the characteristics of other flavoring ingredients (Grotz and Munro, 2009). The desirable effect of enhanced sucrose content on taste was reported in other legumes, such as cowpeas (Vigna unguiculata L. Walp.) and butterbean (Phaseolus lunatus L.). Genotypes of mature cowpeas with sucrose content of 6% DW compared to those with 2% DW were consistently chosen by a professional taste panel due to enhanced flavor (Hall et al. 2003). Furthermore, small additions of sucrose (5-10% DW) to the fiber rich butterbean enhanced the taste and acceptance by a diabetic taste panel, while still maintaining a low glycemic index (Vorster et al., 1987). Therefore, improving the sweetness of mature and immature faba bean may attract new consumers, thus improving market potential. Similarly, identification of mature
seeds low in \( \alpha \)-galactoside content may be desirable to the faba bean industry, since the compounds are usually considered anti-nutritional at high concentrations.

A lack of screening for GFS and \( \alpha \)-galactoside content of both immature and mature seeds from a diverse panel of faba bean germplasm limits the potential to select genotypes with an improved LMWC profile. Recent advances in soybean (\textit{Glycine max} L. Merr.) breeding programs have successfully identified several germplasm accessions with high sucrose and low raffinose and stachyose content, which contribute to developing improved edamame soybeans for specialty markets (Hou et al., 2009; Mozzoni et al., 2013).

Limited information is available to confirm if a relationship between LMWC profile and faba bean seed weight exists. A significant correlation would be valuable for indirect selection of LMWCs through selection for seed size. Therefore, the purpose of this research was to furnish a preliminary assay of LMWCs of both the immature and mature stages of faba bean seed development. Large- and small-seeded types obtained from commercial sources and accessions maintained by the USDA-ARS Western Regional Plant Introduction Station in Pullman, WA would serve to broadly characterize LMWCs within faba bean seeds. These results will serve as a benchmark for further screening, selection, and ultimate improvement of LMWCs.

**MATERIALS AND METHODS**

*Plant material*

A diverse assortment of forty faba bean entries, including commercially available cultivars, open pollinated heirlooms, and germplasm accessions from the USDA-ARS Western
Regional Plant Introduction station were included to represent the range of seed sizes within the species (Table 6.1).

A representative sample of air dried mature seeds or immature seeds were selected from field-grown plants at Washington State University’s Whitlow Farm (WF) in Pullman, WA (46°44’3.2”N-117°7’25.8”W) and dehulled. Immature seeds were collected between 30 and 50 DAF (Lattanzio et al., 1986; Heim et al., 1993; Borisjuk et al., 1995, Frias et al., 1996). Due to high plant to plant variability individual samples (three pods from five plants) were bulked prior to analysis. We attempted to collect at the appropriate stage of seed development (late cotyledon stage) when the sucrose concentration of seed coat and embryo are reported to be highest (Weber et al., 1996). Given that the percent dry matter content of the tissue corresponds with the developmental stage of the seed and potentially affects the LMWC profile, percent water content of the samples is presented for reference (Table 6.1).

To further examine the relationship between percent water content and development of seed LMWCs the cvs. Sweet Lorane and Jubilee Hysor were sampled weekly for five weeks in 2014, starting 16 days after flowering. For this time series, pods and whole seeds were grown and sampled as previously described.

Sample preparation

Mature and immature tissues were ground and extracted following the same protocol, except that immature seeds and pods were freeze-dried to estimate percent water content. LMWCs from mature seed coats were not presented due to low metabolite concentrations.
LMWC extraction and analysis followed Knudsen and Li (1991), with the exception that water was used as the mobile phase instead of 0.015 N Na$_2$SO$_4$. Briefly, the extraction was run in triplicate using 200 mg samples of flour and 4 ml of ETOH (50%, v/v), plus 1 ml of ribitol as an internal standard that were sonicated at 15 min intervals for 1 hr at room temperature. The supernatant was then decanted after centrifugation (3000 g, 10 min). The extraction was then repeated using 3 ml, and then again with 2 ml of ETOH (50%, v/v), but only refluxed for 5 min each cycle. The supernatants from the extraction series were pooled and diluted to 10 ml with ETOH (50%, v/v). An aliquot (1 ml) from this stock was diluted with an equal volume of ETOH (90%, v/v), stored at -20°C for 1 hr, and centrifuged (10000 g for 10 min) to precipitate proteins. The supernatant was dried at 25°C (Thermo Scientific DNA 110 Savant™ SpeedVac™) and redissolved in 1 ml of ultrapure water (EMD Milipore Co.; Billerica, MA, U.S.A.) for high performance liquid chromatography (HPLC) analysis.

**HPLC**

An Agilent 1100 HPLC with an Agilent 1260 Infinity Refractive Index Detector (Agilent Technologies) was used to identify and quantify individual LMWCs. The mobile phase was water with a flow rate of 0.5 ml min$^{-1}$. An Aminex HPX-87N (300 x 7.8 mm) resin-based column (Bio-Rad Laboratories, Inc.; Hercules, CA, U.S.A.) in the sodium form was used to separate LMWCs at 80°C. Quantification and assignment of peaks was based on standard curves of pure glucose, sucrose, fructose, verbascose, stachyose, raffinose, and ribitol (Sigma-Aldrich Co.; St. Louis, MO, U.S.A.). Other regents were of analytical grade. LWMCs were expressed on a percent dry weight basis.
LMWC contents of mature and immature seeds were compiled and analyzed using ANOVA and pairwise comparisons following Tukey’s honest significant difference test ($P \leq 0.05$) of PROC MIXED (SAS, 2008). Analyses were conducted according to a completely randomized design; $n=3$. Significant entry differences were tested once significant seed type (mature seed and seed coat and cotyledon-embryo of immature seed) and seed weight class ($<41$, 42-85, and $>85$ g 100 seeds$^{-1}$) category differences were identified.

RESULTS AND DISCUSSION

A broad distribution of faba bean lines encompassing the minor, equina, and major botanical varieties (Table 6.1) were compiled and analyzed for LMWCs. Mature seed size ranged from 26.2 to 172 g 100 seeds$^{-1}$ exemplifying the wide range across this species. As expected, pod size, at the immature or vegetable stage, corresponded with seed size. The large (var. major) seed class entries had a mean pod fresh weight of 9.4 g, medium (var. equina) 4.7 g, and small (var. minor) 2.8 g. Seed number per pod and percent seed coat tended to follow a similar separation by seed class, however, the distinction was not as strong as with pod weight. Further characterization of these morphophysiological correlations will help guide selection efforts.

The LMWC profiles of the three seed classes were generally characterized by seed tissue type. Across seed tissues, immature cotyledons (12.9%) and seed coats (8.3%) had a higher mean percent sucrose than dry seeds (2.4%). While there was no difference among seed classes for the sucrose concentration of immature seed coats, both immature cotyledons and mature seeds of the large seeded class were higher than either medium or small classes (Fig. 6.1).
In agreement with other research, the main LMWCs of mature seeds apart from sucrose are the α-galactosides verbascose and stachyose (Fig. 6.2) (Lattanzio et al., 1986; Quemener, 1988; Dini et al., 1989). Less than 1% DW total α-galactosides were present within immature seeds, in agreement with Frias et al., (1996). The mono-saccharides glucose and fructose were not readily detectable within mature seeds; however, there was a considerable concentration within immature seeds. The mean concentrations of glucose, fructose, and sucrose from immature seeds for all seed classes and types were higher than that observed by Frias et al., (1996), likely due to the diversity of the entries included herein.

We also separately analyzed seed coats and cotyledon+embryonic axis, i.e., embryo, which resolved differences between the two. It appears that monosaccharides of seed coats, across entries, have a higher mean concentration than cotyledons, which was opposite the trend found for sucrose. Lower relative sucrose concentration of seed coat vs. embryo would be expected based on the loading and unloading of translocated photoassimilate across the testa during seed morphogenesis (Wolswinkel, 1992; Patrick and Stoddard, 2010). For both glucose and fructose, the seed coats from the largest seed class also had the highest mean concentration compared to other seed classes or tissues. Although the difference was biologically small (0.5 vs. 1.5% DW) this may help to support the development of larger seed (Weber et al., 1996).

Additional sampling during seed development confirmed slightly higher glucose, fructose, and sucrose concentrations of the larger seeded Jubilee Hysor compared to the smaller seeded Sweet Lorane during the most rapid growth phase of the seeds. Here again, biological differences were small, 1.7% for glucose, 1% for fructose, and 2.5% for sucrose, at the second sampling. However, the largest differences were observed at the third sampling (30 days after
flowering), corroborating the results of Weber et al. (1996). Furthermore, the concentration of seed sucrose was correlated with fructose ($r = 0.85, P<0.0001$) and glucose ($r = 0.83, P<0.0001$) content. Based on the characteristic duration of sugar metabolism between the two cultivars, the prolonged decline in sucrose concentration of Jubilee Hysor (Fig. 3) indicates a physio-metabolic and possibly genetic component that should be further explored.

As a result of relatively low $\alpha$-galactoside content of immature seed and lack of separation between entries for either seed coat or cotyledon tissues, concentrations of individual entries were not presented. The evolution of total $\alpha$-galactosides was slow, only increasing in concentration at the fifth sampling when embryos were deep yellow in color. The total $\alpha$-galactoside concentration of Jubilee Hysor and Sweet Lorane mirrored their final seed concentrations, yet the sucrose concentrations of both were appreciably higher, indicating additional physiological development until seed maturity (Lahuta et al., 2000).

Although individual saccharides possess characteristic sweetness intensities (Wrolstad, 2012), fructose, glucose, and sucrose concentrations were combined (GFS) for both immature cotyledon and seed coat tissues, because rank orders were not affected (Fig. 6.4). There was a normal distribution of GFS across entries for both seed coat and cotyledon tissues. Seed coat and cotyledon concentrations were not always correlated, however. Peruvian Red Cheek, Copper, and Superaguadulce Morocco had elevated levels and Sweet Lorane, F$_3$:F$_5$, and PI 655348 had among the lowest GFS content across tissue types. Interestingly, Peruvian Red Cheek, Copper, and Superaguadulce Morocco were among the largest seeded entries (>100 g 100seeds$^{-1}$), while PI 655348, Sweet Lorane, and F$_3$:F$_5$ were separated by small, medium, and large classes,
respectively. The relationship between seed size and GFS content appeared to be stronger for cotyledons than for seed coats (Fig. 6.5).

The range in mean percent water content of immature cotyledons from the large (83.3%), medium (79.8%), and small (73.2%) seed classes was likely the result of the size and duration of the developing sink (seed), which influenced dry matter accumulation (Table 6.1) (Weber et al., 1996). Smaller seeds likely transitioned from simple sugar to starch accumulation earlier than for larger seeded types (Barratt, 1992), as a result of characteristic initial seed coat invertase activity and rate of cell division (Weber et al., 1996). Since unloading of sucrose into the developing sink is driven by a concentration gradient maintained by its hydrolysis (Hay and Porter, 2006), larger sink organs would require more sucrose import over a longer duration than smaller organs. Through the time series, sucrose followed this extended trend (Fig. 6.3), and percent moisture of the single harvest resembled that of the third sampling in the time series, where differences between seed size in GFS was most notable.

A general impression from field-grown plants was that the large seeded types often took longer to mature than smaller seeded types. The relationship between mature seed size and simple sugar/starch content of immature seed, observed herein, should be studied physiologically at the molecular level across a similar range of genotypes. Unfortunately, a consistent relationship between GFS of mature and immature seeds was not observed.

Total LMWC of mature seeds across entries ranged from 2.6 to 10.4%—a wider distribution than the results of Barratt (1992) and Guillon and Champ (2002). We would expect an even larger range through further characterization of diverse germplasm accessions.
Due to a lower range in sucrose values of mature seeds, it would be difficult to resolve the difference between entries, except for the highest and lowest concentrations. Similar to the assertion of Barratt (1992), larger seeds tended to have both higher sucrose and total α-galactoside content (Fig. 6.6). The exception was the large seeded De Monica, which had one of the lowest total α-galactoside contents. This was ~30% of Oakland Purple (purple seed), which had the highest α-galactoside content (Fig. 6.7). Additionally, De Monica had a reasonably high GFS concentration for both immature seed coat and cotyledon making this cultivar well suited for dual purpose vegetable and pulse consumption (Fig. 6.4). The small seeded Sweet Lorane and Hiverna/2 also had low total α-galactoside concentrations, but showed contrasting GFS concentrations of immature seeds. None of the entries tested here were as low as cv. Beryl (1.4% DW) a low α-galactoside field bean (Lattanzio, 1986).

Apart from identifying the animal-plant importance of α-galactosides (Pridham et al., 1969; Obendorf and Górecki, 2012), and inducing low α-galactosides specifically (Hitz et al., 2002), we should be aware of the systems biochemistry (Westerhoff et al., 2010) scenario that is physiology. An integrative component or systems thought will be necessary if we are to possibly understand the metabolic network of seed biology and its role in supporting life. While selecting low α-galactoside genotypes may have the benefit of increasing consumption, the potential negatives of seed longevity and seed vigor should also be considered (Górecki, 2001). Horbowicz and Odendorf (1994) observed that a total α-galactoside to sucrose ratio >1 improved seed longevity (>10 years), whereas <1 resulted in a storability half-viability period of <10 years.

*Heritability of LMWCs*
Based on the multiple interactions between LMWCs and their representative quantitative distributions, it appears that selection would be complicated without sophisticated breeding designs coupled with analytical instrumentation. Further research should therefore focus on elucidating the heritability of LMWCs of faba bean and its relationship with seed size, similar to that done in lentil (Frias et al., 1999). Based on this preliminary screening, the F$_3$:5 breeding population derived from a cross between ‘Extra Precoce Violetto’ and ‘Hiverna/2’ provided some insight. Sucrose and total α-galactoside concentrations between mature seed of F$_3$:5 and ‘Extra Precoce Violetto’ were quite similar, as well as the % seed coat of immature seed from Hiverna/2 and F$_3$:5, which suggests these traits are highly heritable. The concentration of GFS between immature seeds of F$_3$:5 and either parent were not clearly distinguishable, however.

We must also be aware of the possible disparity of individuals within heterogeneous entries and between testa and embryo of the same individual. For example, two samples of Oakland Purple based on seed coat color were more often distinguishable, suggesting that this open-pollinated heirloom is also genetically, as well as phenotypically, heterogeneous. In addition to potential polymorphic alleles within a panmictic population, genotype differences between cotyledon and maternal seed coat tissues would make characterization and comparison, in order to make selections, difficult at best. Therefore, the role of the maternal seed coat in controlling carbohydrate state of the developing filial embryo should be considered in future studies (Wolswinkel, 1992; Weber et al., 1996; Frias et al., 1999; Kosina et al., 2009).

This preliminary study of LMWCs from a broad collection of faba bean entries suggests that there exists a wide distribution for each metabolite, which tends to correlate with seed size. Selection for larger seed has likely had an indirect effect of increasing the concentration of
individual sugars (Weber et al., 1996), although the specifics (intentional or unintentional selection) remain speculative (Cleveland and Soleri, 2007). Developing inbred lines and/or mapping populations would help to further understand the genetic basis of the carbohydrate metabolism of developing faba bean seeds. Ultimately, selection for lines with specific quality characteristics will aid in improving the culinary acceptability and functionality of the crop.
REFERENCES


TABLES AND FIGURES

Table 6.1. Mature seeds were measured for 100 seed weight (g) and seed classes set as small (<41 g), medium (42-85 g), and large (>85 g). The mean fresh weight per pod (without seeds) from a representative sample of immature pods from each entry was determined. The proportion of seed coat, percent water content of seed coats, and cotyledons+germ from immature seed samples were determined once freeze-dried.
<table>
<thead>
<tr>
<th>Variety</th>
<th>100 seed weight (g)</th>
<th>Pod weight (g)</th>
<th>Seed coat (%) of whole seed</th>
<th>% moisture of immature seeds at sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 614810 Diana</td>
<td>26.2</td>
<td>2.4</td>
<td>39.4</td>
<td>67.7</td>
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<td>PI 614809 Herz Freya</td>
<td>28.8</td>
<td>2.3</td>
<td>40.7</td>
<td>75.8</td>
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<td>37</td>
<td>3.9</td>
<td>33.7</td>
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</tr>
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<td>39</td>
<td>2.6</td>
<td>48.1</td>
<td>80.6</td>
</tr>
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<td>40.2</td>
<td>1.4</td>
<td>23.5</td>
<td>74.0</td>
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<td>40.2</td>
<td>1.4</td>
<td>47.4</td>
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<td>40.6</td>
<td>2.6</td>
<td>35.7</td>
<td>73.9</td>
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<td>51.6</td>
<td>3.0</td>
<td>17.5</td>
<td>76.5</td>
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<td>45.3</td>
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<td>115.2</td>
<td>4.1</td>
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<td>92.2</td>
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<td>De Monica</td>
<td>116.4</td>
<td>11.2</td>
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<td>79.6</td>
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<td>12.9</td>
<td>50.0</td>
<td>86.5</td>
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<tr>
<td>Oakland purple (purple seed)</td>
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<td>5.7</td>
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1Seed Savers Exchange, 2Prairie Garden Seeds, 3Thompson and Morgan, 4backyardgardener.com, 5Gourmet Seed, 6Peace Seeds, 7Territorial Seed, 8USDA-ARS Western Regional Plant Introduction Station, 9Dr. Wolfgang Link, Georg-August-University, 10Breeding population, 11Adaptive Seeds
Figure 6.1. Mean percent sucrose (se±) of 40 faba bean entries separated into three seed classes (small <41 g, medium 42-85 g, and large >85 g 100 seed\(^{-1}\)) and seed types (immature cotyledon+germ, immature seed coat, and mature seed).
Figure 6.2. Mean glucose, fructose, raffinose, stachyose, and verbascose concentrations (se±) of 40 faba bean entries separated into three seed types (immature cotyledon+germ, immature seed coat, and mature seed).
Figure 6.3. Sucrose concentrations of developing pods and seeds from two faba bean cultivars with contrasting seed size; Sweet Lorane (SL) and Jubilee Hysor (JH). Reproductive organs were sampled (S) once a week for five weeks 16 days after the initiation of flowering. Error bar represents the standard error of the means.
Figure 6.4. Total glucose, fructose, and sucrose (GFS) concentrations (se±) of 40 faba bean entries separated into immature cotyledon+germ and immature seed coat tissues.
Figure 6.5. Scatter plot of immature cotyledon+germ and seedcoat total glucose, fructose, and sucrose (GFS) concentrations from 40 faba bean entries by 100 seed weight. Regression lines are included for each tissue.
Figure 6.6. Scatter plot of total α-galactoside (raffinose, stachyose, and verbascose) and sucrose concentrations from mature seed of 40 faba bean entries by 100 seed weight. Regression lines are included for each metabolite.
Figure 6.7. Total α-galactoside (raffinose, stachyose, and verbascose) and sucrose concentrations (±SE) from mature seed of 40 faba bean entries.

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<th>Total α-galactosides (% DW)</th>
<th>Sucrose (% DW)</th>
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<td>PI 655345</td>
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<tr>
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<td>Bergeron</td>
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<tr>
<td>Scout</td>
<td>PI 655348</td>
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<td>PI 469126</td>
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<td>Ianto’s Yellow</td>
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<td>PI 510593</td>
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<td>De Monica</td>
<td>Peruvian Red Cheek</td>
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<tr>
<td>Oakland Purple (purple seed)</td>
<td>Ianto’s Return</td>
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<tr>
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<td>Superguadulce Morocco</td>
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<td>Polar</td>
<td>F3:F5</td>
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<td>Long Pod Major</td>
<td>Delle Cascine</td>
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<td>Delle Cascine</td>
<td>Oakland Cascine</td>
</tr>
<tr>
<td>Jubilee Hysor</td>
<td>Oakland Purple (purple seed)</td>
</tr>
</tbody>
</table>
Inheritance of height, leaf chlorophyll, and stem, hilum, and flower color mutations

ABSTRACT

Identification of phenotypic markers associated with positive mutations will support plant breeders in improving agronomic characteristics of *Vicia faba* L. An inheritance study was initiated using a single putative hybrid from a population of PI 655345 homozygous for *dw1* and *zt1*. Segregation analysis of the F₂ and F₃ showed that both alleles fit the expected 3:1 and 9:3:3:1 distribution for a monohybrid and dihybrid cross, respectively. Furthermore, red stem and hilum color were found to be segregating independently. However, red stem was difficult to score because the intensity was modified by background genetics and failed to fit the expected ratio when the *zt1* locus was also segregating. Presence of recombination between *dw1* and an unknown mutant locus controlling leaf color was also observed within a genetic distance of <10cM. Tall height:dark foliage and short height:light foliage recombinant family phenotypes were not as strongly expressed as in the parents. Due to limited progeny size a definitive recombination frequency between height and leaf color was not resolved.

Individual plants from a single accession, PI 655345-‘Maris Bead’, were identified as white flowered and either dwarf (<50 cm) or normal height (>80 cm) during the 2010 season. ‘Maris Bead’ was selected by Dr. Bond for its small seed size (500 mg 1000 seeds⁻¹) and use as a specialty market feed for pigeons in the UK. ‘Maris Bead’ is also daylight sensitive, only flowering once daylight exceeds 12 h, and is drought prone compared to other faba bean varieties.
(Dr. Stoddard, per. comm.). Maris Bead is typically of normal height (Pilbeam et al., 1990) and has wild type flowers (Dr. Stoddard, per. comm.). However the GRIN accession is white flowered and segregating for height, even though the contributor, Adrian Russell, remembers it with a wild type flower and height, further suggesting that this accession was not true to type. Possibly it is related to cv. Veritas, which has both white flower and dwarf phenotype (Bozzini and Chiaretti, 1999), or cv. Staygreen, a white flowered and possibly staygreen type (De Vries, 1978). The PI number, rather than the cultivar name, will be used hereafter as reference.

In 2011, the progeny of three dwarf individuals and one individual with normal height expressed varying distributions of dwarf:normal height and light:dark leaf color phenotypes (Fig. A1.1). In 2012, PI 655345 derived progeny [dwarf:dark homozygote and a purported heterozygote (tall:light F1 derived from a dwarf:dark)] were compared to another white flowered accession (PI 655330) (Fig. A1.2) at their seedling stage. Dwarf individuals from the segregating PI 655345 population resembled their sib dwarf counterparts and showed similar chlorophyll content as PI 655330. The tall group, however, exceeded the height of PI 655330 and showed a drastic reduction of chlorophyll content as compared to PI 655330.

Dwarfism has been known for some time in faba bean (Darlington, 1929) to be controlled by a single recessive gene (dw1) and to result in shorter internodes and total height (Erith, 1930; van Norel and Hoogendoorn, 1989; Bozzini and Chiaretti, 1999). This phenotype is quite different than the determinant ti phenotype, which is more suitable for agricultural production (De Costa et al., 1997). Generally, individuals expressing the dwarf phenotype can also appear to have darker and thicker leaves than their normal height sibs (Pelton, 1964). Independent chlorophyll mutations have also been observed independent of mutations in height (Sjödin, 1971).
In 2011, about 50% of the progeny from the dwarf individuals (PS11066-68) were tall, likely the result of foreign pollen (Fig. A1.1). Furthermore, all progeny from the normal height parent (PS11048) were tall, suggesting this individual was homozygous dominant for normal height. Leaf color was more variable in 2011, but based on seedling characteristics of select individuals in 2012, it appeared that these two traits might be controlled by separate genes that are closely linked (Table A1.1). However, definitive proof was lacking because outcrossing was not controlled. Therefore, an objective was set to clarify if there was linkage between height and leaf color and if dark leaf color was dominant (Sjödin, 1971) or recessive to light leaf color. We were able to do this by identifying a heterozygote for these two traits followed by self-pollination and scoring of the F$_2$ and F$_3$ inbred progeny families.

It was unknown whether the dwarf phenotype of PI 655345 was the result of a deficiency in brassinosteriod (Fukuta et al., 2004; Fukuta et al., 2006) or gibberellin biosynthesis (van Norel and Hoogendoorn, 1989). Although both mutations result in shortened epicotyls, brassinosteriod deficient plants have a glossy leaf and reduced seed size than ones deficient in gibberellin biosynthesis. Since mutations in the sensitivity to gibberellic acid are more common (Poulsen, 1981), we applied gibberellic acid to dwarf seedlings and observed their sensitivity as a response in height.

Positive mutations are ones that improve on some characters valued by agriculture (Sjödin, 1971). The semi-dwarf trait has been used extensively by faba bean breeders to improve lodging resistance and yield stability (van Norel and Hoogendoorn, 1989). Increased chlorophyll content may also improve photosynthetic efficiency, and therefore yield, although this remains to be seen. The phenotypic and eventual molecular characterization of the dwarf and dark-green foliage traits could enable breeders to create high yielding, lodging resistant, and semi-dwarf...
faba beans amenable to mechanized harvesting (Satovic et al., 1996). Therefore, the inheritance of these morphological traits and their linkages were followed within the PI 655345 derived population.

MATERIALS AND METHODS

In 2011, phenotypic screening for height and leaf color traits took place in the field when plants were approaching maturity. In 2012, screening was effective at the seedling stage under greenhouse conditions. Ten seeds of PI 655330, ten seeds of a dwarf individual selected from PI 655345, and 105 seeds of a putative heterozygote from PI 655345 were germinated and scored for height at the first and second internodes, as well as for chlorophyll content (SPAD-502 meter, Minolta Camera Co., Ltd., Osaka, Japan) of the first three leaves. Since it was unknown if the putative hybrid PI 655345 outcrossed, we decided to discontinue this population and start with a single individual with normal height (Fig. A1.5) from the dwarf white flowered PI 655345 parent. After further investigation this individual also expressed a red stem color at the juvenile stage similar to a mutation reported by Sjödin (1971), as well as a wild type flower color. This individual was transplanted to an insect-proof cage and self-pollinated seed collected at harvest.

In 2013, the F$_{1:2}$ seed was sown in the greenhouse and scored for height and leaf, stem, and flower color. The segregation ratio for monogenic traits would be expected to follow the classic 3:1 ratio. For all four traits, without linkage, we would expect a 81:27:27:27:9:9:9:9:3:3:3:3:1 ratio based on the general rule that $2^n$ gives the number of terms, $3^n$ provides the expected number of individuals expressing the dominant phenotype at all loci, and $4^n$ the total number of individuals of the combination series, where $n$=the number of loci in question. So for this example: $n$=4, $2^4$=16, $3^4$=81, and $4^4$=256. Since it was clear that this population did not conform to Mendel’s law of combination (independent assortment), as only
eight of the 16 classes were observed, chi-square tests were used to estimate the goodness-of-fit to Mendelian ratios (3:1, 9:3:3:1, etc.) for all traits and combinations (Harris, 1912; Hayes et al., 1955).

The chi-square test ($\chi^2$) for a 3:1 segregation ratio follows the general formula $\chi^2 = \Sigma (O-C)^2/C$, where $\Sigma$ refers to the summation of each observed recessive and dominant frequency (O) and expected frequency (C=expected ratio based on total observations). The chi square statistic can then be validated or falsified depending on the $\chi^2$ statistic according to the associated $P$ value and degrees of freedom (number of classes=2 for monohybrid). Goodness-of-fit tests were used to assess 9:3:3:1 segregation ratios of all dihybrid combinations following the formula $\chi^2 = (16(a^2+3b^2+3c^2+9d^2)/9N)-N$, where a, b, c, and d are the observed frequencies of each phenotype (a= A_B_, b= A_bb, c= aaB_, d= aabb) and N=total observations. A 9:3:3:1 segregation ratio should not be rejected if there was no linkage between the two loci. If a 9:3:3:1 segregation ratio is rejected, however, linkage is likely. In this case, percent recombination or genetic distance is estimated following the formula $[(b+c)/(a+b+c+d)]*100$.

Further progeny testing (Briggs and Knowles, 1967; Kidwell, 1970) was performed on F_2 individuals with particular phenotypes in hope of improving F_1 results. Scoring of progeny from a dominant phenotype parent would identify heterozygosity or homozygosity of that locus and allow estimation of recombination. Inbred progeny homozygous recessive for a particular allele, however, should be uniform and resemble the parent. In addition, a subset of F_2 individuals were open-pollinated and scored for outcrossing based on homozygous recessive traits.

To test for the dw1 dwarfing gene, a seedling sensitivity to GA_3 assay was used following van Norel and Hoogendoorn (1989). Briefly, GA_3 (400 ppm) was applied via irrigation to dwarf seedlings and compared to a non-treated control after three weeks.
RESULTS AND DISCUSSION

The genetics of the dwarf-tall and light-normal-dark leaf color phenotypes were inconclusive when populations were allowed to open-pollinate. Therefore, an outcrossed (hybrid) individual from a population homozygous recessive for both traits was selected and self-pollinated under controlled conditions. From this tall:normal leaf color heterozygote 124, $F_{1:2}$ seeds were collected, germinated, and scored for both traits. Two additional traits were also scored (green:red stem and wild type:white flower color) since the $F_1$ appeared heterozygous for these as well. Based on phenotypic seedling scores all four traits exhibited monofactorial segregation ratios (Table A1.2). Tall was dominant to dwarf, normal leaf color was dominant to dark green, red stem was dominant to green, and wild type flower was dominant over white.

Dihybrid segregation of height:leaf color, stem:flower color, and leaf color:stem color did not follow the expectations of independent assortment. Recombination frequencies highlighted height:leaf color and stem:flower color, linked at 7.26 cM, while leaf and stem color were independent. This discrepancy between goodness-of-fit and recombination equations may be explained by gametic competition resulting in segregation distortion (Lambrides et al., 2004).

Another interesting result was that only eight of the 16 possible phenotypic classes were observed within the $F_2$. Judging by the classes without representatives, the white flower genotype is pleiotropic over red stem and green stems are severely antagonistic to wild type flowers, regardless of height. The white flowered allele $zt1$ is known to be pleiotropic over anthocyanin pigmentation at the whole plant level, including the leuco-anthocyanin content of the testa (tannins) (Crofts et al., 1980; Bond and Poulsen, 1983; Bond, 1987). Sjödin (1971) reported that the red stem color was determined by anthocyanin production, which could explain the absence of the white:red stem phenotype observed here. To validate this hypothesis, the individuals with
red stems and wild type flowers were sown and assessed for possible flower:stem color segregates, but none were obtained.

The other major disparity involved combining the pure green stem and wild type flower traits. Only one of the four expected classes (normal height: normal leaf color) were observed with green stems and wild type flowers. To investigate this further, the two individual seedlings with green stems and wild type flowers in the F2 were scored at flowering for inheritance in the F3. Green stem color did not appear to be stably inherited, as the expression of red stem color varied with age and intensity. Very few individuals, overall, were completely absent of red stem color at flowering except when zt1 was present (Table A1.3). Progeny from heterozygous red stem:wild type flower parents were phenotyped with pure green stems:wild type flowers at varying frequency. Therefore, it remains to be proven that complete absence of stem anthocyanin combined with a wild type flower is heritable.

Fisher (1956) was instrumental in explaining that variation in discontinuous characters is the result of both modification via genotypic organization and interaction with the surrounding environment. In this way, qualitative traits can be scored into relatively clear groups, although variation within persists. Height is a classic example of this phenomenon, but stem color also exhibited this quantitative variation. Possibly scoring at the seedling stage or use of a colorimetric assay would improve precision, but the apparent presence of modifiers did not allow clear distinction except for the most extreme phenotypes.

Chlorophyll content also showed a normal distribution within classes (Fig. A1.3), making clear distinctions difficult. Individual F2 plants with either lighter or darker foliage than normal were progeny tested, confirming the relationship between dark leaf color and dwarf height, as well as light leaf color and normal height. However, dwarf individuals with lighter than typical
leaf color (34-39) did not consistently produce light colored progeny. Mean chlorophyll content of the individual families ranged from 44 to 51, whereas tall light color individuals (<40) produced families that ranged from 35 to 44. In comparison, none of the dwarf light color individuals produced progeny as low as the typical wild type plants, but there did appear to be some separation among the dwarf light color families. Conversely, normal height individuals with above average leaf color (40-42) produced means between 38 and 43, which were typically higher than other families derived from lighter color parents, but did not approach the leaf color of dwarf families (>50).

Similar to the F2, F3 families derived from F2 individuals segregating for flower color and normal and dwarf height produced the rare dwarf:light leaf color and tall:dark leaf color recombinants (Table A1.4). When combined, they produced similar genetic mapping distances. The dwarf individual with the lowest chlorophyll content was 38 and the highest chlorophyll content from a normal height individual was 57, exceeding F2 extremes. In comparison, the lightest F3 individual was 25 and the darkest 63. By growing out the F3 generation the total number of these rare individuals increased slightly, but none of the families produced as many as in the F2 generation. This is predicted based on the fact that heterozygosity is highest for the F1 and decreases by half for each subsequent generation (Briggs and Knowles, 1967).

In addition to the leaf color:height recombinants, a few individuals showed severe leaf yellowing of the youngest leaves that appeared to be an independent mutation than that of the dark-light green leaf color scored (Fig. A1.5). This mutation is described as *viridis* by Sjödin (1971) who stated that normal leaf color is dominant over mutants. Unfortunately, population sizes of dwarf (<50) and tall (<100) individuals were inadequate to further characterize this putative mutant leaf color allele. Scoring at the seedling stage does not appear to correlate with
scoring later in development, as the lightest leaf color individuals did not show this phenotype. Ultimately, increasing seed set during inbreeding (Coyne and Greene, 2002) will be necessary for any successful progeny testing of closely linked or mutant alleles.

The use of dwarfing genes to reduce lodging, increase chlorophyll content and retention, and aid in mechanized harvesting is dependent on the intensity of the height reduction as affected by the genetic background (van Norel and Hoogendoorn, 1989; Bozzini and Chiaretti, 1999). The dwarfing allele studied here is likely \( dw^{1} \), since the application of gibberellic acid stimulated internode elongation similar to the wild type (Fig. A1.5) (Pelton, 1964). Individuals homozygous for \( dw^{1} \) ranged from 20-30 cm at maturity, reducing their application to commercial agriculture. Single plants derived from parents heterozygous for \( dw^{1} \) produced mid-range heights (40-50 cm) and some F\(_3\) families were uniform for mid-parent height, suggesting a semi-dwarf height is heritable. The use of these dwarfing mutants is one way to alter aspects of growth, i.e., darker leaf color, thicker leaves, seed set, and maturity.

It did not appear that the high chlorophyll normal height individuals were stay-green mutants, as senescence proceeded independently and was likely influenced by environmental variables and polygenic control of maturity. For dwarf individuals, however, it did appear that maturity was delayed in association with darker leaf color and later flowering compared to taller sib families. Further investigation is necessary to validate if stay-green, dwarf height, and flowering time are physiologically/genetically linked as it has been observed in wheat (Thomas and Ougham, 2014).

The outcrossing rate of faba bean has been reported to be from 20 to 60% (Robertson and Saxena, 1993) to between 4 and 84% (Nassib and Khalil, 1982; Suso and Moreno, 1999). Some of this discrepancy can be attributed to the ability to accurately score intra-accession outcrossing...
and abundance of pollinators, which would tend to underestimate true outcrossing (Metz et al., 1994).

We found outcrossing rates that were relatively high compared to those found in the literature, given that the materials herein were F2 individuals, likely displaying substantial autofertility (Wright, 1977; Link, 1990). For example, three individuals with normal height and white flower color, i.e., homozygous recessive for flower color, had an outcrossing rate, i.e., wild type flowered progeny, of 33, 55, and 88%. These results verify the variability in outcrossing rate found for white flowered genotypes: De Vries (1978) <20%, Bond (1968) >50%, and Hu et al., (2011) 0-82.6%. Three individuals that were homozygous for the dw1 allele showed outcrossing rates, i.e., normal height progeny, of 21, 50, and 60%. Furthermore, two individuals homozygous for the recessive clear hilum trait produced 60 and 88% of their progeny with black hilum.

Summary

The dw1 allele that is associated with dwarf plant height and dark leaf color is likely a composite locus of multiple genes. Plant height and leaf color were found to be closely linked, with both light leaf color dwarf and dark leaf color tall individuals recovered from a segregating population. Moreover, modifying factors resulted in both traits exhibiting a quantitative distribution that appeared heritable by the F3 generation. Linkage between stem and flower color was likely the result of pleiotropy of the zt1 allele over anthocyanin metabolism. Unfortunately, the combination of white flowers, semi-dwarf height, clear hilum, and dark leaf color was not recovered in any F3 family, yet individuals with this phenotype were present. Due to significant outcrossing rates, maintaining these recessive traits would be difficult without isolation and probable inbreeding depression.
REFERENCES


Bozzini, A. and D. Chiaretti. 1999. The genetic improvement of the Mediterranean faba bean (Vicia faba L.) II. Transfer into Mediterranean lines of progressive traits found in other gentic pools: seed quality associated with pure white flower. J. Genet. & Breed. 53:201-206.


TABLES AND FIGURES

Table A1.1. Chi-square tests ($\chi^2$) for fit of segregation and recombination frequencies from a putative hybrid (without pollination restriction) for height and leaf color traits.

<table>
<thead>
<tr>
<th>2012: PI 655345 open pollinated F₂(F₁:DW/CH)</th>
<th>Expected (C)</th>
<th>Observed (O)</th>
<th>$\chi^2$ = $\Sigma$(O-C)/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dwarf-a &lt;2cm first internode &lt;3cm second internode</td>
<td>26.25</td>
<td>32</td>
<td>$\chi^2$ = 1.68</td>
</tr>
<tr>
<td>Tall-A</td>
<td>78.75</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll-dark- b &gt;40 chlorophyll content</td>
<td>26.25</td>
<td>40</td>
<td>$\chi^2$ = 19.81</td>
</tr>
<tr>
<td>Chlorophyll-light-B</td>
<td>78.75</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>56= A_B_</td>
<td>17= A_bb</td>
<td>3= aaB_</td>
<td>29= aabb</td>
</tr>
<tr>
<td>Goodness of fit 9:3:3:1</td>
<td>$\chi^2$ = (16(a²+3b²+3c²+9d²)/9N)-N</td>
<td>$\chi^2$ = 91.39</td>
<td></td>
</tr>
<tr>
<td>% recombination</td>
<td>[(Recombinants)/(total)]*100</td>
<td>19.05</td>
<td></td>
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</table>
Table A1.2. Chi-square tests ($\chi^2$) for fit of segregation and recombination frequencies from inbred progeny of a putative hybrid for height and leaf, stem, and flower color traits.

<table>
<thead>
<tr>
<th>2013: PI 655345 Outcrossed dark dwarf (F$_1$: Height 14cm; CHL 31.8; red stem; WT) F$_2$ population(controlled selfing)</th>
<th>Expected (C)</th>
<th>Observed (O)</th>
<th>$\chi^2=\Sigma[(O-C)^2/C]$</th>
<th>$\chi^2$ (df=1, $\alpha=0.05$)&lt;3.84 Do not reject $H_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dwarf-a &lt;2cm first internode &lt;3cm second internode</td>
<td>26.25</td>
<td>36</td>
<td>$\chi^2=1.08$</td>
<td></td>
</tr>
<tr>
<td>Tall-A</td>
<td>78.75</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll-dark- $b$ &gt;40 chlorophyll content</td>
<td>26.25</td>
<td>32</td>
<td>$\chi^2=0.04$</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll-light-$B$</td>
<td>78.75</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red stem-$C$</td>
<td>78.75</td>
<td>89</td>
<td>$\chi^2=0.69$</td>
<td></td>
</tr>
<tr>
<td>Green stem-$c$</td>
<td>26.25</td>
<td>35</td>
<td>$\chi^2=1.8$</td>
<td></td>
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<tr>
<td>White flower-$d$</td>
<td>26.25</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type flower-$D$</td>
<td>78.75</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57= $A_B_C_D_-$</td>
<td>3= $A_{bb}C_D_-$</td>
<td>9= $A_B_{cc}D_-$</td>
<td>20= $A_B_{cc}dd$</td>
<td></td>
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<tr>
<td>26= $aabbC_D_-$</td>
<td>3= $aaB_{cc}dd$</td>
<td>3= $aaB_C_D_-$</td>
<td>3= $aabbccdd$</td>
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<tr>
<td>Goodness of fit 9:3:3:1</td>
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<tr>
<td>$\chi'^2=(16(a^2+3b^2+3c^2+9d^2)/9N)-N$</td>
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<td>$\chi'^2$ (df=3, $\alpha=0.05$) &lt; 7.82 Do not reject $H_0$</td>
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<tr>
<td>% recombination [(Recombinants)/(total)]*100</td>
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<td>a/d=39.52</td>
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<td>b/c=49.19</td>
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<td>b/d=41.94</td>
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<td>c/d=7.26</td>
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<td>Conclusions: A &amp; B are independent of C &amp; D.</td>
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</table>

Crossing over

Tall-Light/Dwarf-Dark $\rightarrow$ Tall-Dark=3; Dwarf-Light=6
Red stem-WT/Green stem-White flower $\rightarrow$ Red-White=0; Green-WT=9
Table A1.3. Segregation analysis of F₃ families from F₂ plants with the rare green stem (g):wild type flower (WT):Tall (T) phenotype or putative red stem (R):WT:T or short (S) phenotypes.

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>Parent phenotype</th>
<th>R-WT-T</th>
<th>R-WT-S</th>
<th>G-WT-S</th>
<th>G-WT-T</th>
<th>G-W-T</th>
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<td>R-WT-T</td>
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<td>R-WT-S</td>
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<td>10</td>
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<tr>
<td>PS14023</td>
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<td>8</td>
<td>3</td>
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<td>PS14027</td>
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Table A1.4. Chi-square tests ($\chi^2$) for fit of segregation and recombination frequencies from combined inbred progeny of F$_2$ individuals identified as putative hybrids for height and leaf, stem, and flower color traits.

<table>
<thead>
<tr>
<th>2014: Combined progeny scores from 11 heterozygous F$_2$ individuals</th>
<th>Expected (C)</th>
<th>Observed (O)</th>
<th>$\chi^2=\Sigma[(O-C)^2/C]$</th>
<th>$\chi^2$ (df=1, $\alpha=0.05$) &lt; 3.84 Do not reject $H_0$</th>
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<tr>
<td>Dwarf- $a$ &lt;20cm at flowering</td>
<td>131</td>
<td>116</td>
<td>$\chi^2=2.5$</td>
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<tr>
<td>Tall-A &gt;20cm at flowering</td>
<td>395</td>
<td>411</td>
<td></td>
<td></td>
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<tr>
<td>Chlorophyll-dark- $b$ &gt;45 chlorophyll content</td>
<td>131</td>
<td>115</td>
<td>$\chi^2=2.8$</td>
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<tr>
<td>Chlorophyll-light- $B$ &lt;45 chlorophyll content</td>
<td>395</td>
<td>412</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red stem-C</td>
<td>131</td>
<td>400</td>
<td>$\chi^2=0.2$</td>
<td></td>
</tr>
<tr>
<td>Green stem-c</td>
<td>395</td>
<td>127</td>
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<tr>
<td>White flower-d</td>
<td>131</td>
<td>124</td>
<td>$\chi^2=0.6$</td>
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<tr>
<td>Wild type flower-D</td>
<td>395</td>
<td>403</td>
<td></td>
<td></td>
</tr>
<tr>
<td>287 = A_B_C_D_ \hspace{1cm} 2 = A_bbccdd \hspace{1cm} 21 = A_bbC_D_ \hspace{1cm} 3 = aabbccD_ \hspace{1cm} 101 = A_B_ccdd</td>
<td></td>
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<tr>
<td>74 = aabbC_D_ \hspace{1cm} 6 = aaB_ccdd \hspace{1cm} 18 = aaB_C_D_ \hspace{1cm} 15 = aabbccdd</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goodness of fit 9:3:3:1</td>
<td>\hspace{1cm} $\chi^2=(16(a^2+3b^2+3c^2+9d^2)/9N)-N$</td>
<td>No fit \hspace{1cm} No fit</td>
<td>\hspace{1cm} No fit…chance?</td>
<td></td>
</tr>
<tr>
<td>Do not reject $H_0$</td>
<td>$\chi^2$ (df=3, $\alpha=0.05$) &lt; 7.82</td>
<td>Fit \hspace{1cm} Fit \hspace{1cm} Fit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% recombination [(Recombinants)/(total)]*100</td>
<td>a/b=8.9 \hspace{1cm} a/c=36.9 \hspace{1cm} a/d=37.6 \hspace{1cm} b/c=38.3 \hspace{1cm} b/d=38.9 \hspace{1cm} c/d=0.57</td>
<td>Conclusions: A &amp; B linkage is independent of C &amp; D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crossing over</td>
<td>Tall-Light/Dwarf-Dark $\rightarrow$ Tall-Dark=23; Dwarf-Light=24 \hspace{1cm} Red stem-WT/Green stem-White flower $\rightarrow$ Red-White=0; Green-WT=3</td>
<td></td>
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</tbody>
</table>
Figure A1.1. Mature height and chlorophyll content of individual PI 655345 plants in 2011. Separate plots originated from single plant selections in 2010.
Figure A1.2. Seedling height and chlorophyll content of progeny from a homozygote dwarf individual of PI 655345, a normal height PI 655330, and a heterozygote for height of PI 655345. The putative heterozygote is further separated into dwarf and tall phenotypic classes.
Figure A1.3. Bean plot (A) and histogram (B) of chlorophyll content frequency distributions from the dwarf and normal height phenotypic classes of the F$_2$ and the dwarf (DW):white flower (W), tall (T):W, DW:wild type flower (WT), and T:WT phenotypic classes across F$_3$ families.
Figure A1.4. Bean plot (A) and histogram (B) of chlorophyll content frequency distributions from individuals of select F₃ families: 8 is all normal height and leaf color and 17, 24, and 19 are all dwarf and derived from F₂ individuals with below average chlorophyll content (<40).
Figure A1.5: (Clockwise from top left) Putative heterozygote for height and stem, flower, and leaf color; dwarf and normal height seedlings; possible Viridis mutation; flat of GA$_3$ treated seedlings on left and untreated on right.
Mass selection for faba bean tolerant to drought-like conditions

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most drought sensitive cool-season pulses (Dantuma et al., 1983; Day and Legg, 1983; Link et al., 1999; Ricciardi et al., 2001; Xia, 1994; Khan et al., 2010). However, water deficits during growth and development and terminal drought in Mediterranean-like environments are common throughout much of its geographical production range, possibly contributing to yield instability (Kahn et al., 2007; Torres et al., 2012).

Genotypes with drought resistance or drought escape and avoidance mechanisms should exist, however. Moreover, climate change is expected to increase the frequency and intensity of droughts (Cutforth et al., 2007). Therefore, identification of genotypes with yield stability in water-limiting conditions will be crucial for sustaining agricultural production in marginal environments.

Escaping heat and drought in faba bean is commonly achieved via the crop management strategies of early spring or autumn sowing (Lawes, 1978; Chaves et al., 2002). These practices generally outperform late spring sowing through earlier flowering, higher branching, and less pod shedding (McVetty et al., 1986; Herzog, 1989; Mwanamwenge et al., 1998; Thalji and Shalaldeh, 2006). Appropriate phenology is perhaps the second most important aspect of adaptation to environments with terminal drought (Chaves et al., 2002). Since much of the Pacific Northwest falls under a terminal drought cropping system, drought escape through early flowering, pod set, and maturity are traits of interest (Saxena et al., 1993), as well as avoidance
via reduced water loss through stomata and maintenance of water uptake via the root system (Torres et al., 2012).

Drought avoidance may be a component of drought escape, however, this is most important where transient droughts are common (Kahn et al., 2007). The mechanisms underlying tolerance to soil water deficit stress are complex but include dehydration avoidance (maintenance of tissue water potential via stomatal regulation/cuticle/leaf area and water scavenging) and recovery (oxidative response) (Turner, 1980; Chaves et al., 2002). Generally, small thick leaves are adapted to high light and temperature environments, optimizing carbon assimilation by minimizing transpiration losses and maximizing heat dissipation (Chaves et al., 2002). Therefore, a faba bean plant with a smaller, or lanceolate leaf phenotype, common of Mediterranean genotypes, may be more tolerant to water deficit than the larger oval leaves of northern European cultivars like Maris Bead (Dr. Stoddard, per. comm.).

A shorter plant has been shown to avoid drought stress by using less water (lower transpiration rate) than larger plants; however, yield has a negative correlation with height under high moisture conditions (Loss et al., 1997; Amede et al., 1999). Optimizing sink and source activity without compromising either is a perennial issue facing plant breeders who aim to increase production in marginal environments (Wardlaw, 1990).

Drought tolerant faba bean cultivars are difficult to breed for, due to the seasonal variation and intensity of drought stress (Kahn et al., 2007). Link et al. (1999) used shelters that prevented rainfall, however, this technique becomes costly with large populations. The USDA Central Ferry (CF) Research Farm in Central Ferry, WA is an ideal field environment for testing terminal drought response of faba bean. The CF location has a Chard silt loam soil, which is
lighter than the soils typically found on the Palouse. Light soils are known to exacerbate drought symptoms (Jensen et al., 2010). Furthermore, CF is on average warmer and receives less rainfall than Pullman and requires irrigation for most crop production. These factors coincide with when the crop is most drought sensitive (during flowering, pod set, and pod fill), making the selection of drought and heat tolerance ideal, given adequate genetic variation and sufficient selection pressure (Bond et al., 1994; Cutforth et al., 2007). Ceccarelli (1994) showed direct selection in the presence of stress to be more effective in improving yield than indirect selection for correlated traits in the absence of stress. Therefore, an experiment was initiated to directly select for a population that is adapted to the terminal drought conditions present at CF.

Mass selection involving single plant selections and bulking their progenies is an effective breeding methodology for improving yield and stability of a diverse faba bean collection (Nassib and Khalil, 1982), but is limited in improving traits from elite populations with low heritability (Hawtin, 1982). The main advantage of mass selection is the maintenance of genetic diversity, while improvement through natural and artificial selection. The danger of mass selection is that superior individuals are likely hybrids with low heritability for overdominant traits (Muehlbauer et al., 1988; Falconer and Mackay, 1996; Link et al., 2010). Indeed, drought tolerance in faba bean responds to heterosis and is reduced upon inbreeding (Khan et al., 2010). Population diversity is one way to exploit heterosis for drought tolerance.

MATERIALS AND METHODS

Assessing water use, water potential, stomatal characteristics, leaf temperature, and carbon isotope discrimination (Kahn et al., 2007) were beyond the scope of this study. Leaf temperature, as an indicator of stomatal conductance and leaf water potential (Hay and Porter,
through using a handheld infrared thermometer (Torres et al., 2012), was found to
distinguish between wilting and turgid individuals, however clear distinction among more
tolerant individuals will require further investigation. The main objective of this research was to
screen for soil moisture deficit adapted lines and disseminate these as a mass-reservoir
population or gene pool for future evaluation in drought prone environments (Duc et al., 2010).
To do this, a source population was created by bulk harvesting 466 USDA WRPIS accessions
spring-sown at the Central Ferry (CF) Research Farm in Central Ferry, WA, in 2010. A portion
of this harvest was then spring-sown at CF for three consecutive years (2011-13) without
irrigation. Mass selection was practiced each year selecting for early maturing individuals with
above average pod set. The selection index was approximately 10%. Sowing was on 9 April
2011, 4 April 2012, and 2 April 2013 and planted with a Hege 120 planter with four rows spaced
35 cm apart. Planting density was low (40 kg ha\(^{-1}\)) to maximize evapotranspiration (Kahn et al.,
2007), increase moisture stress (Robertson and Saxena, 1993), and to streamline single plant
selections.

Treflan (a,a,a- Trifluoro-2,6- dinitro- N,N-dipropyl-p-toluidine; Dow Chemical) was
applied pre-emergent to control monocot weeds. From planting to harvest, plants solely relied on
stored soil moisture. A block of winter-type faba bean was added in 2014 to compare
phenological development and stress tolerance.

**RESULTS AND DISCUSSION**

Heat and drought conditions were sufficient to differentiate tolerant and susceptible
plants at CF through the duration of the trial (Fig. A2.1). During the final cycle of selection
plants with no pods to over 20 pods were observed. Yield was perceptibly lower than adjacent
irrigated trials. Furthermore, early flowering appeared to be related to pod set and single plant yield as was observed in other trials (Chapter 4). The block of winter-type field bean was later to flower and mature and was notably taller at maturity than the mass selected population. Subsequently, fewer plants with more than 10 pods were found, confirming speculations made in previous studies (Chapter 4) with spring-sown winter-type materials that winter-type faba bean genotypes are ill suited for short season environments.

Common mechanisms of resistance to heat and drought are through escape or accelerated phenology, avoidance through reduced canopy temperature, and tolerance through metabolic protectants (Wery et al., 1993). Wery et al. (1993) divided the grain legume life cycle into five basic phases: phase 1 is from emergence to flowering; phase 2 is flowering; phase 3 is grain fill; phase 4 is physiological maturity; and phase 5 is harvest. Drought stress during phase 1 leads to a loss of biomass, while in phase 2-3 reduces seed and pod number along with seed size. During the final season of selection, seedlings (phase 1), with the most extreme drought symptoms, failed to flower or set seed, while seedlings without symptoms were often selections at harvest.

It remains unclear why a number of plants remained turgid, while adjacent neighbors wilted. Possibly resistant individuals had improved osmoregulation, transpiration efficiency, or acquired untapped soil moisture due to a more vigorous root system. Individuals with severe wilting symptoms had a foliage temperature >30°C, while plants with turgid leaves measured consistently below 30°C using an infrared thermometer. Soil surface temperature at this sampling was ~57°C. Leaf temperature, or its depression below atmospheric temperature, is a potential indicator of plant water deficit, since stomatal closure results in decreased
transpirational cooling and increased leaf temperature relative to well watered plants (Khan et al., 2010). Unfortunately, intra and interplant variation limited the use of leaf temperature as a screening tool within drought tolerant individuals and could only distinguish what was already obvious to the eye. The use of an infrared thermometer to distinguish between selected replicated families could, however, be a cost effective and simple method to make further selections from this initial population.

Surprisingly, there appeared to be more severe wilting of seedlings within the mass selected than the winter-type population. However, in general, leaf size and number appeared to be less for individuals from the mass selected than from the winter-type population. Commonly, large thin leaves with a low specific leaf area are susceptible to fire (Kahn et al., 2007), or have the inability to maintain leaf turgor during drought and heat stress, resulting in leaf abscission. Fire could be a result of oxidative damage associated with the Mehler reaction and the inability to keep leaves cool (Chaves et al., 2002). Due to a prolonged vegetative phase and possible synergism of osmoprotection against freezing and drought conditions the winter-type population may have had drought tolerance at the seedling stage because of a greater production of roots at the expense of vegetative development.

Early drought stress would explain the reduced height (Husain et al., 1990) of even the most tolerant plants in comparison with adjacent irrigated plots. Seedlings with severe wilting often failed to elongate and flower explaining the lack of root growth. It is known that as drought progresses, stomata close for increasing longer periods of the day in response to ABA translocated from the root system and leaf turgor and/or water potential in response to low humidity and reduced evapotranspiration (Chaves et al., 2002). This reaction reduces water loss,
but also depresses gas exchange and the photosynthetic machinery that assimilates carbon allowing for continued development. Possibly from an early developmental stage the seedlings that did not tap into enough stored moisture failed to maintain growth, while more vigorous seedlings did.

While stomatal conductance, epicuticular wax, and rooting depth (50-90 cm for faba bean) are important in drought avoidance (Khan et al., 2010), trade-offs between yield potential and fitness based on phenology (Stoddard, 1993) need to be taken into consideration. This study has highlighted the importance of vigorous stand establishment as a primary importance prior to later developmental phenomenon. Seedling vigor may have genetic components, which selection could act upon, and environmental components, which depending on the environment of the maternal parent, would be less functional and more problematic. In all likelihood, the individuals selected *en masse* were hybrids exhibiting heterosis for seedling vigor. Therefore, the heritability for the perceived water use efficiency would be expectedly low and destabilized by inbreeding (Bond et al., 1994).

Further progeny testing of individuals from this mass selected population would characterize heterozygotes and the heritability for seedling vigor and water use efficiency. Alternatively, the mass selected population could be made available to researches interested in selecting faba bean for drought stressed conditions; i.e., soil water availability <70% of field capacity (Hebblethwaite, 1982). A diverse array of seed colors and types remains to be exploited within this population (Fig. A2.2).
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115:270-278.


FIGURES

Figure A2.1. Drought stressed and normal seedlings.
Figure A2.2. Seeds of mass selected population after three seasons of terminal drought conditions.
White flowered allelism: Zero tannin alleles in the NPGS faba bean collection

INTRODUCTION

White flowered accessions were discovered during the characterization of the WRPI NPGS faba bean collection. Two accessions consisted of all white flowered plants and 11 accessions were segregating for white and wild-type flowers, i.e., colored wings. The wild-type flowers are pleiotropic over the presence of tannins. Tannins are anti-nutritional interfering with the digestion of protein (Bond, 1976) and inhibit cellulase action within ruminants (Lawes, 1978). The tannin concentration of seed coats range from 4 to 8% for wild-type flowered genotypes (Duc et al., 1995). It has been reported that two independent recessive genes, zt1 (Vicia faba major L. origin) and zt2 (Vicia faba minor L. origin), control the absence of seed coat tannins and block the synthesis of anthocyanin, or anthocyanin precursors, producing white flowered plants (Cabrera, 1988; Metz et al., 1992). These zero tannin alleles are used to improve the nutritional value of faba bean for food and feed (van Norel, 1985).

The objective of this study was to identify the zero tannin alleles (zt1 or zt2) within the thirteen identified white flowered accessions through a complementation study. Based on available literature, the two zero tannin alleles zt1 and zt2 were expected to be allelic (Crofton et al., 2000). Thus crossing zt1 x zt1 would result in progeny with white flowers. However zt1 x zt2 or zt2 x zt1 would give progeny with a wild-type flower, since ZT1zt2 x ZT1ZT2 or ZT1ZT2 x ZT1zt2 would complement one another, i.e., ZT1zt1 & ZT2zt2. The majority of cultivars, especially of English origin (‘Express Long Pod’ and ‘Giant White Windsor’) and their
descendants (accessions from Quebec), were expected to possess the \( zt1 \) allele, as \( zt2 \) is less common.

**MATERIALS AND METHODS**

To test for allelism between identified white flowered accessions, crosses were made between PI 415044 and all other white flowered accessions (Table A3.1). It was unknown if PI 415044 had the \( zt1 \) or \( zt2 \) gene. Both \( zt1 \) and \( zt2 \) are complementary so when crossed should produce a wild type flower phenotype (Crofts et al., 1980). If the F₁ produced a white flowered phenotype the parents may have the same allele or are allelic at the same locus. If the F₁ does not possess either \( zt1 \) or \( zt2 \) then a novel complementary gene may be responsible.

**RESULTS AND DISCUSSION**

We were not able to distinguish any of the white flowered accessions as all crosses produced white flowered progeny. Since, \( zt1 \) is more commonly utilized than \( zt2 \) (Crofton et al., 2001) it is assumed that all white flowered NPGS accessions carry the \( zt1 \) allele.

Hybridization between known sources of the \( zt2 \) allele or the utilization of SCAR markers (Gutierrez et al., 2007) would help to further identify the zero tannin genes and determine if additional loci are present in the collection.
REFERENCES


Table A3.1. NPGS accession, name, and origin of white flowered faba bean.

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<th>Accession #</th>
<th>Name</th>
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</tr>
<tr>
<td>PI 415044</td>
<td>325</td>
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<td>PI 433531</td>
<td>Grosse Windsor</td>
<td>Quebec, Canada</td>
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<td>PI 433537</td>
<td>Triple Blanche</td>
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<td>PI 433539</td>
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</tr>
<tr>
<td>PI 469118</td>
<td>Express Long Pod</td>
<td>UK</td>
</tr>
<tr>
<td>PI 469120</td>
<td>Giant White Windsor</td>
<td>UK</td>
</tr>
<tr>
<td>PI 469128</td>
<td>NO. 170</td>
<td>Morocco</td>
</tr>
<tr>
<td>PI 469143</td>
<td>Tenquchene</td>
<td>Iran</td>
</tr>
<tr>
<td>PI 469200</td>
<td>Threefold White <em>zt1</em></td>
<td>UK</td>
</tr>
<tr>
<td>PI 655330</td>
<td>Eureka <em>zt1</em></td>
<td>Kenya</td>
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<td>PI 655331</td>
<td>NEB 247</td>
<td>Kenya</td>
</tr>
<tr>
<td>PI 655345</td>
<td>Maris Bead ‘dwarf’</td>
<td>New Zealand</td>
</tr>
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<td>PI 655345</td>
<td>Maris Bead ‘tall’</td>
<td>New Zealand</td>
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</table>
CHAPTER SEVEN

GENERAL CONCLUSIONS

The inclusion of winter-type faba bean into the current cropping systems of southeastern WA, USA will require additional cultivar development over a broad environmental context. This dissertation has shown the potential for autumn sowing winter-type faba bean, but has also highlighted significant agronomic barriers to successful adoption. Breeding for winter-hardiness, earlier development, and disease resistance are feasible, but will require resources limited by consumer demand.

The faba bean germplasm maintained by the USDA-ARS Western Regional Plant Introduction Station is one available source of traits for regional adaptation. Bulk selection has improved the winter-hardiness of populations and individual accessions derived from this germplasm, while promoting phenotypic diversity. These materials, made available to the public, are amenable to selection from a diverse array of quality traits and selection for adaptation to diverse environmental contexts. Released populations are recommended for areas not exceeding an air temperature of -25°C or a soil temperature at the seed of -7°C during the winter. Moreover, faba bean, grown for seed, should be limited to areas that rarely exceed an air temperature of 25°C during the flowering period (May-July).

It appears that the large seeded var. Major faba bean genotypes are, in general, less winterhardy than var. Equina or Minor. Possibly this is due to the relationship between seed size and glucose, fructose, and sucrose content, or other metabolites resulting in freezing injury. Further cold acclimation studies elucidating the relationship between seed size and percent moisture and metabolite dynamics of the seed through germination and early development are necessary to validate this theory. Comparing winterhardy, small seeded northern European
materials with moderately hardy, large seeded cultivars such as Ianto’s Return (172 g 100 seed\(^{-1}\)) may be informative.

The physiological and genetic underpinnings of winter-hardiness in faba bean remain relatively unknown limiting progress by breeders. Exploiting heterosis in the form of synthetic cultivars remains the most effective technique for optimizing both winter-hardiness and yield of faba bean. Hybridization between northern European and Mediterranean gene pools will likely provide useful materials adapted to southeastern WA conditions. The Extra Precoce Violettto x Hiverna/2 population provided insight into how early flowering and maturity, coupled with sufficient winter-hardiness, can produce yields over 8 t ha\(^{-1}\). These traits, as well as numerous others, were observed within the germplasm derived bulk populations and will require further investigation into their use.

Bulk population management schemes remain efficient and effective at creating repositories of potentially useful alleles, while producing moderate genetic gain, given sufficient source diversity.